QUANTITATIVE BIOIMAGING qbi CONFERENCE

2019 Quantitative BioImaging Conference

January 8 – 11, 2019

Le Couvent des Jacobins

4-6, rue d'Echange
35000 Rennes, France
Phone: +33 (0)2 99 45 90 50

Table of Contents

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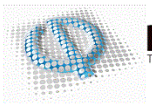

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Marketing and Workshop Presenters

Hamamatsu Photonics Workshops

Wednesday (9 January) 10:40 to 17:00 in Horizons room
Thursday (10 January) 11:15 to 17:00 in Horizons room

Registration for the Hamamatsu workshop can be found at:

https://www.hamamatsu-news.de/promo/qbi18/

Oxford NanoImaging Marketing Session

Wednesday (9 January) 16:25 to 16:40 in Lecture Room 1 (NEF)

QuantaCell Marketing Session

Thursday (10 January) 16:15 to 16:20 in Lecture Room 1 (NEF)

GdR ImaBio Sponsor Session

Friday (11 January) 16:35 to 16:40 in Lecture Room 1 (NEF)

France-BioImaging Sponsor Session

Friday (11 January) 16:20 to 16:35 in Lecture Room 1 (NEF)

GE Healthcare Marketing Session

Thursday (10 January) 16:00 to 16:15 in Lecture Room 1 (NEF)

Conference Exhibitors

Le Couvent des Jacobins Floor Plan

Level 0

Cloakroom

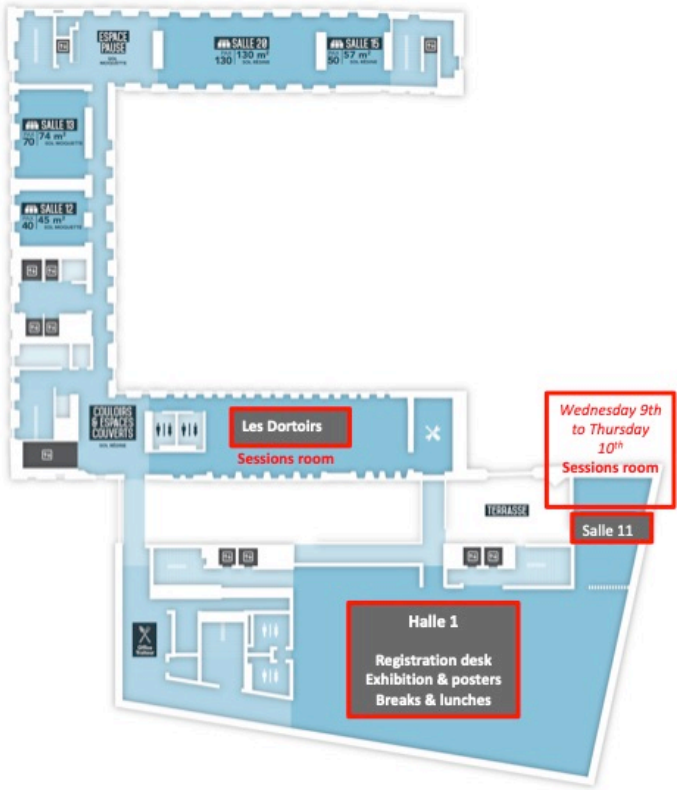
Lecture Room 1 (NEF)

Level 1

Lecture Room 2 (Dortoirs)

Lecture Room 3 (Salle 11)
Wednesday and Thursday Sessions

Foyer (Hall 1)
Reception/Registration Desk
Exhibitor's Area
Poster Area
Food/Catering Area



Level 2

Hamamatsu Workshop (Horizons)
Wednesday and Thursday

Lecture Room 3 (Horizons)
Friday Sessions

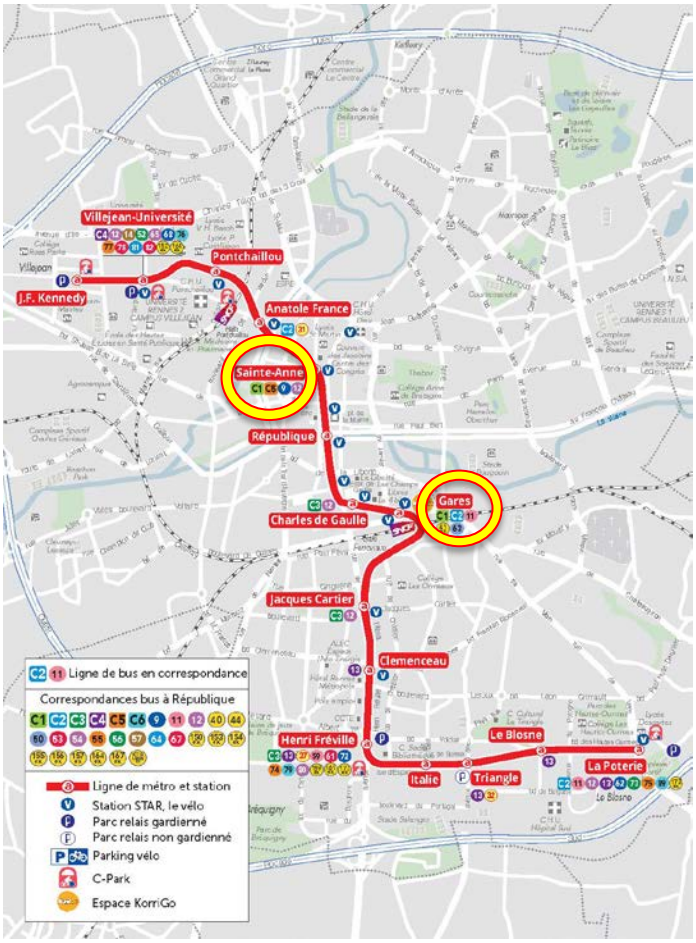
Bus and Metro Information

Le Couvent des Jacobins, the Sainte-Anne Metro Stop, and Surrounding Area

From the [Rennes Tourist Office]

The Metro A line has 15 stations located near several of the city's key locations with Sainte-Anne, less than a 1 minute walk from Le Couvent des Jacobins (see link for Metro line A map below).

The Metro A line is the easiest way to access the Jacobins convent from the station. It takes about 10mn.



Bus routes C1, C5, 9 and 12 also stop at Sainte-Anne making public transport between the conference site and several locations throughout Rennes possible (see link for Map and schedules by bus route below).

Metro and Bus Fares

1 hour ticket: €1.50
Book of 10 tickets: €14.50
Group tickets: €6.60
(More information on tickets can be found on the STAR website: https://www.star.fr/titres-et-tarifs/detail-des-titres-et-tarifs/au-trajet/)

NOTE: Due to a strike movement on the STAR network, the bus route mentioned above are running at reduced service levels in December 2018 and might continue at reduced service into January 2019. The Metro service is currently unaffected.

Metro line A map:
https://www.tourisme-rennes.com/uploads/file/2016/02/plan-metro-rennes.pdf

Map and schedule information by bus route:
https://www.star.fr/se-deplacer/fiches-horaires-et-plans/

Conference Registration and Badge Pickup

For your security, an access control will be carried out at the entrance of the Couvent des Jacobins conference center.

Please show your printed REGISTRATION CONFIRMATION email to the security guards in order to access to the QBI 2019 conference.

Workshop Registration – Tuesday (8 January)

08:00 to 16:00
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer, Hall 1 - Registration Area (Niveau 1)
4-6, rue d'Echange
35000 Rennes
France

Primary Conference Registration – Tuesday (8 January)

17:15 to 21:15
Overlap with Opening Reception (18:15 to 20:30)
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer, Hall 1 - Registration Area (Niveau 1)
4-6, rue d'Echange
35000 Rennes
France

Continued Conference Registration – Wednesday to Friday (9-11 January)

08:00 to 20:30
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer, Hall 1 - Registration Area (Niveau 1)
4-6, rue d'Echange
35000 Rennes
France

Scientific Organizing Committee QBI 2019

Chairs

Raimund Ober (College Station / Southampton)

Charles Kervrann (Rennes)

Members

Martin Booth (Oxford)

Chris Calderon (Boulder)

Edward Cohen (London)

Susan Cox (London)

Joerg Enderlein (Göttingen)

David Grunwald (Worcester)

Charles Kervrann (Rennes)

Diane Lidke (Albuquerque)

Keith Lidke (Albuquerque)

Sripad Ram (San Diego)

Jens Rittscher (Oxford)

Jean Salamero (Paris)

Mark Tsuchida (San Francisco)

Daniel Wüstner (Odense)

E. Sally Ward (College Station / Southampton)

Quantitative BioImaging Society

Meeting to discuss future plans and events for the QBI Society

Thursday (10 Junuary) at 20:30

Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Room 11 (Niveau 1)

Session Chair: Raimund Ober

Meals and Refreshments

The following meals and refreshments are provided free of charge to workshop and conference attendees.

Tuesday (8 January)

10:40 to 11:00
Coffee Break
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

16:45 to 17:15
Coffee Break
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

Wednesday (9 January)

10:10 to 10:40
Coffee Break
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

12:25 to 13:45
Lunch
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1) - Food Area

15:15 to 16:45
Coffee Break and Poster Session
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

18:45 to 22:45
Opening Reception and Poster Viewing
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

Thursday (10 January)

10:40 to 11:10
Coffee Break
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

12:10 to 13:30
Lunch
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1) - Food Area

14:50 to 16:20
Coffee Break and Poster Session
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

Friday (11 January)

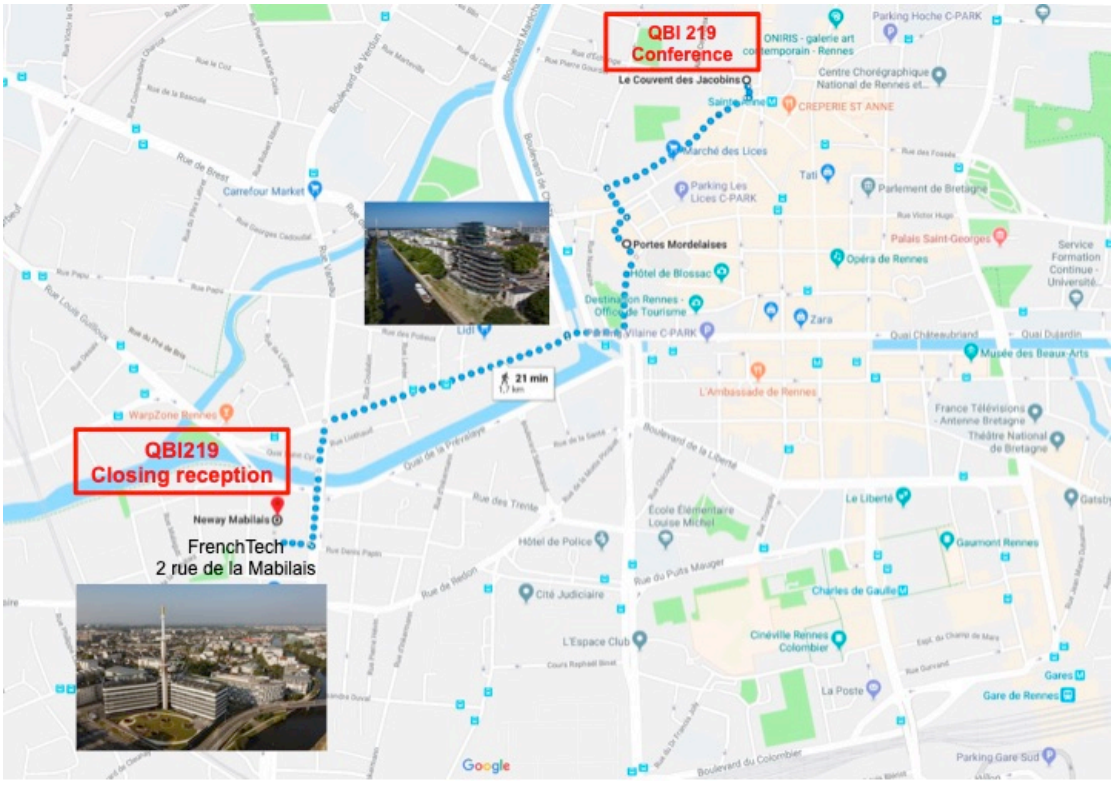
10:10 to 10:40
Coffee Break
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

12:10 to 13:30
Lunch
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer, (Hall 1) - Food Area

15:15 to 16:45
Coffee Break and Poster Session
Le Couvent des Jacobins - Centre des Congrès de Rennes Métropole
Foyer (Hall 1)

19:00 to 23:00
Closing Gala Dinner Reception
La French Tech - Le Pool (Le Mabilay, 2, rue de la Mabilais, Rennes)

Map : Le Couvent des Jacobins (Venue) to La French Tech (Closing Reception)



Conference Information

Internet Wi-Fi Access

Sessions Rooms	Network: couventdesjacobins Connection time: 30 minutes
Foyer (Hall 1)	Login: QBI_2019 Password: QBI_2019

Cloakroom/Baggage Room

Atrium on Level 0	Open for conference attendees	Tuesday (8 January) 08:30 to 20:30 Wednesday (9 January) 08:00 to 22:15 Friday (11 January) 08:00 to 18:45

Posters

Posters should be in vertical format, and the maximal dimensions should be 3.28 feet wide x 4.92 feet height (1.0m wide x 1.5m high). The conference organization will supply materials for mounting posters on the boards. Each poster will be mounted on the appropriately numbered board corresponding to the submitted abstract number.

Set up	Wednesday (9 January) starting at 08:00
Take down	Friday (11 January) beginning at 16:45 and no later than 18:10

Keynote Speakers

Michael Elad

(Computer Science; Technion, Israel Institute of Technology- Technion City, Haifa, Israel)

"Michael Elad works in the field of signal and image processing, specializing in particular on inverse problems and sparse representations. Prof. Elad has authored hundreds of technical publications in leading venues, many of which have led to exceptionally high impact. He is the author of the 2010's book 'Sparse and Redundant Representations: From Theory to Applications in Signal and Image Processing', which is a leading publication in this field." (source: Technion)

Dr. Elad's presentation, 'Sparse modeling of data and deep learning', is scheduled for 08:30 on Wednesday, 9 January.

Frederick Maxfield

(Weill Cornell Medical College - New York, New York, USA)

"The central focus of the laboratory is the development and use of new optical microscopy and biophysical techniques to study the properties of living cells. We use digital imaging devices, confocal microscopes, multiphoton microscopy, automated microscopy systems, and image processing computers to analyze processes occurring at specific sites within cells. Using these tools, we study the distribution and movement of various types of molecules in cells. We are interested both in the basic mechanisms regulating the movement of molecules through cells as well as the role that these processes play in specific diseases." (source: Weill Cornell)

Dr. Maxfield's presentation, 'Using microscopy to dissect endolysosomal trafficking and function' is scheduled for 08:30 on Thursday, 10 January.

Ernst HK Stelzer

(Buchmann Institute for Molecular Life Sciences; Goethe-Universität Frankfurt am Main, Germany)

"A major goal of the Physical Biology Group (AK Stelzer) is to pursue experiments in the life sciences under close-to-natural conditions. Hence, its members favor primary cell lines and try to avoid experiments with cultured cell lines, they favor three-dimensional cell cultures over two-dimensional cell monolayers that are cultivated on hard and flat surfaces and they try to maintain the three-dimensional context of plants, cell clusters, tissues sections and small animal embryos. In consequence, many specimens are relatively large, optically dense and require advanced methods in terms of preparation, maintenance, visualization, data handling and data analysis." (source: Goethe-Universität)

Dr. Stelzer's presentation, 'Analyzing three-dimensional biological specimens observed with light sheet microscopy under near-natural conditions', is scheduled for 09:50 on Thursday, 10 January.

Rainer Heintzmann

(Institute of Photonic Technology; Friedrich Schiller Universität - Jena, Jena, Germany)

“Rainer Heintzmann's research focusses on imaging cellular function at high resolution. His group develops techniques to measure multidimensional information in small biological objects such as cells, cellular organelles or other small structures of interest. A further interest is in computer-based reconstruction methods.” (source: Friedrich Schiller)

Dr. Heintzmann’s presentation, ‘Structured Illumination, past and present development’, is scheduled for 08:30 on Friday, 11 January.

Invited Speakers

Pierre Bon [Laboratoire Photonique Numérique et Nanosciences] - Talence, France
Edward Cohen [Imperial College] - London, UK
Hans-Ulrich Dodt [Medical University of Vienna and Vienna University of Technology] - Vienna, Austria
Kevin Eliceiri [University of Wisconsin at Madison] - Madison, Wisconsin
Seth Flaxman [Imperial College London] - London, UK
Spencer Freeman [University of Toronto] - Toronto, Canada
Thomas Huser [Biomolecular Photonics Group] - Bielefeld, Germany
Khuloud Jaqaman [UT Southwestern Medical Center] - Dallas, Texas
Ludger Johannes [Institut Curie] - Paris, France
Florian Jug [Max Planck Institute of Molecular Cell Biology and Genetics] - Dresden, Germany
Yannis Kalaidzidis [Max Planck Institute of Molecular Cell Biology and Genetics] - Dresden, Germany
Friedemann Kiefer [Max Planck Institute for Molecular Biomedicine] - Muenster, Germany.
Judith Klumperman [University Medical Center Utrecht] - Utrecht, The Netherlands
Gregory R. Johnson [Allen Institute of Cell Science] - Seattle, Washington
Julien Mairal [INRIA, Grenoble] - Grenoble, France
Jean-Christophe Olivo-Marin [Institut Pasteur] - Paris, France
Steve Pressé [Arizona State University] - Tempe, Arizona
Bernd Rieger [Delft University of Technology] - Delft, Netherlands
Jonas Ries [European Molecular Biology Laboratory] - Heidelberg, Germany
Silvio Rizzoli [University Medical Center] - Goettingen, Germany
Daniel Sage [EPFL] - Lausanne, Switzerland
Anne Sentenac [Institut Fresnel] - Marseille, France
Ernst Stelzer [Buchmann Institute for Molecular Life Sciences] - Frankfurt, Germany
Per Uhlén [Karolinska Institutet] - Stokholm, Sweden
Geert van den Bogaart [Groningen Biomolecular Sciences and Biotechnology Institute] - Groningen, Netherlands
Simon Walker-Samuel [University College] - London, UK
Daniel Wüstner [Syddansk Universitet] - Odense, Denmark
Marino Zerial [Max Planck Institute of Molecular Cell Biology and Genetics] - Dresden, Germany
Christophe Zimmer [Institut Pasteur] - Paris, France

Workshops

1. Adaptive Optics

This workshop will review the practice and implementation of adaptive optics in microscope systems. This will be relevant to researchers who are interested in using adaptive optics in custom built microscopes of various modalities. For this workshop, Martin Booth will present 'Practical aspects of adaptive optics for microscopes' (8 January at 08:30 to 10:45 in Lecture Room 2/Dortoir).

2. Machine Learning

The presentations by Seth Flaxman (8 January at 11:00 to 12:30 and 13:30 to 14:30 in Lecture Room 2/Dortoir) will introduce the theme of machine learning:

- Supervised vs unsupervised learning: k-nearest neighbors, k-means clustering
- From linear to nonlinear methods: transformations, featuring engineering, and the kernel trick
- Bias vs variance, and how to deal with the problem of overfitting
- Regularization, lasso, ridge regression, and the elasticnet
- Ensemble methods
- Gaussian processes

The presentation by Julien Mairal (8 January at 14:30 to 15:45 in Lecture Room 2/Dortoir) will cover techniques recently introduced in machine learning and optimization to deal with large amounts of data. The focus will be on regularized empirical risk minimization problems, which consists of minimizing a large sum of functions, and cover also stochastic optimization techniques for minimizing expectations. Concepts covered will include introductions to advanced themes in machine learning:

- Stochastic gradient descent techniques with variance reduction
- Nesterov's acceleration
- Quasi-Newton techniques

Also included will be variants that allow dealing with nonsmooth regularization such as the l1-norm, which is useful for sparse estimation in high dimension

The presentation by Christophe Zimmer (8 January at 15:45 to 16:45 in Lecture Room 2/Dortoir) will introduce themes of deep learning:

- What deep learning can do?
  - Image classification
  - Other Applications
- How deep learning works.
  - Forward propagation
  - Convents
  - Training
  - Babysitting neural nets
- DIY deep learning with Keras.

3. 3D Single Molecule Microscopy Data Analysis

For this workshop, Daniel Sage will present 'Single-molecule localization microscopy: performing 3D super-resolution reconstruction using open-source software' (8 January at 17:15 to 19:15 in Lecture Room 2/Dortoir).

Minisymposia

1. **Microscopy in BioPharma**

This session will focus on the recent explosive growth of digital microscopy technologies in biotech, biopharma and life-sciences industries especially in the preclinical and clinical space. This year, a plenary lecture (10 January at 09:50 to 10:30 in Lecture Room 1/NEF) and the minisymposium session (10 January at 16:20 to 18:00 in Lecture Room 1/NEF) will focus on 3D imaging and quantitative analysis of cleared tissues. The talks will revolve around sample preparation, 3D imaging and 3D image analysis of cleared samples for studying the vasculature, drug delivery and immune-cell distribution in tumors.

Pleanary Speaker

Ernst Stelzer (Johann Wolfgang Gothe-Universität Frankfurt am Main) 'Analyzing three-dimensional biological specimens observed with light sheet microscopy under near-natural conditions'

Minisymposium Speakers

Per Uhlén (Karolinska Institute) 'Volumetric imaging of whole-tumors reveal cancer malignancy'

Friedemann Kiefer (Max Planck Institute for Molecular Biomedicine) 'Analyzing development, functions and pathofunction of the vascular systems using light sheet microscopy'

Simon Walker-Samuel (University College London) 'Combining optical imaging of cleared tissue with mathematical modeling and in vivo imaging to predict drug delivery and therapeutic response'

David Rousseau (Laboratoire LARIS, Université d'Angers, Angers, FR) 'Deep learning based detection of cells in 3D Light sheet fluorescence microscopy'

2. **Structured Illumination: a Review of the State of the Art**

Structured illumination is powerful technique to improve resolution in microscopy. While it had a major impact on the field significant current research points to the potential for major advances in the future. This theme will include a keynote lecture (11 January at 08:30 to 09:20 in Lecture Room 1/NEF), contributed presentations, and minisymposium speakers (11 January at 10:40 to 12:10 in Lecture Room 1/NEF) presenting an overview of the state of the art.

Keynote Speaker

Rainer Heintzmann (Institute of Physical Chemistry Jena University) 'Structured Illumination, past and present development'

Minisymposium Speakers

Thomas Huser (University of Bielefeld), 'High-speed, real-time reconstructed structured illumination microscopy of living cells'

Anne Sentenac (CNRS Institut Fresnel), 'Random Illumination Microscopy: using uncontrolled speckles to improve the resolution of fluorescence microscopy'

Jean-Christophe Olivo-Marin (Institut Pasteur), 'A signal processing view of Structured Illumination Microscopy'

3. **Machine Learning in Bioimaging Analysis and Microscopy**

Machine learning is one of the main themes of this year's conference. The conference starts with a workshop on machine learning (8 January at 11:00 to 16:45 in Lecture Room 2/Dortoir), followed by keynote and plenary lectures (9 January at 08:30 to 09:40 in Lecture Room 1/NEF), contributed presentations and this minisymposium (9 January at 10:40 to 11:40 in Lecture Room 1/NEF).

Keynote and Pleanary Speakers

Michael Elad (Technion) 'Sparse modeling of data and deep learning'

Florian Jug (Max Planck Institute of Molecular Cell Biology and Genetics) 'Content-aware image restoration for light and electron microscopy'

Workshop Speakers

Christophe Zimmer (Institut Pasteur), 'Introduction to deep learning'

Julien Mairal (INRAI), 'Large-scale optimization for machine learning'

Seth Flaxman (Imperial College London), 'Machine learning workshop: an introduction to machine learning and a taste of advanced methods'

Minisymposium Speakers

Gregory R Johnson (Allen Institute for Cell Science), 'Label-free prediction of subcellular organization: capturing variation and integrating observations'

Steve Presse (Arizona State University), 'Unraveling the Rules of Life a few photons at a time'

Christophe Zimmer (Institut Pasteur), 'Computational boosts to single molecule localization microscopy'

4. **Quantitative Tools to Define Subcellular Trafficking Processes**

Microscopy tools play a central tool in cell biology. However, the impact of microscopy has been significantly higher in subfield where images could be taken in one focal plane such as the plasma membrane. This minisymposium addresses the problem of subcellular trafficking which is key to the functions of cells. As this is genuinely 3D problem developments have been more limited. This theme includes both a plenary speaker (10 January at 08:30 to 09:20 in Lecture Room 1/NEF) and a minisymposium to present different related cell biological questions and methods to address them (10 January at 11:15 to 12:15 in Lecture Room 1/NEF).

Plenary Speaker

Fred Maxfield (Weill Cornell Medical College) 'Using microscopy to dissect endolysosomal trafficking'

Minisymposium Speakers

Ludger Johannes (Institut Curie), 'Lectin-driven and glycosphingolipid-dependent construction of endocytic pits'

Daniel Wüstner (Syddansk Universitet), 'Fluorescence studies of sterol transport between cellular membranes'

Geert van den Bogaart (Groningen Biomolecular Sciences and Biotechnology Institute), 'Quantitative visualization of SNARE complex formation in living cells'

5. **Software Design for Image Analysis**

Over several years software design for microscopy and image analysis has played an important role in the QBI conference. This year we continue this theme with a plenary speaker (9 January at 14:25 to 14:55 in Lecture Room 1/NEF) and a dedicated minisymposium session (9 January at 16:45 to 18:45 in Lecture Room 1/NEF).

Plenary Speaker

Kevin Eliceiri (University of Wisconsin-Madison) 'The ImageJ ecosystem: an open platform for biomedical image analysis'

Minisymposium Speakers

Winfried Wiegraebe (Allen Institute for Cell Science), 'Why to automate your microscope and how to do it'

Mark Bates (Max Planck Institute for Biophysical Chemistry), 'Why to automate your microscope and how to do it'

Pair Talks

Interdisciplinary research is at the heart of modern science. Applying quantitative approaches to important problems in cell biology is a valuable example. QBI has over time featured pair talks that bring together a biologist and a quantitative scientist who have collaborated on challenging biological problem.

Pair Talk Speakers:

Marino Zerial and Yannis Kalaidzidis (both of Max Planck Institute of Molecular Cell Biology and Genetics), 'Structure and dynamics of endosomes analyzed by imaging-based methods'
Wednesday (9 January) 13:45 to 14:25 in Lecture Room 1 (NEF)

Spencer Freeman (Hospital for Sick Children - Toronto), 'Transmembrane pickets corral bystanders in the plasma membrane by tethering to the submembrane cytoskeleton'
and
Khuloud Jaqaman (UT Southwestern Medical Center), 'Uncovering transients in single molecule motion: application to cell surface proteins'
Thursday (10 January) 13:30 to 14:10 IN Lecture Room 1 (NEF)

Tutorials

At QBI we present from time to time overviews and tutorial lectures on topical research areas to introduce the attendees to a field of interest. At this year's QBI conference, we have the following tutorial presentations:

Tutorial Speakers:

Judith Klumperman (University Medical Certer Utrecht) 'Correlative light – electron microscopy: approaches, applications and outlook'
Thursday (10 January) 09:20 to 09:50 in Lecture Room 1 (NEF)

Edward Cohen (Imperial College London) 'A tutorial in spatial statistics for microscopy data analysis'
Friday (11 January) 09:40 to 10:10 in Lecture Room 1 (NEF)

QBI Student/Post-doc Chapter

We are excited to introduce the student-postdoc chapter of the Quantitative Bioimaging conference, with the goal of increasing the engagement and active participation of current and future young investigators in this field. We believe this chapter would provide a platform for students and postdocs to learn and present their work, while also engaging in chapter activities to enhance their professional skills and network with the leaders in the field. This would be a great opportunity to get involved and make an impact as budding young investigators.

We will be having an inaugural meeting of the QBI student-postdoc chapter on the 9th of January during the opening reception at the QBI conference (Location will be announced closer to the meeting) marking the inception of the student-postdoc chapter, with the following agenda:

- Welcome address
- Chapter goals
- Guest speaker
- Upcoming activities and participation
- Floor open for Q & A
- Registration

Participation is open to all interested students and postdoc QBI registrants. Given that this chapter is in its infancy, any suggestions for this and future meetings are welcome. Please contact studentpostdocchapter@quantitativebioimaging.com if you have any suggestions.

Best Poster Award

Students presenting a poster are eligible for the Best Student Poster Presentation Award. An independent jury will assess the poster presentations and determine the awardee. The winner will be given the opportunity to present his/her work in a plenary lecture at the next QBI conference. The 2019 Best Poster Award is sponsored by Institut de Recherches Servier.

Award Lecture – 2018 Winner:	Klaus Yserentant and Felix Braun "Measuring the absolute degree of labeling for self-labeling protein tags"
	Thursday (10 January) at 10:30 in Lecture Room 1 (NEF)
2019 Best Poster Announcement:	Friday (11 January) at 18:30 in Lecture Room 1 (NEF)

Program

Tuesday (8 January)

	Start	End	Location
Registration (max capacity 150 per workshop)	08:00	16:00	Foyer (Hall 1)
Workshop: Adaptive Optics 193-Practical Aspects of Adaptive Optics for Microscopes Martin Booth, Aurélien Barbotin, Mantas Žurauskas *University of Oxford, Oxford, GB*	08:30	10:45	Lecture Room 2 (Dortoir)
Coffee Break	10:45	11:00	Foyer (Hall 1)
Workshop: Machine Learning 226-Machine Learning Workshop: An Introduction to Machine Learning and a Taste of Advanced Methods Seth Flaxman *Imperial College London, Oxford, GB*	11:00	12:30	Lecture Room 2 (Dortoir)
Lunch Break	12:30	13:30	(on own)
Workshop: Machine Learning continued 226-Machine Learning Workshop: An Introduction to Machine Learning and a Taste of Advanced Methods Seth Flaxman *Imperial College London, Oxford, GB* 227-Large-Scale Optimization for Machine Learning Julien Mairal *Inria, Montbonnot, FR* 228-Introduction to Deep Learning Christophe Zimmer *Institut Pasteur, Paris, FR*	13:30	16:45	Lecture Room 2 (Dortoir)
Coffee Break	16:45	17:15	Foyer (Hall 1)
Workshop: 3D Super Resolution Microscopy 217-Single-Molecule Localization Microscopy: Performing 3D Super-Resolution Reconstruction Using Open-Source Software Daniel Sage *EPFL, Lausanne, CH*	17:15	19:15	Lecture Room 2 (Dortoir)
Registration Desk Opens: Main Conference	17:15	21:15	Foyer (Hall 1): Registration Area
Opening Reception	18:15	20:30	Foyer (Hall 1)

Wednesday (9 Jan)

	Start	End	Location
Registration Desk Opens	08:00	20:30	Foyer (Hall 1): Registration Area
Opening Remarks	08:15	08:30	Lecture Room 1 (NEF)
Lecture (Machine Learning theme) 177-Sparse Modeling of Data and Deep Learning Michael Elad *Technion – Israel Institute of Technology, Haifa, IL*	08:30	09:10	Lecture Room 1 (NEF)
Lecture (Machine Learning theme) 168-Content-Aware Image restoration for Light and Electron Microscopy Florian Jug *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE*	09:10	09:40	Lecture Room 1 (NEF)

Lecture (Single Molecule Microscopy theme) 157-Fixation and Sample Processing Issues for Super-Resolution Microscopy Silvio Rizzoli *University Medical Centre Goettingen, Goettingen, DE*	09:40	10:10	Lecture Room 1 (NEF)
Coffee Break	10:10	10:40	Foyer (Hall 1)
Hamamastu Photonics Workshop 1	10:40	17:00	Workshop (Horizons)
Minisymposium: Machine Learning in BioImage Analysis and Microscopy 236-Label-Free Prediction of Subcellular Organization: Capturing Variation and Integrating Observations Chawin Ounkomol, Sharmishtaa Seshamani, Forrest Collman, Mary (Molly) Malechar, Gregory R Johnson *Allen Institute for Cell Science, Seattle, US* 166-Unraveling the Rules of Life a Few Photons at a Time Steve Pressé *Arizona State University, Tempe, US* 207-Computational Boosts to Single Molecule Localization Microscopy Christophe Zimmer *Institut Pasteur, Paris, FR*	10:40	11:40	Lecture Room 1 (NEF)
Session: Single Molecule Microscopy 146-Data Fusion for Localization Microscopy Bernd Rieger *TU Delft, Delft, NL* 145-Nuclear Pores as Universal Reference Standards for Quantitative Microscopy Jonas Ries *EMBL, Heidelberg, DE* 066-Artefact Free High Density Localization Microscopy Analysis Richard John Marsh *King's College London, London, GB*	11:40	12:25	Lecture Room 1 (NEF)
Session: Optical Methods Developments 120-Super-Resolution Imaging of Unlabeled Living Cells Using Superoscillatory Polarization Contrast Edward T F Rogers, Shmma Quraishe, Katrine Rogers, Tracey Newman, Peter Smith, Nikolay Zheludev *University of Southampton, Southampton, GB* 085-Single-Shot Fluorescence Holography for 3D Tracking in Live Cells Matz Liebel, Jamie Ortega Arroyo, Vanesa Sanz, Johann Osmond, Romain Quident, Niek van Hulst *ICFO – The Institute of Photonic Sciences, Castelldefels, ES* 106-Simple and Compact Microscope for Time-Lapse Phase and Fluorescence Imaging Based on Chromatic Aberration Ondrej Mandula, Cédric Allier, Sophie Morales, Jean-Philippe Kleman, Francoise Lacroix, Lionel Hervé *CEA-Leti, Grenoble Cedex 9, FR* 111-Speed OPIOM (Out-of-Phase Imaging after Optical Modulation) For Quantitative Multiplexed Fluorescence Imaging Against Autofluorescence Under Ambient Light Ruikang Zhang, Raja Chouket, Marie Aude Plamont, Zsolt Kelemen, Agathe Espagne, Alison Tebo, Arnaud Gautier, Lionel Gissot, Jean-Denis Faure, Ludovic Jullien, Vincent Croquette, Thomas le Saux *Ecole Normale Superieure, Paris, FR* 191-Methods and Applications of Quantitative Dynamic Full-Field Coherence Tomography Jules Scholler, Kassandra Groux, Claude Boccara, Kate Grieve *ESPCI, Paris, FR*	10:40	11:55	Lecture Room 2 (Dortoir)

Session: Biological Applications of Optical Developments 11:55 12:25 Lecture Room 2 (Dortoir)

038-LIESS-FCS: Spatially Resolved Diffusion Modes Measured by STED Nanoscopy
Falk Schneider, Dominic Waithe, Erdinc Sezgin, Christian Eggeling
University of Oxford, Oxford, GB

108-Adaptive Optics Allows 3D STED-FCS Measurements in the Cytoplasm of Living Cells
Aurélien Barbotin, Silvia Galiani, Iztok Urbančič, Christian Eggeling, Martin Booth
University of Oxford, Oxford, GB

Lunch (provided) 12:25 13:45 Foyer (Hall 1): Food Area

Pair Talk 13:45 14:25 Lecture Room 1 (NEF)

181-Structure and Dynamics of Endosomes Analyzed by Imaging-Based Methods
Marino Zerial and Yannis Kalaidzidis
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE

Lecture (Software Development theme) 14:25 14:55 Lecture Room 1 (NEF)

187-The ImageJ Ecosystem: An Open Platform for Biomedical Image Analysis
Kevin W Eliceiri
University of Wisconsin at Madison, Madison, US

Lecture (Past Chair lecture) 14:55 15:15 Lecture Room 1 (NEF)

027-About the Optimal Optical Transfer Function of a Microscope
Jörg Enderlein
Georg August University, Goettingen, DE

Coffee Break 15:15 16:45 Foyer (Hall 1)

Poster Session 1 15:15 16:45 Foyer (Hall 1): Poster Area

Single Molecule Microscopy Methods Development
Optical Methods Development
Applications of Single Molecule Microscopy
Biological Applications of Optical Developments
Single Molecule Microscopy Experimental Approaches

Marketing Session: Oxford NanoImaging 16:25 16:40 Lecture Room 1 (NEF)

Minisymposium: Software Design for Microscopy Hardware Control 16:45 18:45 Lecture Room 1 (NEF)

158-Why to Automate Your Microscope and How to Control It
Winfried Wiegraebe and Mark Bates
Allen Institute for Cell Science, Seattle, US and
Max Planck Institute for Biophysical Chemistry, Goettingen, DE

Session: Applications of Single Molecule Microscopy 16:45 17:30 Lecture Room 2 (Dortoir)

042-Unraveling Molecular Arrangement of Synaptic Proteins With Multiple Color Super Resolution Microscopy and Statistical Object Distance Analyse with Icy Soda
Thibault Lagache, Philippe Bun, Stephane Dallongeville, Alexandre Dufour, Jean-Christophe Olivo-Marin, Thierry Galli, Lydia A Danglot
Institute of Psychiatry & Neurosciences of Paris, Paris, FR

080-Multiscale Computational Modeling of Chromatin Three-Dimensional Structure Using iPALM Microscopic High-Resolution Images
Zofia Anna Parteka, Dariusz Plewczyński
Centre of New Technologies, University of Warsaw, Warsaw, PL

081-A Quantitative Look at Cellular Oligonucleotide Delivery
Leontien van der Bent, Natalia Feiner, R. Alis Olea, Johanna Berndt, Derick Wansink, Lorenzo Albertazzi, Roland Brock
Radboud University Medical Center, Nijmegen, NL

Session: Single Molecule Microscopy 17:30 18:30 Lecture Room 2 (Dortoir)

034-Imaging Complex Protein Machines by High-Throughput Localization Microscopy
Joran Deschamps, Markus Mund, Jonas Ries
EMBL, Heidelberg, DL

067-Multi-Color Cryo-Fluorescence Microscopy
Maximiliaan Huisman, Farzin Farzam, David Grunwald
UMass Medical School, Worcester, US

082-Wide Depth Reproducible Multicolor Bioimaging Through a Combination of 3D Molecule Localization Strategies
Clement Cabriel, Nicolas Bourg, Pierre Jouchet, Guillaume Dupuis, Christophe Leterrier, Aurélie Baron, Boris Vauzeilles, Emmanuel Fort, Sandrine Leveque-Fort
ISMO-CNRS, ORSAY, FR

110-A Tilted Light Sheet for Single-Molecule Super-Resolution Imaging in Thick Cells
Petar N Petrov, Anna-Karin Gustavsson, Yoav Shechtman, Maurice Lee, W. E. Moerner
Stanford University, Stanford, US

Opening Reception and Poster Viewing	18:45	22:15	Foyer (Hall 1)
QBI Student/Post-doc Chapter Inaugural Meeting	18:45	19:30	Location TBD

Thursday (10 January)

	Start	End	Location
Registration Desk Opens	08:00	20:30	Foyer (Hall 1): Registration Area
Lecture (Quantitative Tools for Subcellular Trafficking theme)	08:30	09:20	Lecture Room 1 (NEF)
214-Using Microscopy to Dissect Endolysosomal Trafficking and Function Frederick Maxfield *Weill Cornell Medical College, New York, US*			
Tutorial (Correlative Imaging theme)	09:20	09:50	Lecture Room 1 (NEF)
183-Correlative Light – Electron Microscopy: Approaches, Applications and Outlook Judith Klumperman *University Medical Center Utrecht, NL*			
Lecture (Imaging for BioPharma Applications theme)	09:50	10:30	Lecture Room 1 (NEF)
167-Analyzing Three-Dimensional Biological Specimens Observed With Light Sheet Microscopy Under Near-Natural Conditions Ernst HK Stelzer *Goethe-Universität Frankfurt am Main, Frankfurt am Main, DE*			
Poster Award Lecture	10:30	10:40	Lecture Room 1 (NEF)
116-Measuring the Absolute Degree of Labeling for Self-Labeling Protein Tags Klaus Yserentant, Felix Braun, Siegfried Hänselmann, Wioleta Chmielewicz, Johan Hummert, Florian Salopiata, Dirk-Peter Herten *Heidelberg University, Heidelberg, DE*			
Tribute to Maxime Dahan by Bassam Hajj (Institut Curie Paris)	10:40	10:50	Lecture Room 1 (NEF)
Coffee Break	10:50	11:15	Foyer (Hall 1)
Hamamatsu Photonics Workshop 2	11:15	17:00	Horizons
Minisymposium: Quantitative Tools to Define Subcellular Trafficking Processes	11:15	12:15	Lecture Room 1 (NEF)
162-Lectin-Driven and Glycosphingolipid-Dependent Construction of Endocytic Pits Ludger Johannes *Institut Curie, Paris Cedex 05, FR*			
185-Quantitative Visualization of SNARE Complex Formation in Living Cells Geert van den Bogaart *University of Groningen, Groningen, NL*			
221-Fluorescence Studies of Sterol Transport Between Cellular Membranes Daniel Wüstner *The University of Southern Denmark, Odense, DK*			

Session: Machine Learning in Single Molecule Microscopy and Deconvolution	11:15	12:15	Lecture Room 2 (Dortoir)

015-Deep Learning for Dense and Multicolor Localization Microscopy
Elias Nehme, Eran Hershko, Lucien Weiss, Tomer Michaeli, Yaov Shechtman
Technion, Israel Institute of Technology, Haifa, IL

061-3D ConvNets Improve Macromolecular Localization in 3D Cellular Cryo-Electron Tomograms
Emmanuel Moebel, Charles Kervrann
Inria, Rennes, FR

143-Single Molecule at the Age of Big Data: Probabilistic Pipeline and Unsupervised Learning
Francois Laurent, Alexander Serov, Christian Vestergaard, Jean-Baptiste Masson
Institut Pasteur, Paris, FR

050-Semi-Blind Spatially-Variant Deconvoluted and Focus Estimation in Optical Microscopy by Use of Convolutional Neural Networks
Adrian Shajkofci, Michael Liebling
Idiap Research Institute, Martigny, CH

Lunch (provided)	12:10	13:30	Foyer (Hall 1): Food Area
Pair Talk	13:30	14:10	Lecture Room 1 (NEF)

163-Uncovering Transients in Single Molecule Motion: Application to Cell Surface Proteins
Anthony Vega, Spencer Freeman, Sergio Grinstein, Khuloud Jaqaman
UT Southwestern Medical Center, Dallas, US

194-Transmembrane Pickets Corral Bystanders in the Plasma Membrane by Tethering to the Submembrane Cytoskeleton
Khuloud Jaqaman, Sivakami Mylvaganam, Anthony Vega, Sergio Grinstein, Spencer Freeman
Hospital for Sick Children, Toronto, CA

Session: Application of Image Analysis Approaches	13:30	15:00	Lecture Room 2 (Dortoir)

039-High Content Cell Classification and Tracking Improvement by Morphology and Quantitative Phase Feature Combination
Benoit Wattellier, Sherazade Aknoun, Manuel Yonnet, Fabrice Valentino, Djamel Brahmi, Michel Barlaud, Philippe Pognonec, Thierry Pourcher
Phasics, Saint Aubin, FR

047-Two Dynamic Behaviors of the Microtubules at the Cell Cortex Reveal the Pulling and Pushing Forces That Position the Mitotic Spindle in C. elegans Embryos
Hélène Bouvrais, Danielle Fairbrass, Laurent Chesneau, Nina Soler, Yann Le Cunff, Thierry Pécot, Charles Kervrann, Jacques Pécréaux
Institut de Génétique et Développement de Rennes), Rennes, FR

105-Investigating the Spatio-Temporal Patterning of Neural Stem Cell Activation Events
Sebastien Herbert, Nicolas Dray, Laure Mancini, Willy Supatto, Jean-Yves Tinevez, Jean-Baptiste Masson, Laure Bally-Cuif
Institut Pasteur Paris, Paris, FR

023-Quantitative Analysis of Collagen Microstructure In Vitro and In Vivo by Means of Multi-Phasor Analysis of Second Harmonic Generation Microscopy
Giuseppe Chirico, Laura D'Alfonso, MAddalena Collini, Margaux Bouzin, Riccardo Scodellaro, Laura Sironi
Universita' di Milano-Bicocca, Milano, IT

054-Spatiotemporal Quantification of 3D Cellular Parameters During Leaf Morphogenesis
Faïçal Selka, Thomas Blein, Jasmine Burguet, Eric Biot, Patrick Laufs, Philippe Andrey
INRA, Versailles, FR

115-Segmentation of 3D Images of Plant Tissues at Multiple Scales Using the Level Set Method
Annamaria Kiss, Typhaine Moreau, Vincent Mirabet, Cerasela Iliana Calugaru, Arezki Boudaoud, Pradeep Das
ENS de Lyon, Lyon, FR

Session: Sample Methods Development 14:10 15:05 Lecture Room 1 (NEF)

028-DNA Origami as a Nanoscale Platform for T-Cell Activation
Joschka Peter Hellmeier, Eva Sevcsik
TU Vienna, AT

100-Characterisation of GFP and Derivatives for Super-Resolution Cryogenic Single Molecule Localisation Microscopy
Amy N Moores, Lin Wang, Laura Zanetti-Domingues, Benji Bateman, Marisa Martin-Fernandez
STFC, Didcot, GB

125-Novel Tetrazine Probes for Fluorogenic Protein Labeling
Felix Braun, Philipp Werther, Klaus Yserentant, Achim Wieczorek, Richard Wombacher, Dirk-Peter Herten
Heidelberg University, Heidelberg, DE

Coffee Break 14:50 16:20 Foyer (Hall 1)

Poster Session 2 14:50 16:20 Foyer (Hall 1): Poster Area

Applications in Biopharma
Application of Image Analysis Approaches
Data Analysis Methods Development for Single Molecule Microscopy
Machine Learning
Sample Methods Developments
Instrument Characterization

Marketing Session: GE Healthcare 16:00 16:15 Lecture Room 1 (NEF)

Marketing Session: QuantaCell 16:15 16:20 Lecture Room 1 (NEF)

Minisymposium: Microscopy in BioPharma 16:20 18:00 Lecture Room 1 (NEF)

164-Volumetric Imaging of Whole-Tumors Reveal Cancer Malignancy
Per Uhlen
Karolinska Institutet, Stockholm, SE

170-Combining Optical Imaging of Cleared Tissue With Mathematical Modelling and In Vivo Imaging to Predict Drug Delivery and Therapeutic Response
Simon Walker-Samuel, Paul Sweeney, Angela d'Esposito, Rebecca Shipley
University College London, London, GB

172-Analyzing Development, Function and Pathofunction of the Vascular Systems Using Light Sheet Microscopy
Friedemann Kiefer
European Institute for Mol. Imaging (EIMI), Münster, DE

104-Deep learning based detection of cells in 3D Light sheet with ultramicroscopy
David Rousseau, Ali Ahmad, Pejman Rasti, Carole Frindel, David Sarrut
Laboratoire LARIS, Université d'Angers, Angers, FR

190-Microscopy as a Tool in the Development of Antibody-Based Therapeutics
E. Sally Ward
University of Southampton / Texas A&M Health Science Center, College Station, US

Session: Data Analysis Methods Development for Single Molecule Microscopy 16:20 18:05 Lecture Room 2 (Dortoir)

136-1 Nanometer Precision by Bayesian Grouping of Localizations
Mohamadreza Fazel, Bernd Rieger, Ralf Jungmann, Keith A Lidke
University of New Mexico, Albuquerque, US

055-4Pi Single Molecule Localization With an Experimental PSF Model That Achieves Theoretical Minimal Uncertainty
Yiming Li, Jonas Ries
European Molecular Biology Laboratory, Heidelberg, DE

155-Simultaneous Detection of 3D Orientation and 3D Spatial Localization of Single Emitters for Super Resolution Structural Imaging
Miguel A Alonso
Institut Fresnel, Marseille, FR

122-Photon Count Estimation in Single-Molecule Imaging
Rasmus Østergaard Thorsen, Bernd Rieger, Sjoerd Stallinga, Christiaan Hulleman
TUDelft, Rotterdam, NL

046-Polygon-Based Colocalization Analysis for Multicolor Single-Molecule Localization Microscopy Data
Florian Levet, Jean-Baptiste Sibarita
Interdisciplinary Institute for NeuroScience, Bordeaux, FR

014-Preprocessing Structured Background Signals to Improve Super-Resolution Fluorescence Images
Siewert Hugelier, Tomas Lukes, Peter Dedecker, Paul Eilers, Cyril Ruckebusch
KU Leuven, Heverlee, BE

033-An Automated Bayesian Pipeline for Rapid Analysis of Single- Molecule Binding Data
Carlas Sierd Smith, Karina Jouravleva, Maximiliaan Huisman, Samson M. Jolly, Phillip D. Zamore, David Grunwald
University of Massachusetts Medical School, Worcester, US

Dinner Break (on own)	18:30	20:30	on own
QBI Society Meeting	20:30	22:00	Room 11, Level 1

Friday (11 January)

	Start	End	Location
Registration Desk Opens	08:00	18:00	Foyer (Hall 1): Registration Area
Lecture (Structured Illumination theme)	08:30	09:20	Workshop (Horizons)
184-Structured Illumination, Past and Present Development Rainer Heintzmann *Institute of Physical Chemistry, Heidelberg, DL*			
Lecture (Optics theme)	09:20	09:40	Lecture Room 1 (NEF)
161-Measuring the Phase and Intensity of the Light: From Quantitative Label-Free Imaging to Fluorescence 3D Super-Resolution Deep in Tissues Pierre Bon *CNRS - Univ. Bordeaux - IOGS, Bordeaux (Talence), FR*			
Tutorial (Spatial Statistics)	9:40	10:10	Lecture Room 1 (NEF)
148-A Tutorial in Spatial Statistics for Microscopy Data Analysis Edward Cohen *Imperial College London, London, UK*			
Coffee Break	10:10	10:40	Foyer (Hall 1)
Minisymposium: Structured Illumination: a Review of the State of the Art	10:40	12:10	Lecture Room 1 (NEF)
171-A signal processing view of Structured Illumination Microscopy Jean-Christophe Olivo-Marin *Institut Pasteur - CNRS 3691, Paris, FR*			
091-Noise Controlled Image Reconstruction for Structured Illumination Microscopy Sjoerd Stallinga *Delft University of Technology, Delft, NL*			
127-Robust Adaptive Optics for Structured Illumination Microscopy Fluorescence Imaging Mantas Žurauskas, Martin Booth *University of Oxford, Oxford, GB*			
154-High-Speed, Real-Time Reconstructed Structured Illumination Microscopy of Living Cells Thomas Huser *University of Bielefeld, Bielefeld, DL*			
186-Random Illumination Microscopy: Using Uncontrolled Speckles to Improve the Resolution of Fluorescence Microscopy Anne Sentenac *CNRS Institut Fresnel, Marseille Cedex, FR*			
Session: Applications in Cell Biology and Biophysics II	10:40	12:10	Lecture Room 2 (Dortoir)
020-A Detailed Analysis of the Mobility of Synaptic Proteins Reveals Principles of Protein Segregation in the Synapse and in the Axon Sofiia Reshetniak, Sven Truckenbrodt, Silvio Rizzoli *University Medical Center Goettingen, Goettingen, DE*			
032-Investigation of the Cross-Talk Between Desmosomes and Tetraspanin-Enriched Microdomains Maryam Arab, Iqra Arif, Jeremy Pike, Natalie Poulter, Martyn Chidgey, Elena Odintsova *University of Birmingham, Edgbaston, Birmingham, GB*			

088-The TCR is Randomly Distributed in the Plasma Membrane of Resting T Cells
Andreas M Arnold, Benedikt Rossboth, Gerhard J Schütz
TU Wien, Wien, AT

099-Multimodal Microscopy Reveals Stiffness-Dependent Nanoscale Remodeling of Different Actin Modules During Cell Protrusion
Alessandra Cambi
Radboud UMC, RIMLS, Nijmegen, NL

153-Functional Insights into the Intrinsically Disordered Protein α-Synuclein
Amin Fakhree, Christian Blum, Mireille Claessens
University of Twente, Enschede, NL

169-Three-Dimensional Single Molecule Imaging, With Cellular Context, of an Engineered FcRn Antagonist Using Remote Focusing Multifocal Plane Microscopy
Sreevidhya Ramakrishnan, Sungyong You, Jerry Chao, Anish V Abraham, E. Sally Ward, Raimund J Ober
Texas A&M University, College Station, US

Lunch (provided) 12:10 13:30 Foyer (Hall 1): Food Area

Session: Structured Illumination 13:30 14:30 Lecture Room 1 (NEF)

084-Structured illumination for Single Molecule Localization Microscopy at Depth
Pierre Jouchet, Clement Cabriel, Nicolas Bourg, Marion Bardou, Guillaume Dupuis, Christian Poüs, Emmanuel Fort, Sandrine Leveque-Fort
CNRS, Orsay Cedex, FR

121-Super-Resolved Live Imaging for a Wide Range of Biological Applications Using Random Illumination Microscopy (RIM)
Simon Labouesse, Emmanuel Martin, Renaud Poincloux, Sylvain Cantaloube, Tong Li, Christian Rouvière, Magali Suzanne, Nathalie Campo, Sophie Allard, Mathieu Pinot, Marc Allain, Jérome Idier, Anne Sentenac, Thomas Mangeat
CBI, Toulouse, FR

124-Novel Method for Structured Illumination Retinal Imaging
Yann Lai-Tim, Laurent Mugnier
ONERA, Chatillon Cedex, FR

130-Structured Illumination - Localization Microscopy
Stefan Wieser
ICFO - The Institute of Photonic Sciences, Castelldefels, ES

Session: Machine Learning in High Throughput Imaging 14:30 15:15 Lecture Room 1 (NEF)

165-Use of Deep Learning in High-Throughput Flow Imaging Microscopy Applications
Christopher P Calderon
University of Colorado, Denver, US

036-Advanced Imaging Flow Cytometry
Andreas Kleiber, Thomas Henkel
Leibniz Institute of Photonic Technology, Jena, DE

159-High and Deep Imaging Flow Cytometry: a Potential Diagnostic Tool for Hematological Disorders
Anne E Carpenter, Minh Doan
Broad Institute of MIT and Harvard, Cambridge, US

Session: Spatial Statistics 13:30 14:15 Lecture Room 2 (Dortoir)

053-Modelling the Spatial Distribution of the Type 6 Secretion System in Pseudonomas Aeruginosa
Scott Ward, Edward Cohen, Niall Adams
Imperial College London, London, UK

064-Mapping and Comparing Spatial Distributions in Biological Imaging
Jasmine Burguet, Philippe Andrey
INRA, Versailles, FR

078-Cluster Analysis of the Cell Membrane
Jerome Boulanger, Leila A Muresan
MRC-LMB, Cambridge, US

Session: Single Molecule Counting and Cluster Analysis 14:15 15:15 Lecture Room 2 (Dortoir)

017-In Vitro Verification of a Molecular Counting Technique Based on Fluorophore Blinking Statistics Using DNA Origami
Daniel Felipe Nino, Daniel Djayakarsana, Nafiseh Rafiei, Anton Zilman, Josh Milstein
University of Toronto, Mississauga, CA

052-Measuring Ligand Surface Density on Various Substrates Using Fluorescence Fluctuation Microscopy
Dwiria Wahyuni, Martial Balland, Olivier Destaing, Irène Wang, Antoine Delon
Université Grenoble Alpes, Grenoble, FR

071-Overcoming Blinking Artifacts in Nanocluster Detection With Two-Color Storm
Magdalena Schneider, Andreas Arnold, Florian Baumgart, Gerhard J Schütz
Vienna University of Technology, Vienna, AT

129-Quantifying Protein Oligomerization in Living Cells: a Systematic Comparison of Fluorescent Proteins and Application to Developmental Cell-Cell Fusion
Valentin Dunsing, Madlen Luckner, Boris Zühlke, Roberto Petazzi, Andreas Herrmann, Salvatore Chiantia
University of Potsdam, Berlin, DE

Session: Applications in Cell Biology and Biophysics 13:30 14:15 Lecture Room 3 (Horizons)

056-Micro-Fluctuations of the Length of the Mitotic Spindle Reveal its Mechanics and Its Dynamics During Cell Division
Benjamin Mercat, Xavier Pinson, Yann Le Cunff, Jonathan Fouchard, Hadrien Mary, Sylvain Pastezeur, Yannick Gachet, Sylvie Tournier, Hélène Bouvrais Jacques Pécréaux
IGDR, Rennes, FR

074-3D-EM-ISH – a Novel Tool to Visualize Specific Chromatin Regions
Blazej H Ruszczycki
Polish Academy of Sciences, Warszawa, PL

103-Combining 3D Image Analysis and Computer Modeling to Understand Cell Division Patterns in Plant Early Embryogenesis
Julien Moukhtar, Elise Laruelle, Alain Trubuil, Katia Blecram, David Legland, Zhor Khadir, Jean-Christophe Palauqui, Philippe Andrey
INRA, Versailles, FR

Session: Software 14:15 15:15 Lecture Room 3 (Horizons)

051-Combining Annotation of Movies and Learning for Fate Prediction From Early Embryo Development Observation
Alain Trubuil, Alline Reis, Véronique Duranthon, Brigitte Le Guienne, Nathalie Le Brusq, Soundouss Messoudi, Guillaume Brocart
INRA, Jouy en Josas, FR

077-u-track 3D: A Tracking Framework to Quantify, Observe and Contextualize Intracellular Dynamics in Three Dimensions.
Philippe Roudot, Wesley R. Legant, Kevin Dean, Ana David, Daniel Gerlich, Reto Fiolka, Eric Betzig, Gaudenz Danuser
UT Southwestern Medical Center, Dallas, US

089-PartSeg - GUI for Image Processing Algorithm in Python
Grzegorz Bokota, Nirmal Das, Adriana Magalska, Subhadip Basu, Jacek Sroka, Dariusz Plewczynski
University of Warsaw, Warszawa, PL

144-A Human-in-the-Loop Approach to Image Analysis
Mohamed El Beheiry, Sebastien Doutreligne, Fabien Reyal, Jean-Baptiste Masson
Institut Curie, Paris, FR

Coffee Break 15:15 16:45 Foyer (Hall 1)

Poster Session 3 15:15 16:45 Foyer (Hall 1): Poster Area

Applications in Cell Biology and Biophysics
Single Molecule Counting and Cluster Analysis
Image Analysis Methods Development
Software
Tracking
Spatial Statistics
Digital Pathology
Structured Illumination

Sponsor Session: New R&D Microscopy Advances in France-BioImaging by Jean Salamero 16:20 16:35 Lecture Room 1 (NEF)

GdR Imabio Session 16:35 16:40 Lecture Room 1 (NEF)

Complex Diffusion Analysis Challenge
Cyril Favard
GdR Imabio, FR

Session: Image Analysis Methods Development 16:45 18:00 Lecture Room 1 (NEF)

016-How Accurately Two or More Fluorophores can be Spectrally Resolved in a Fluorescence Microscope?
Sripad Ram
Pfizer, Inc., San Diego, US

010-Object Detection Networks for Localization and Classification of Cells in Fluorescence Microscopy Acquisition and Analysis.
Dominic Waithe
University of Oxford, Oxford, GB

101-Automatic Registration for Multidimensional Correlative Microscopies With Error Assessment and Detections of Deformations
Perrine Paul-Gilloteaux
CNRS, Nantes, FR

118-Membrane Curvature Estimation Method for Cryo-Electron Tomography
Maria Kalemanov, Javier F. Collado, Wolfgang Baumeister, Rubén Fernández-Busnadiego, Antonio Martínez-Sánchez
Max Planck Institute of Biochemistry, Martinsried, DE

142-Accordion-Like Collagen Fibrils Suggested by P-SHG Image Modeling: Implication in Liver Fibrosis
Denis Rouede, Emmanuel Schaub, Jean-Jacques Bellanger, Frederic Ezan, François Tiaho
University of Rennes 1, Rennes, FR

Session: Tracking	16:45	18:00	Lecture Room 2 (Dortoir)

058-Local Maximum Likelihood Estimation for Time-Varying Single Particle Tracking Models
Boris I Godoy, Nicholas Vickers, Sean Andersson
Boston University, Boston, US

043-3D Motion Estimation in 3D Light Microscopy Image Sequences: Application to Cell Migration
Sandeep Manandhar, Patrick Bouthemy, Philippe Roudot, Charles Kervrann
Inria, Rennes, FR

068-Non-Equilibrium Forces Govern Anomalous Dynamics of Subcellular Domains in Mammalian Cells
Lorenz Stadler, Konstantin Speckner, Matthias Weiss
University of Bayreuth, Bayreuth, DE

079-The Topography of Biological Membranes Artefactually Creates the Appearance of Anomalous Diffusion: a Remedy
Jeremy Adler, Ingela Parmryd, Robin Strand, Ida Sintorn
Uppsala University, Uppsala, SE

192-Fisher Information Matrix for Molecules With Stochastic Trajectories
Milad Rafiee Vahid, Bernard Hanzon, Raimund J Ober
Texas A&M University, College Station, US

Session: Digital Pathology	16:45	18:00	Lecture Room 3 (Horizons)

093-3D Fluorescence Whole Slide Imaging Using Confocal Multi-Line Scanning
Leon van der Graaff, Sjoerd Stallinga
Delft University of Technology, Delft, NL

097-Medical Image Analysis: Brain Tumor Grade Classification
Kawtar EL Karfi, Azeddine AIbrahimi
Medical and Pharmacy School, Mohammed V University, Rabat, Morocco, Temara, MA

133-Robust Discrimination of Antigen Stain in Histopathology
Laura Nicolas-Saenz, Paula Martin-Gonzalez, Patricia Garoz, Felipe Calvo, Sara Guerrero-Apizua, Arrate Muñoz-Barrutia
Universidad Carlos iii, Madrid, ES

137-QuPath – a Tool for Quality Assurance, Enhanced User Training, and Improved Reproducibility of Image Analysis in Histopathology Research
Zbigniew Mikulski, Katarzyna Dobaczewska, Angela Denn, Sara A McArdle
La Jolla Institute for Immunology, La Jolla, US

176-Segmenting 3D Vascular Networks With Deep Learning
Natalie Holroyd, Claire Walsh, Monica Sidarous, Eoin Finnerty, Rebecca Shipley, Simon Walker-Samuel
University College London, London, GB

Conferencing Closing	18:10	18:30	Lecture Room 1 (NEF)
Poster Award Announcement – sponsored by Institut de Recherches Servier	18:30	18:45	Lecture Room 1 (NEF)
Closing Gala Dinner Reception	19:00	23:00	La French Tech – Le Pool (Le Mabilay, 2 rue de la Mabilais, Rennes)

Poster Sessions

9 Jan (Wed)

A dynamic reconstruction of the endocytic machinery in yeast from static superresolution images (# 131 , Board: 41)
Yu-Le Wu, Philipp Hoess, Markus Mund, Joran Deschamps, Jonas Ries
European Molecular Biology Laboratory, Heidelberg, DE

Adaptive Holographic Region of Interest Illumination with Oblique Angles for use in Single Molecule Localization Microscopy (# 216 , Board: 47)
Alexander Jügler, Jan Becker, Patrick Then, Ronny Förster, Rainer Heintzmann
Leibniz-Institute of Photonic Technology, Jena, DE

Biological Deep Thermal Imaging with Fluorescence Lifetime of Rare-Earth-Based Ceramics Particles that Emit Near-Infrared Light in the Second Biological Window (# 223 , Board: 54)
Takumi Chihara, Masakazu Umezawa, Keiji Miyata, Shota Sekiyama, Naoki Hosokawa, Kyohei Okubo, Masao Kamimura, Kohei Soga
Tokyo University of Science, Katsushika, JP

Correlative STED and SMLM, without demons (# 232)
Kirti Prakash
University of Cambridge, Cambridge, GB

Design of an "orthogonality-breaking" polarimetric confocal microscope to study intracellular architecture dynamics (# 128 , Board: 5)
Stephanie Dutertre, Mehdi Alouini, Julien Fade, Emilie Gillier, Cyril Hamel, Sébastien Huet, Gilles Le Marchand, Rebecca Smith, Paulami Ray, Marc Tramier
Univ Rennes, RENNES, FR

Improved single molecule localization from dual-objective microscopes with ZOLA-3D (# 206 , Board: 1)
benoit lelandais, MICKAEL LELEK, christophe Zimmer
institut pasteur, paris, FR

Intracellular dry-mass density measurements of bacteria using quantitative phase microscopy (# 76 , Board: 25)
Enno Oldewurtel, Sven van Teeffelen
Institut Pasteur, Paris, FR

Label-free refractometry and pathogen detection by super-critical angle fluorescence (# 196 , Board: 14)
Boris Ferdman, Lucien Weiss, Onit Alalouf, Yonathan Haimovich, Yoav Shechtman
Technion – Israel Institute of Technology, Adi, IL

Mapping fluid and single-cell dynamics in a controlled thermal landscape (# 87 , Board: 36)
Jaime Ortega Arroyo, Matz Liebel, Bernard Ciraulo, Stefan Wieser, Niek van Hulst, Romain Quidant
ICFO - The Institute of Photonic Sciences, Casteldefels, ES

Nanostructured substrates for super-resolution imaging (# 212)
Maia Brunstein, Anne Talneau, Minh-Chau Nguyen, Pacal Berto, Anne-Laure Fehrembach, Anne Sentenac, Martin Oheim
Université Paris Descartes, Paris, FR

PRACTICAL IMPLEMENTATION OF THE OFF-AXIS HOLOGRAPHIC MICROSCOPE (# 235 , Board: 38)
Radim Kolar, Vratislav Cmiel, Larisa Baiazitova, Ivo Provaznik
Brno university of technology, Brno, CZ

Probing cells dynamics with quantitative Dynamic Full-Field OCT (# 19)
Kassandra Groux, Jules Scholler, Kate Grieve, Claude Boccara
ESPCI, Paris, FR

Quantitative phase imaging of adherent mammalian cells: a comparison of three different techniques (# 63 , Board: 19)
Cédric Allier, Julien Savatier, Serge Monneret, Yves Usson, Ondrej Mandula, Lionel Hervé, Pierre Blandin, Sophie Morales
CEA-LETI, Grenoble, FR

RSMLM: An R-package for pointillist based analysis of single molecule localization microscopy data (# 209 , Board: 23)
Jeremy Pike, Iain B Styles
University of Birmingham, , United Kingdom

Single molecule nucleocytoplasmic transport dynamics in live cells (# 117 , Board: 9)
Seoungjun Lee
King's College London, London, GB

Structured illumination in laser scanning microscopy for super-resolved multiphoton imaging: theory and simulations of scanning SIM (# 215 , Board: 28)
Eli Slenders, Marcel Ameloot, M vandeVen
Hasselt University, Hasselt, BE

Temperature Sensing of Deep Abdominal Region in Mice by Using Over-1000 nm Near-Infrared Luminescence of Rare-Earth-Doped NaYF4 Nanothermometer (# 224)
Shota Sekiyama, Masakazu Umezawa, Shuhei Kuraoka, Takuji Ube, Masao Kamimura, Kohei Soga
Tokyo University of Science, Katsushika, JP

The problem of orientation-induced mislocalizations of single emitters in cryo-fluorescence microscopy (# 160 , Board: 32)
Oleksii Nevskyi, Jörg Enderlein, Ingo Gregor
Georg August University Goettingen, Goettingen, DE

Towards self-driving super-resolution microscopes for live-cell imaging (# 240 , Board: 45)
Dora Mahecic, Suliana Manley
EPFL, Lausanne, CH

10 Jan (Thu)

Automated brain region recognition in fluorescent tissue slices using deep learning (# 237)
Michaël Barbier, Hervé Maurin, Emiel Holvoet, Rony Nuydens, Peter Larsen, Peter Horvath, Winnok De Vos
University of Antwerp, Antwerpen, BE

CNN based semi-supervised FIB-SEM image segmentation via propagation of learned information from a small subset of a single image stack (# 86 , Board: 30)
Kevin G Loftis, Kevin Stoltz, Guillaume Thibault, Jessica L Riesterer, Young Hwan Chang, Joe Gray
Oregon Health & Science University, Portland, US

Calibrating a microscope by learning its diversity (# 21)
Valentin Debarnot, Pierre Weiss, Thomas Mangeat, Paul Escande
ITAV, CNRS, Toulouse, FR

Deep learning based detection of cells in 3D Light sheet fluorescence microscopy (# 104 , Board: 39)
Ali Ahmad, Pejman Rasti, Carole Frindel, David Sarrut, David Rousseau
Laboratoire LARIS, Université d'Angers, ANGERS, FR

Deep learning for dense 3D single molecule localization microscopy (# 203 , Board: 49)
Elias E Nehme, Daniel Freedman, Tomer Michaeli, Yoav Shechtman
Technion - Israel Institute of Technology, Haifa, IL

Deep learning for quantitative bi-exponential fluorescence lifetime imaging (# 12 , Board: 7)
Jason T. Smith, Ruoyang Yao, Sez-Jade Chen, Nattawut Sinsuebphon, Alena Rudkouskaya, Margarida Barroso, Pingkun Yan, Xavier Intes
Rensselaer Polytechnic Institute, Troy, US

Establishing the theoretical density/SNR limits for SMLM analysis leads to the design of UNLOC a parameter-free and fast computing ImageJ plugin (# 107 , Board: 12)
Sébastien Mailfert, Nicolas Bertaux, Didier Marguet
Centre d'Immunologie de Marseille Luminy, Marseille cedex 09, FR

Experimentally-generated ground truth for tracking lymphocyte populations in image-based immunotherapy screens (# 102 , Board: 34)
Joseph Boyd, Zelia Gouveia, Franck Perez, Thomas Edgar Walter
MINES Paristech, Paris, FR

Image based modeling of leaf development (# 37 , Board: 43)
Mohamed Oughou, Patrick Laufs, Eric Cunha, Eric Biot, Nicolas Arnaud, Philippe Andrey, Jasmine Burguet
INRA, Nanterre, FR

Image profiling exposes a pharmacological window for modifiers of neuronal network connectivity (# 230 , Board: 3)
Marlies Verschuuren, Winnok De Vos
University of Antwerp, Antwerp, BE

PyMOL Molecular Graphics System for Immunoglobulin Cross-Reactivity Analysis (# 233 , Board: 56)
Nasreddine SAIDI
Institut Pasteur Tunis, Tunis, TN

Quantifying molecular confinement using sSTED-FCS: The case of lipid trapping during HIV-1 assembly in living T cells (# 213 , Board: 26)
Cyril Favard, Jakub Chojnacki, Christian Eggeling, Delphine Muriaux
CNRS, Montpellier, FR

Quantitative evaluation of a next generation scientific CMOS (sCMOS) camera for high fidelity imaging (# 114 , Board: 21)
Shigeo Watanabe, Takafumi Higuchi, Teruo Takahashi, Katsuhide Ito
Hamamatsu Photonics K.K., Hamamatsu-city, JP

Scalable analysis of ultra-terabyte brain images with deep learning (# 73 , Board: 17)
Giacomo Mazzamuto, Francesco Orsini, Matteo Roffilli, Paolo Frasconi, Francesco Pavone, Ludovico Silvestri
European Laboratory for Non-linear Spectroscopy, Sesto Fiorentino, IT

Segmentation of adjacent cells in a C. elegans embryo using a multi-compartment active contour (# 40)
Anaïs Badoual
Ecole Polytechnique Fédérale de Lausanne, Prilly, CH

Semi-quantitative analysis of the trace metallic elements with synchrotron radiation X-ray fluorescence analysis and the experimental concentration standard specimens (# 22 , Board: 52)
Motohiro Uo, Takahiro Wada
The University of Tokyo, Bunkyo-ku, JP

3D stochastic process simulation for better interpretation of molecular dynamics related to cell wall biogenesis observed with TIRF microscopy (# 72 , Board: 6)
Charles Kervrann, Alain Trubuil, Cyrille Billaudeau, Rut Carballido-Lopez, Yunjiao LU
INRA, Jouy-en-Josas, FR

3D tracking of endocytic events using lattice light sheet microscopy (# 92 , Board: 8)
Cesar Augusto VALADES CRUZ, Alison Forrester, Christian Wunder, Charles Kervrann, Jean SALAMERO, Ludger Johannes
Institut Curie, Paris, FR

A mechano-imaging method to quantify intracellular biophysics (# 140 , Board: 48)
Aleix Boquet-Pujadas, Elisabeth Labruyere, Jean-Christophe Olivo-Marin
Institut Pasteur, Paris, FR

A pyramidal PDE-constrained approach to quasi-static optical elastography (# 141 , Board: 51)
Aleix Boquet-Pujadas, Jean-Christophe Olivo-Marin
Institut Pasteur, PARIS, FR

Adaptive optics flood-illumination ophthalmoscope with structured illumination capabilities (# 123 , Board: 16)
Yann Lai-Tim, Laurent Mugnier
ONERA, Chatillon Cedex, FR

Analysing and quantifying intracellular particles movements in 3D LLSM data (# 109 , Board: 44)
Antoine Salomon, Vincent Briane, Myriam Vimond, Cesar Augusto VALADES CRUZ, Charles Kervrann
Inria Rennes, Rennes, FR

Automated inhibitor screening of aurka kinase activity based on a FRET biosensor (# 13 , Board: 13)
Florian Sizaire, Gilles Le Marchand, Giulia Bertolin, Sandrine Ruchaud, Claude Prigent, Otmane Bouchareb, Olivier Chanteux, Marc Tramier
Institut de Génétique et de Développement de Rennes, Rennes, FR

Automatic detection of tumoral tissue in hepatocellular carcinoma digital slides using deep convolutional networks (# 65 , Board: 53)
Qinghe Zeng, Nicolas Lomenie, Julien Calderaro, Christophe Klein
INSERM U1138 - Centre de Recherche des Cordeliers, Nantes, FR

Biophysical characterization of the interactions between Influenza A virus matrix protein M1 and host plasma membrane (# 112 , Board: 24)
Salvatore Chiantia
University of Potsdam, Potsdam / Golm, DE

Brownian motion using a piezo actuated microscope stage (# 26 , Board: 4)
Nicholas Vickers, Sean Andersson
Boston University, Quincy, US

Challenges in structured illumination microscopy (# 30)
Verena Richter, Mathis Piper, Michael Wagner, Herbert Schneckenburger
Aalen University, Aalen, DE

Complex diffusion analysis challenge (# 208 , Board: 11)
Maxime Woringer, Hugues Berry, Dominique Bourgeois, Cyril Favard, Ignacio Izeddin
Institut Pasteur, , France

Computational pipeline for registration of 3D images of fixed specimens to 4D time-lapse recordings of developing biological systems (# 138)
Manan Lalit, Mette Handberg-Thorsager, Florian Jug, Pavel Tomancak
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE

Determining biophysical parameters in living cells with Icy (# 198 , Board: 10)
Maria Manich, Aleix Boquet-Pujadas, Nancy Guillén, Jean-Christophe Olivo-Marin
Institut Pasteur, Paris, FR

High-resolution 2d+t imaging of calcium signaling microdomains in cardiac myocytes (# 132 , Board: 27)
Mouna Abdesselem
KU Leuven, Leuven, BE

Icy: A powerful and user friendly platform for Bio image analysis. (# 211)
Fabrice de Chaumont, Stephane Dallongeville, Jean-Christophe Olivo-Marin
Institut Pasteur, Paris, FR

Imaging release of siRNAs from vesicles damaged by membrane-disrupting drugs (# 239 , Board: 33)
Hampus Du Rietz, Hampus Hedlund, Anders Wittrup
Lund University, Lund, SE

Large scale feature extraction and image classification for high content screening microscopy (# 113 , Board: 57)
Laurent Thomas, Gunjan Pandey, Jens H.Westhoff, Franz Schaefer, Jochen Gehrig
Uniklinikum Heidelberg and Acquifer, Heidelberg, DE

Measuring ligand 2D/surface density on various substrates using fluorescence fluctuation microscopy (# 49 , Board: 35)
Dwiria Wahyuni, Irène Wang, Antoine Delon
Université Grenoble Alpes, , France

Mechanistic investigation of mEos4b blinking suggests a strategy to reduce track interruptions in sptPALM (# 25 , Board: 2)
Daniel Thédié, Dominique Bourgeois, Elke De Zitter, Peter Dedecker, Virgile Adam
IBS (Institut de Biologie Structurale), Grenoble, FR

Mesoscopic organisation of polarity proteins in epithelial cells (# 210 , Board: 29)
Edo Dzafic, Leila A Muresan
Cambridge University, Cambridge, GB

Near real time analysis of stress fiber formation in stem cells (# 31 , Board: 55)
Lara Hauke, Benjamin Eltzner, Carina Wollnik, Stephan Huckemann, Florian Rehfeldt
Georg August Universität Göttingen, Göttingen, DE

Protein copy number estimation in biological samples with high fluorescent background (# 75 , Board: 40)
Johan Hummert, Klaus Yserentant, Wioleta Chmielewicz, Dirk-Peter Herten
Heidelberg University, , Germany

Protein counting in T-cell receptor microclusters (# 139 , Board: 42)
Wioleta Chmielewicz, Dirk-Peter Herten, Oliver T. Fackler
Heidelberg University, Heidelberg, DE

Protein counting in localisation microscopy (# 62 , Board: 37)
Daniel Varga, Miklós Erdélyi
University of Szeged, Szeged, HU

Quantitative 3D geometry for large scale connectomics data (# 126 , Board: 46)
Minh-Son Phan, Katherine Matho, Lamiae Abdeladim, Jean Livet, Emmanuel Beaurepaire, Anatole Chessel
Ecole Polytechnique Paris-Saclay, Palaiseau, FR

SIMTOOLBOX 2.0: AN OPEN-SOURCE TOOLBOX FOR STRUCTURED ILLUMINATION MICROSCOPY (# 220)
Jakub Pospíšil, Tomáš Lukeš, Pavel Křížek, Martin Ovesný, Vojtěch Terš, Karel Fliegel, Miloš Klíma, Guy M. Hagen
UNI-Bielefeld, Bielefeld, DE

Simulator of benchmarking image datasets for time-lapse lightsheet microscopy (# 134)
Vladimír Ulman
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE

Software framework for Advanced Single Molecule Data Analysis (# 218)
Anish V Abraham, Dukwhan David Kim, Jerry Chao, E. Sally Ward, Raimund J Ober
Texas A&M University, College Station, US

Spatial analysis of protein or lipid distributions on the surface of spherical beads (# 135)
Anastasia Solomatina, Alice Cezanne, Marino Zerial, Ivo F. Sbalzarini
MPI-CBG, Dresden, Germany, Dresden, DE

Studying oscillatory behavior in asymmetric division of Caenorhabditis elegans embryo with fluorescence miscroscopy (# 24 , Board: 15)
Anca Caranfil, Charles Kervrann, Yann Le Cunff, Jacques Pécréaux
INRIA Rennes - Bretagne Atlantique, France, RENNES, FR

Time resolve intensity photo bleaching (TRIP) as a tool for quantify bound /unbound fraction of lamin A protein in vivo. (# 229 , Board: 31)
Eugene Brozgol, Irena Bronshtein, Iatamar Kantor, Yuval Garini
Bar Ilan University, גבעת שמואל, IL

Ultrastructure of endo-lysosomes during cholesterol efflux studied by combined fluorescence and cryo-soft X-ray tomography (# 35 , Board: 18)
Alice Dupont Juhl, Frederik W. Lund, Maria Louise Vestbjerg Jensen, Maria Szomek, Gitte Krogh Nielsen, Christian W. Heegaard, Peter Guttmann, Stephan Werner, James McNally, Gerd Schneider, Sergey Kapishnikov, Daniel Wüstner
The University of Southern Denmark, Årslev, DK

Use of X-ray micro computed tomography imaging to analyze the morphology of wheat grain through its development (# 60 , Board: 22)
Thang Duong Quoc Le, Christine Girousse, David Legland, Anne-Laure Chateigner Boutin
INRA Angers-Nantes, Nantes, FR

Using time-lapse microscopy to unravel the stochastic nature of Listeria monocytogenes infection (# 44 , Board: 20)
Josephine C Moran, Andrew Berry, James Bagnall, Pawel Paszek, Ian Roberts
University of Manchester, Manchester, GB

List of posters grouped based on topics

Poster session 1	**Abstract IDs**
Single Molecule Microscopy Methods Development	206, 209, 216
Optical Methods Development	128, 196, 215, 235, 240
Applications of Single Molecule Microscopy	117, 131
Single Molecule Microscopy Experimental Approaches	160, 212
Biological Applications of Optical Developments	19, 63, 76, 87, 223, 224, 232
Poster session 2	
Applications in Biopharma	230, 237
Machine Learning	12, 73, 86, 102, 104, 203
Application of Image Analysis Approaches	37, 40, 233
Sample Methods Developments	22
Instrument Characterization	21, 114
Data Analysis Methods Development for Single Molecule Microscopy	107, 213
Poster session 3	
Tracking	25, 26, 72, 92, 198, 208
Applications in Cell Biology and Biophysics	13, 24, 35, 44, 60, 112, 132, 210, 229, 239
Spatial Statistics	135
Single Molecule Counting and Cluster Analysis	49, 62, 75, 139
Image Analysis Methods Development	109, 126, 140, 141
Digital Pathology	65
Software	31, 113, 134, 138, 211, 218, 220
Structured Illumination	30, 123

Object detection networks for localization and classification of cells in fluorescence microscopy acquisition and analysis.

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KEYWORDS: fluorescence, microscopy, machine learning, computer vision, cell biology.

In this work we demonstrate the application of object detection networks for the classification and localization of cells in fluorescence microscopy. We benchmark two leading object detection algorithms across multiple challenging 2-D microscopy datasets as well as develop and demonstrate an algorithm which can localize and image cells in 3-D, in real-time. Object detection networks are well-known high performance networks famously applied to the task of identifying and localizing objects in photography images, here we show their application and efficiency for localizing cells in fluorescence microscopy. In the field of photography, object detection algorithms are typically trained on many thousands of images, which can be prohibitive within the biological sciences due to the cost of imaging and annotating large data. We show that with some careful considerations that it is possible to achieve very high performance with datasets with as few as 25 images present, with a dependence on the visual complexity of the cells being imaged. Through using this approach, it is possible for relatively non-skilled users to automate detection of a variety of cell classes using microscopy and opens up new avenues for automation of conventionally manual microscopy acquisition pipelines.

Deep Learning for Quantitative Bi-Exponential Fluorescence Lifetime Imaging

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Fluorescence lifetime imaging (FLI) has become an invaluable tool in the biomedical field by providing unique nanometer-range information about the specimen of interest. Applications of FLI range from super-resolution microscopy to whole body imaging using visible and near-infrared (NIR) fluorophores. However, quantifying lifetime can still be a challenging task especially in the case of bi-exponential applications. In such cases, model based iterative fitting are typically employed but necessitate setting up multiple parameters *ad hoc* and can be both time consuming and computationally expensive. These facts have limited the universal appeal of the technique and methodologies can be specific to certain applications/technology or laboratory bound.

Herein, we propose a novel approach based on the use of deep learning (DL) to quantify bi-lifetime Förster Resonance Energy Transfer (FRET). Our deep neural network outputs three images – consisting of both lifetimes and the fractional amplitude contribution. The network is trained using synthetic voxels of temporal point spread functions (TPSFs) and subsequently validated using experimental FLI microscopic (FLIM) and macroscopic data sets (MFLI). Our results demonstrate that the use of deep learning is well suited to quantify wide-field bi-exponential fluorescence lifetime accurately at speeds necessary for real-time analysis.

Keywords: fluorescence lifetime imaging, Förster Resonance Energy Transfer, NIR, deep neural network, temporal point spread function

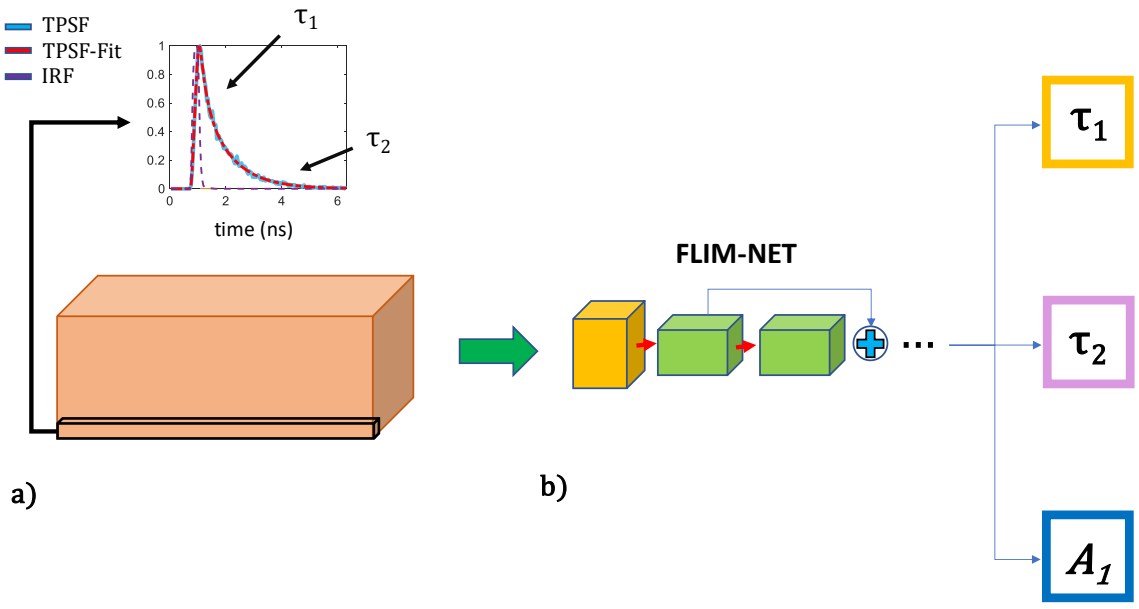

Figure 1. A simplistic depiction of a representative input data voxel containing fluorescence time decays at every pixel (a) and an illustration representing our fully-convolutional bi-exponential fitting DNN deemed "FLIM-NET", which produces three prediction maps simultaneously from the input (b).

AUTOMATED INHIBITOR SCREENING OF AURKA KINASE ACTIVITY BASED ON A FRET BIOSENSOR

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KEYWORDS: AURKA, FRET biosensor, FLIM, High-Content-Screening

AURKA gene encodes a multifunctional serine/threonine kinase involved in the cell cycle and plays a key role during cell division. Overexpression of AURKA is a major hallmark of several solid tumors rising from epithelial tissues. So far, no inhibitor of this oncogene has been FDA-approved and therefore it is of great importance to identify new molecules. To be functional, AURKA switch to an activated form through autophosphorylation on the T288 residue leading to a change of conformation.

Our team has developed a FRET (Forster Resonance Energy Transfer) based biosensor for Aurora A consisting of the whole kinase flanking by two fluorophores (Figure 1). We showed that the change of conformation of Aurora A when activated brings closer the fluorophores increasing FRET efficiency and that the biosensor is as functional as the endogenous protein [1]. We have also developed a microscope call fast-FLIM (Fluorescence Lifetime Imaging Microscopy) that allows us to image and measure fluorescence lifetime with a higher speed than conventional techniques. As fluorescence lifetime is inversely correlated with FRET efficiency, we are able to track and to image the activation of AURKA within the cells.

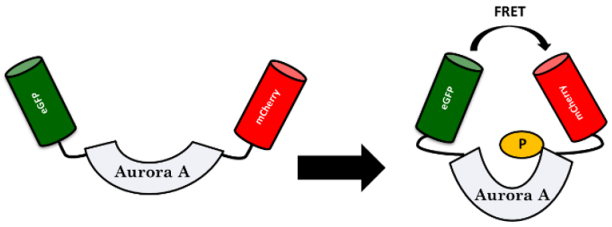

Figure 1. A FRET biosensor of AURKA activation.
The phosphorylation on the T288 residue of the kinase leads to a change of conformation gathering GFP and mCherry and increasing FRET efficiency. By measuring the fluorescence lifetime of GFP, we can have access to FRET efficiency and thus to the activation state of AURKA.

Our project aims to establish an innovative methodology to perform HCS (High Content Screening)-FLIM. In collaboration with the start-up Inscoper, we have improved our fast FLIM system and implemented a solution that can be used for Multi-Dimensional Acquisition. We are now able to screen automatically and rapidly a 96-well plate by acquiring a high number of random positions in each well. Thus, we measure and analyze FRET efficiency for each single cell expressing the biosensor indicating the activation state of the AURKA kinase. This system will allow us to screen potential inhibitors of AURKA kinase activity.

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Preprocessing structured background signals to improve super-resolution fluorescence images

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Super-resolution wide-field fluorescence microscopy can provide structural information at the nanoscale and dynamic insight about biological processes in live cell samples. In general, the available information in super-resolution images is related to the density of emitters, with more emitters leading to more information. To obtain a high spatial resolution on short time sampling, and potentially probe dynamic processes in live cells, high-density labeling is required. This results in many emitters being simultaneously active and their emissions strongly overlapping in each frame. Additionally, high-density imaging often suffers from the significant presence of structured (heterogenous[1]) fluorescence backgrounds that may be associated to auto-fluorescence, scattering or photodegradation. These phenomena not only mask the blinking of the exogenous fluorophores, with a distortion of the signal of the emitters of interest as a result, but they also reduce the nanoscale resolution and introduce significant artifacts in the super-resolved image. Here we propose to investigate the use of signal and image smoothing procedures to handle structured fluorescence background signals at a pre-processing step. The aim is to accurately separate the signal of the fluorescent emitters of interest from the heterogeneous background signals. For this purpose, smoothing approaches are flexible and solid alternatives to data fitting methods where one would model photobleaching with multi-exponential decays[2], on the one hand, and filtering approaches, such as temporal median filters[1] and spatial filtering methods[3], which do not rely on any statistical or phenomenological model, on the other hand.

In the temporal mode, one can remove the general trend of the underlying pixel signal by using different approaches, ranging from three-way approaches[4], where one would indirectly profit from the multi-exponential nature of the data, to Whittaker smoothing[5], where the intrinsic smoothness of the photodegradation signal is used to our advantage. The latter is probably the most efficient and the most flexible tool, and it can be extended to the spatial dimension, using an asymmetric 2D P-splines approach[6]. This allows clearly spatially structured background signals to be separated with ease from the signals of the fluorescent emitters. In addition, simultaneously working in the spatial and temporal dimensions can also be investigated, and takes into account the advantages of both approaches. We show that with proper pre-processing of the data, the final results obtained after super-resolution are clearer, sharper and have a better resolution. Moreover, several other steps of the analysis pipeline become unnecessary. We demonstrate the performance and accuracy of the proposed approaches both on simulations and experimental data in the presence of strong heterogeneous fluorescence backgrounds of different nature.

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DEEP LEARNING FOR DENSE AND MULTICOLOR LOCALIZATION MICROSCOPY

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KEYWORDS: deep learning, super-resolution microscopy, localization microscopy, particle tracking

Deep learning has become an extremely effective tool for image classification and image restoration problems. Here, we address two fundamental problems of localization microscopy using machine learning: high emitter density, and color determination. Modern microscopy can produce images of biological specimen at very high (super) resolution, by precisely determining the positions of numerous blinking light emitting molecules over time. To achieve fast acquisition time, a high density of molecules is required, which poses a significant challenge in terms of image processing. Existing approaches use elaborate algorithms with many parameters that require tuning and a long computation time. Here, we report an ultra-fast, precise, and parameter-free method for super-resolution microscopy that utilizes deep-learning: by feeding the computer images of dense molecules along with their correct positions, it is trained to automatically produce super-resolution images from blinking data [1].

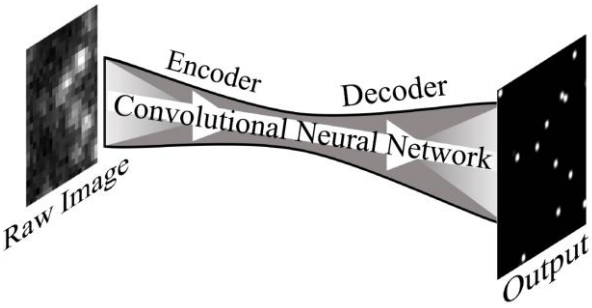

Figure 1. DeepSTORM concept. A set of low-resolution diffraction limited iamges of stochastically blinking emitters is fed into the network to produce reconstructed high-resolution images. The outpus are summed to generate the final SR image [1].

Next, we demonstrate how neural networks can exploit the chromatic dependence of the point-spread function to classify the colors of single emitters imaged on a grayscale camera. While existing single-molecule methods for spectral classification require additional optical elements in the emission path, e.g. spectral filters, prisms, or phase masks, our neural net correctly identifies static as well as mobile emitters with high efficiency using a standard, unmodified single-channel configuration. Finally, we demonstrate how deep learning can be used to design phase-modulating elements that, when implemented into the imaging path, result in further improved color differentiation between species [2].

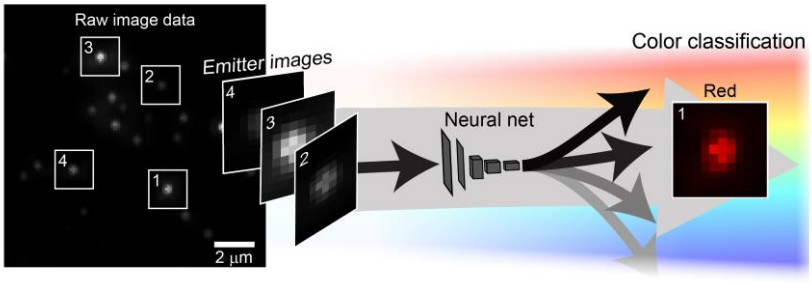

Figure 2. A neural net is trained to distinguish between the colors of different emitters, relying on inherent chromatic aberrations in the PSF [2].

[1] E. Nehme, L.E. Weiss, T. Michaeli, and Y. Shechtman, "*Deep-STORM: Super Resolution Single Molecule Microscopy by Deep Learning*", Optica 4, 458-464 (2018).

[2] E. Hershko, L.E. Weiss, T. Michaeli, Y. Shechtman, "*Multicolor localization microscopy by deep learning*", arXiv: 1807.01637 (2018).

How accurately two or more fluorophores can be spectrally resolved in a fluorescence microscope?

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KEYWORDS: Spectral unmixing, Multispectral imaging

Multicolor fluorescence imaging is widely used to visualize the spatial distribution of distinct protein targets in cells and tissues. Due to the broad emission spectra of fluorophores, traditional microscope configurations can typically image a limited number (~4-5) of fluorescent labels simultaneously. This raises a fundamental question on spectral resolvability which concerns with how accurately two or more spectrally close fluorophores can be resolved when imaged through a fluorescence microscope. Here, this question is addressed by adopting a general stochastic model for multicolor imaging data and deriving the Fisher information matrix for the underlying spectral unmixing problem. As a special case, the linear mixing model is investigated which is typically used to model multicolor fluorescence data. By using the Cramer-Rao inequality, a linear unmixing performance bound is introduced that provides a spectral resolution limit for fluorescence imaging and predicts how accurately a pair of fluorescent labels can be spectrally unmixed.

Further, it is shown how the spectral resolution limit can be overcome by exploiting the phenomenon of anti-Stokes shift fluorescence. In addition, the effects of photon statistics, channel addition and channel splitting on the performance bound are investigated. Finally by using the performance bound as a benchmark, the behavior of the least squares and the maximum likelihood estimator based spectral unmixing algorithms are evaluated. For the imaging conditions tested here, it is shown that both estimators are unbiased and that the standard deviation of the maximum likelihood estimator is consistently closer to the performance bound than that of the least squares estimator. The results presented here are based on broad assumptions regarding the underlying data model and are applicable to imaging data acquired with point detectors, sCMOS, CCD and EMCCD imaging detectors.

In vitro Verification of a Molecular Counting Technique Based on Fluorophore Blinking Statistics using DNA Origami

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KEYWORDS: Molecular Counting, Single-Molecule Localization Microscopy, Quantitative Localization Microscopy, DNA Origami

Single-molecule localization microscopy (SMLM) has been a powerful tool for expanding our understanding of cell biology. By taking advantage of the stochastic blinking that fluorophores naturally exhibit and controlling the dynamics of this process, SMLM pushes the level of resolution an order of magnitude beyond the diffraction limit allowing light microscopy to visualize cellular components with enhanced resolution. There is also tremendous interest in using the technique as a quantitative tool to count single molecules. The main challenge of molecular counting in SMLM is largely a result of the multiple and random blinking of fluorophores, which leads to over-counting the number of molecules. In [1], we proposed a method for counting biomolecules based on the blinking statistics of fluorophores captured in a single parameter which we called the characteristic number of blinks. Our method also takes into account the labeling efficiency to the target molecule. We show how our theory may be used by analyzing in silico data, and in vitro data based on DNA origami structures with organic fluorophores. Accurately determining the number of proteins or nucleic acids in a cell has wide-ranging applications: from systems biology, to proteomics/genomics, to fundamental cell biology. As a potential application, we've begun to apply this technique to the problem of accurately determining plasmid copy number in bacteria.

[1] D. Nino, N. Rafiei, Y. Wang, A. Zilman, and J.N. Milstein *Molecular Counting with Localization Microscopy: A Bayesian Estimate Based on Single Fluorophore Statistics,* (Biophys. J. 112, 1777-1785, 2017)

Probing cells dynamics with quantitative Dynamic Full-Field OCT

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KEYWORDS: OCT, cell cultures, 3D reconstruction, multimodal imaging, *ex vivo* samples, intracellular dynamics

Dynamic Full-Field Optical Coherence Tomography (FFOCT), a recent *en-face* microscopic tomography technique that we developed, allows us to capture metabolic information (intracellular dynamics) in *in vitro* samples, such as cell cultures, and in *ex vivo* samples, thus without using any exogenous contrast agent. The metabolic information inside each cell are recorded in the ms range with a 0.5 µm lateral x 1.7 µm axial resolution, over 100 µm depth in fresh tissue explants. Our setup combines three imaging techniques: FFOCT, Dynamic FFOCT and fluorescence imaging system. Combining the setup with our developed-and-applied algorithms, we are able to quantitatively distinguish different structures in living samples and cell cultures.

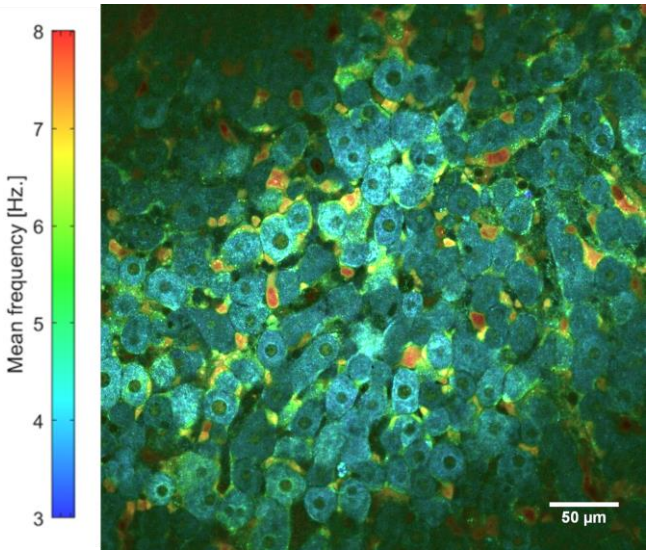

Figure 1. En-face dynamic image of rat liver, at a depth of 20 µm below the surface

With the Dynamic FFOCT, it is possible to register and reconstruct 3D volumes, but also, thanks to stabilized acquisitions, to track cells all-day long, allowing us to monitor their activity. Using these possibilities, we demonstrated the organization and function of cells at different layers in *ex vivo* and *in vivo* samples, and we can follow their behavior, such as migration in a wounded cornea or cells death in Retinal Pigmented Epithelium (RPE) cell culture for instance. With a waterproof insulated cavity, we can maintain an appropriate environment for the sample and so acquire the dynamics on a rat liver during eight hours. We have been able to distinguish red blood cells from Küpffer cells (which appear red on Figure 1) and hepatocytes (which appear blue-green on Figure 1).
Combining our three imaging techniques (fluorescence imaging system, FFOCT and Dynamic FFOCT) allows us to discriminate living cells from dying cells and dead cells by their dynamic signal. This comparison permits us to demonstrate that our method is completely non-contact and non-invasive and introduces a new mode contrast for 2D and 3D samples. Reconstructing 3D volumes of cell cultures and monitor their behavior, such as cancerous cells, could help biologists and oncologists to better understand the cancerous cells' defense mechanisms to survive difficult environment.

A DETAILED ANALYSIS OF THE MOBILITY OF SYNAPTIC PROTEINS REVEALS PRINCIPLES OF PROTEIN SEGREGATION IN THE SYNAPSE AND IN THE AXON

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The synaptic bouton is one of the most intensely studied cellular compartments. Its protein composition has been determined both qualitatively and quantitatively. However, little is known about the mobility of synaptic proteins, and the mechanisms regulating the protein composition of the synaptic bouton are still unclear. In this study we used fluorescence recovery after photobleaching (FRAP) to determine protein mobility in synaptic boutons and axons of primary hippocampal neurons. Analysis of ~50 of the most abundant synaptic proteins showed significant differences between protein mobility in axons and synapses. Additionally, we found that proteins from different functional categories have different mobility behaviors.

To unravel possible mechanisms of such segregation we modeled protein diffusion in an "average" synapse, relying on a realistic 3D space created using data from 3D electron microscopy reconstructions of synapses of primary hippocampal neurons. We then simulated FRAP experiments on the basis of this virtual synapse. By fitting simulated data to experimental results we determined the different diffusion coefficients. We found that for plasma membrane proteins the difference in protein mobility between the synapse and the axon could be explained solely by differences in the geometries of the respective compartments. The more complex behavior of soluble proteins required the introduction of additional variables such as their binding to synaptic vesicles. The parameters we obtained enable us to create the first dynamic model of the synapse at molecular scale.

Calibrating a microscope by learning its diversity

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KEYWORDS: Learning in microscopy, PSF engineering, blind-deblurring.

Microscopy images usually suffer from blur which impairs image interpretation by lowering the effective resolution… In this work, we propose a new calibration technique that learns a low dimensional subspace of potential degradation operators instead of a single one. We focus on the challenging problem of space-varying blurs which are - arguably - the most common in practice. We then design specific blind deblurring approaches relying on the prior knowledge of the blur family. This knowledge strongly improves the problem's identifiability.

During the calibration stage, we first collect a set of images containing fluorescent microbeads under different conditions (e.g. variations of focus, temperature, tilt of the slide,...). A typical example of image using a wide-field microscope is displayed in Fig. 1.
From this information we can reconstruct a family of blurring operators with a product-convolution structure [1]. The reconstruction method consists in decomposing the observed point spread functions in a single orthogonal basis (Fig. 2) and to interpolate the coefficients spatially using radial basis functions (Fig. 3) [2,3]. Each operator is therefore described by these two sets of images, learned using a Tucker-2 tensor decomposition. In addition, we compute the convex hull of the observed operators. Fig. 2 and Fig. 3 show the two bases learned during a calibration stage with 640 calibration images such as that in Fig. 1.

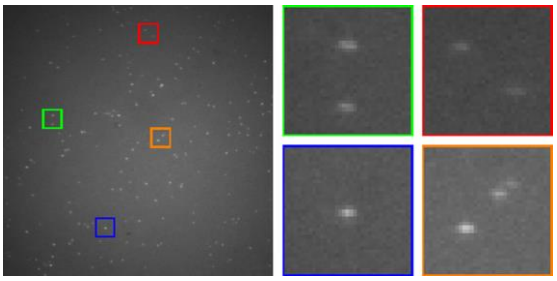

Figure 1: Sampled PSFs.

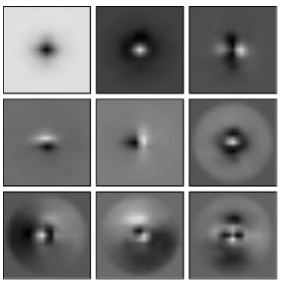

Figure 2: Convolution basis.

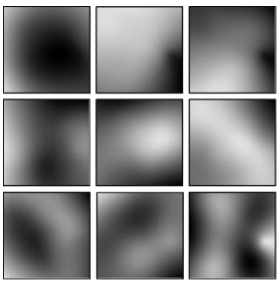

Figure 3: Coefficients basis.

Significant theoretical advances have been achieved recently in the field of blind-deconvolution [4]. Unfortunately the algorithms rely on the knowledge of a low dimensional basis where the sharp image lives and are restricted to convolution operators. We will show how the proposed calibration step allows to extend the scope of these methods to space-varying blurs. We also make those algorithms practical on synthetic and real data.

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[2] F. N. Mboula, J. L. Starck, S. Ronayette, K. Okumura, and J. Amiaux, *Super-resolution method using sparse regularization for point-spread function recovery*, Astronomy & Astrophysics, 575, A86, 2015.
[3] M. Gentile, F. Courbin, and G. Meylan, *Interpolating point spread function anisotropy*, Astronomy & Astrophysics, 549, A1, 2013.
[4] A. Ahmed, B. Recht, and J. Romberg, *Blind deconvolution using convex programming*, IEEE Transactions on Information Theory, 60(3), 1711-1732, 2014.

Semi-quantitative analysis of the trace metallic elements with synchrotron radiation X-ray fluorescence analysis and the experimental concentration standard specimens

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KEYWORDS: SR-XRF, elemental distribution, trace metallic element

In medical diagnosis, the distribution of trace metallic elements is analyzed to determine their effect on various lesions, such as cancers, dysplasia, and inflammations. Synchrotron radiated fluorescence X-ray analysis (SR-XRF) is useful tool to visualize the trace metallic element distribuion in the medical specimens because of its high sensitivity and low damage to specimens. Quantitative analysis of such accumulated metallic elements, using standard specimens, should be conducted to evaluate their effect on tissues. The authors prepared the thin film standard specimens using organometallic compounds and photo-cured methacrylate resins. The resin monomer was prepared by bisphenol A-glycidyl methacrylate (Bis-GMA) and the acetylacetonates of Cr, Fe, Ni, Cu, and Zn were disslved into the monomers to be a specific contents. The mixed monomers were pressed with glass plates and photo-polymerized. Prepared thin films are flexible and both chemically and mechanically durable for repetitive usage. As shown in Figure 1, good linearity between the thickness corrected fluorescence X-ray intensity and the element concentration of Cr, Fe, Ni, and Zn standards.

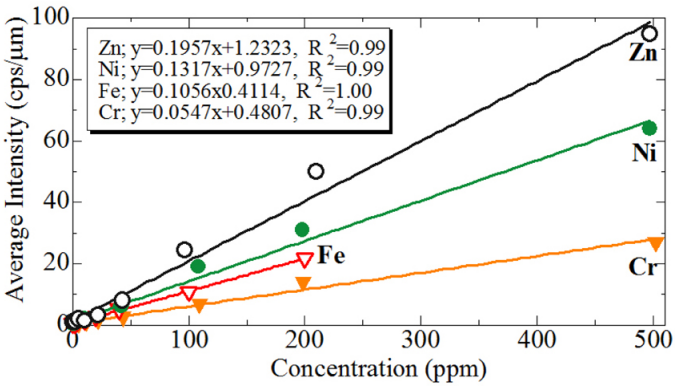

Figure 1. The calibration curves of the prepared thin film standards of Cr, Fe, Ni, and Zn.

Figure 2 shows the distribution image of Ni-ion-spread specimen (freeze-sectioned; 8μm) from the skin surface, onto which a Ni solution was applied. By applying the previous calibration curve, it was revealed that Ni could be evaluated in the skin up to a highest concentration of 700 ppm, whereas the average concentration in the Ni-infiltrated region was approximately 200–300 ppm. Thus, the thin film standards would be applicable for the quantitative estimations of trace metallic elements contained in thin-sectioned biomedical specimens.

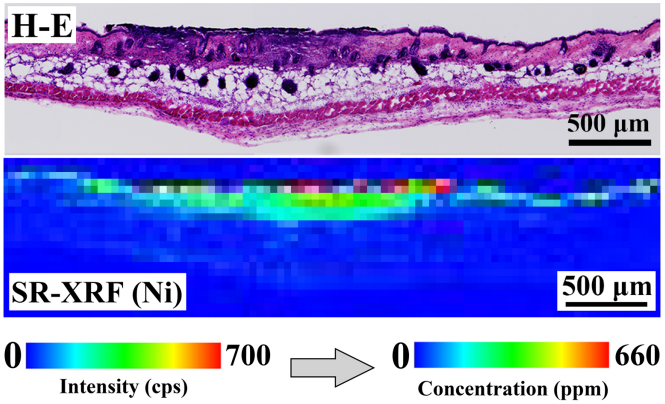

Figure 2. An example of practical quantitative estimation using the case of Ni concentration in Ni-ion-infiltrated mouse skin (24 hrs) [1]

[1] T. Sugiyama, M. Uo, T. Wada et al.: *J. Nanobiotechnology*, 12:51, 1-6, 2014.

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Quantitative analysis of collagen microstructure in vitro and in vivo by means of multi-phasor analysis of second harmonic generation microscopy.

G. Chirico, L. D'Alfonso, M. Collini, M. Bouzin, L. Sironi

The collagen organization in tissues changes following pathological states or stresses and can be monitored by means of Second Harmonic Generation (SHG), a label-free imaging method. Due to its sensitivity to the incident polarization, it can easily provide qualitative microstructural information. We have developed a phasor analysis of the polarization dependent second harmonic generation images that is able to quantitative map the features of the collagen architecture in tissues at the micrometer scale. Two coupled phasor analysis performed on a stack of polarization images on distinct polarization angle range ranges provide the fibril orientation and the local molecular disorder.

This method that we call Microscopic Multiparametric Analysis by Phasor projection of Polarization-dependent SHG (μMAPPS, Radaelli 2017), is a fast, efficient approach that retrieves pixel-by-pixel the collagen fibrils anisotropy and orientation, avoiding direct fitting of the polarization dependent SHG signal. μMAPPS can be applied to study the collagen microscopic organization *ex-vivo* and *in-vivo*. We further developed a clustering algorithm to automatically group pixels with similar microstructural features.
μMAPPS can perform fast analyses of tissues and opens to future applications for *the study of the evolution of stress conditions in 3D cell culture models*.

F. Radaelli, L. D'Alfonso, M. Collini, F. Mingozzi, L. Marongiu, F. Granucci, I. Zanoni, G. Chirico & L. Sironi. μMAPPS: a novel phasor approach to second harmonic analysis for in vitro-in vivo investigation of collagen microstructure. Scientific Reports (2017) 7: 17468.

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Studying oscillatory behavior in asymmetric division of *Caenorhabditis elegans* embryo with fluorescence microscopy

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Keywords: asymmetric cell division, *C.elegans* embryo, modelling oscillatory behavior, Bayesian estimation, fluorescence miscroscopy

Asymmetric cell division is a complex process that is not yet fully understood. A very well-known example of such a division is *C.elegans* embryo's first division. To improve our understanding of this process, we used fluorescence microscopy and mathematical modelling to study *C.elegans* embryo's first division, both on wild type cells and under a wide range of genetic perturbations. Asymmetry is clearly visible at the end of the anaphase, as the mitotic spindle is off-center. The study of the mitotic spindle dynamics is, thus, a useful tool to gain insights into the general mechanics of the system used by the cell to correctly achieve asymmetric division. The overall spindle behavior is led by the spindle poles behavior.

We proposed a new dynamic model for the spindle pole that explains the oscillatory behavior during anaphase and confirms some previous findings, such as the existence of a threshold number of active force-generator motors required for the onset of oscillations. We also confirmed that the monotonic increase of motor activity accounts for their build-up and die-down. By analyzing our model, we determined boundaries for the motor activity-related parameters for these oscillations to happen. This also allowed us to describe the influence of the number of motors, as well as physical parameters related to viscosity or string-like forces, on features such as the amplitude and number of oscillations. Lastly, by using a Bayesian approach to confront our model to experimental data, we were able to estimate distributions for our biological and bio-physical parameters. These results give us insights on variations in spindle behavior during anaphase in asymmetric division, and provide means of prediction for phenotypes related to misguided asymmetric division. This model will be instrumental in probing the function of yet undocumented genes involved in controlling cell division dynamics.

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MECHANISTIC INVESTIGATION OF mEos4b BLINKING SUGGESTS A STRATEGY TO REDUCE TRACK INTERRUPTIONS IN sptPALM

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KEYWORDS: PALM, fluorescent proteins, single-particle tracking, photophysics

Green-to-red photoconvertible fluorescent proteins (PCFPs), such as the EosFP variants, are widely used in PhotoActivated Localization Microscopy (PALM). However, PCFPs tend to blink, that is, repeatedly enter non-emissive states. This behavior is detrimental to PALM experiments: it can induce clustering artifacts, counting errors in quantitative PALM studies, and interrupted tracks in single particle tracking (sptPALM). Furthermore, PCFPs can enter several blinking regimes [1], [2]. Short-lived blinking processes, such as conversion to the triplet state, have been characterized [3], but the nature of long-lived dark states, most detrimental to PALM experiments, has remained unknown. Figure 1 shows a typical time-trace of a single mEos4b molecule during a PALM experiment, with long blinking events lasting up to tens of seconds.

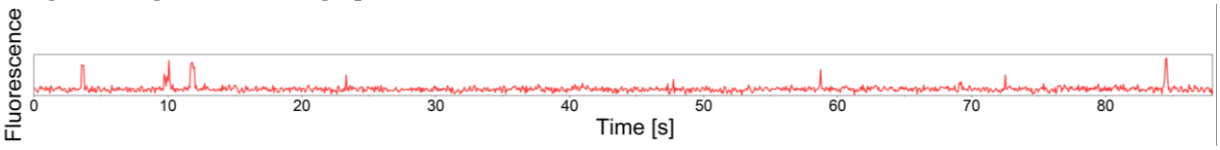

Figure 1. Time-trace of a single mEos4b molecule during a PALM experiment, showing short bursts of fluorescence, separated by repeated, long-lived blinking events.

Here, we studied long-lived dark states in mEos4b and showed that in the red form of the protein, the major long-lived dark state corresponds to a trans protonated state of the chromophore, reminiscent of photoswitching in Reversibly Switchable Fluorescent Proteins (RSFPs). This long-lived dark state exhibits a maximum absorption at around 488 nm, suggesting that short bursts of light at this wavelength during PALM experiments could swiftly revert dark mEos4b to its fluorescent state, hence suppressing long-lived blinking without affecting the photoconversion rate. This strategy allowed extending the tracking time of single-molecules in spt-PALM (Figure 2), and will be demonstrated in this presentation.

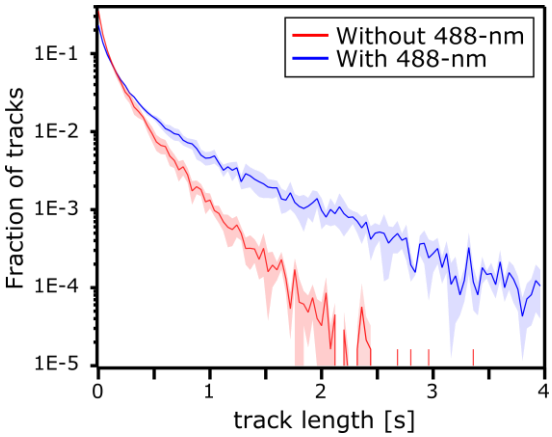

Figure 2. Using 488-nm light allows increasing the number of long tracks in spt-PALM experiments. PDF: Probability Density Function.

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Brownian Motion Using a Piezo Actuated Microscope Stage

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KEYWORDS: single particle tracking; simulation

Small molecules, proteins, and other organic material play a large role in health and disease on a cellular level. To understand cellular function and disease, it is necessary to understand how these molecules transport, bind, undergo conformational changes, and are consumed within cells and tissues. The study of cells at these length scales necessitates the development of methods that can localize and track single particles at the nanometer scale, collectively known as single particle tracking (SPT). However successful these techniques are, it is difficult to compare them directly [1], especially as one is most interested in their performance on a specific experimental setup.

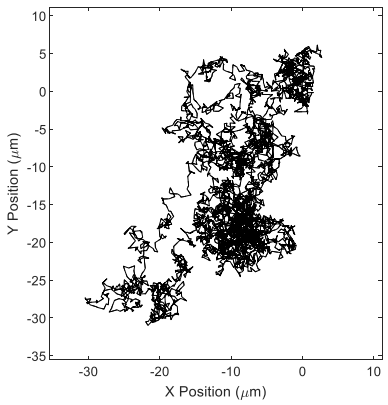

Figure 1. Example Brownian motion (BM) trajectory

In this work, we demonstrate through simulation and experiment the ability of a piezo actuated microscope stage to replicate faithfully a discrete time sampled Brownian motion (BM) trajectory. We envision this work will allow for the characterize the performance of microscopes and localization algorithms used in SPT by providing accurate ground truth as in a simulation but real imaging data as in an experiment.

We consider both simulation and experiment. The piezo-actuated stage was modeled as a first order system, describing the closed-loop performance of the stage. BM trajectories were generated using a discrete time sampled motion model (Figure 1) and then used as an input to the system. The resulting trajectories were compared in two ways (Figure 2): through the Kullback-Leibler (KL) divergence between the single-step increment probability distributions of the input and output trajectories, and through the mean squared displacements (MSD) as a function of time. We find that the motion of the stage follows the diffusion trajectory when the sampling time step of the BM trajectory was greater than three times the system time constant, independent of the diffusion value. Thus, BM over a large range of diffusion constant can be generated at typical frame rates of widefield fluorescence microscopy. When intra-frame motion is required (to capture motion blur), the trajectory must be pre-filtered to maintain faithful trajectory following.

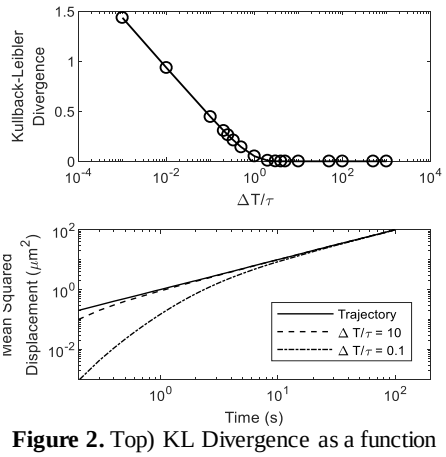

Figure 2. Top) KL Divergence as a function of BM sampling time/system time constant ratio. Bottom) MSD as a function of time for the BM trajectory, widefield frame rate, and intra-frame rate respectively.

Future work includes using other biologically relevant motion models such as confined diffusion and elastic tethering as the motion model, and a comparison of SPT microscope configurations and estimation algorithms through simulated motion with a piezo stage.

[1] Chenouard, Nicolas, et al. "Objective comparison of particle tracking methods." *Nature methods* 11.3 (2014): 281.

ABOUT THE OPTIMAL OPTICAL TRANSFER FUNCTION OF A MICROSCOPE

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KEYWORDS: point spread function, optical transfer function, image deconvolution

We study the question what the optimal optical transfer function (OTF) is for a given type of optical microscope with a given numerical aperture, In other word, we consider which OTF would lead to the best possible spatial resolution and contrast in imaging. This question is important for an optimal deconvolution of a recorded microscopy image. Only by knowing the theoretically optimal OTF one can obtain deconvolution results with maximum spatial resolution and contrast. We present a heuristic criterion how to theoretically find this optimum, and analyze various microscopy types such as wide-field microscopy, confocal scanning microscopy, and image scanning microscopy.

DNA ORIGAMI AS A NANOSCALE PLATFORM FOR T-CELL ACTIVATION

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In the human immune system, the recognition of an antigen by the T-cell receptor (TCR) takes place within the contact area between the T-cell and the antigen-presenting cell (APC). It is thought that the nanoscale spatial distribution of proteins within this contact zone plays an essential role in the initiation of an immune response. Despite extensive studies, the molecular details of this process, in particular the structural requirements for TCR triggering and how nanoscale events are translated into T-cell activation, are still poorly understood.

Here, we use DNA origami decorated with TCR ligands anchored to a planar glass-supported lipid bilayer to assess the effects of local ligand density and arrangement on T-cell activation. Thus, our experimental setup allows for the precise nanoscale arrangement of TCR ligands on the DNA origami scaffold, while at the same time permitting the re-organization of ligand and TCR during T-cell activation. We used either recombinant TCRβ-reactive single chain antibody fragment (scF_v) or MHC class 2 as stimulatory ligands that were placed on the DNA origami at various engineered capture sites in different layouts and densities.

The actual number of ligands per origami was determined using several single molecule fluorescence microcopy methods and atomic force microscopy. The activation of T-cells interfaced with the APC-mimicking surfaces was measured using a Ca^2-sensitive fluorescent dye and the effects of local ligand density, nanoscale ligand arrangement as well as the nature of the ligand were assessed. Further, the rearrangement of TCR and ligand in the process of T-cell activation was monitored by single molecule microscopy.

CHALLENGES IN STRUCTURED ILLUMINATION MICROSCOPY

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KEYWORDS: Live cell imaging, superresolution microscopy, structured illumination, SIM

In addition to Stimulated Emission Depletion (STED) or Single Molecule Localization Microscopy (PALM, STORM and related techniques) Structured Illumination Microscopy (SIM) has proven its super-resolution potential. Lateral resolution of about 100 nm is by a factor 2 below the value given by the Abbe criterion. Light exposure in SIM exceeds that of conventional wide-field microscopy only slightly, making SIM suitable for live cell microscopy, in particular, if long exposure times or repeated measurements are required.

An experimental setup for SIM – based on a spatial light modulator (SLM) has been established previously [1,2]. Preliminary applications include imaging of nanoparticles as well as of the cytoskeleton (using a fluorescent tubulin marker) and mitochondria (using rhodamine 123) of living cells. In all cases an increase of resolution from more than 200 nm to about 100 nm was well documented.

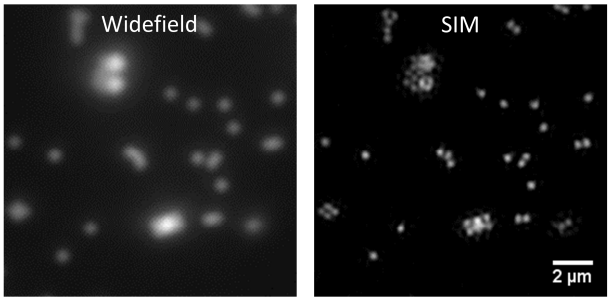

Figure 1. Imaging of fluorescent particles of 200 nm size (λex = 488nm; $\lambda d \geq$ 515 nm).

When using SIM for live cell imaging several features are required or subject of further discussion:

1. The grating constant of the interference pattern should be only slightly above 200 nm in order to profit from the advantage of the SIM method. This requires minimum values of the diffraction angle α as well as high resolution cameras for image detection.
2. The aperture A of the microscope lens should be large enough to permit transmission of 2 illuminating beams.
3. The total light dose of illumination should be limited to about 100 J/cm² corresponding to 1 µJ/µm² to keep cells viable after incubation with a fluorescence marker or transfection with a fluorescent protein.
4. Fast image detection, automatization and synchronization of SLM and camera are necessary for studies of dynamic processes.
5. Modular design and versatility, e.g. combination with light sheet microscopy, is desired for 3D imaging.

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NEAR REAL TIME ANALYSIS OF STRESS FIBER FORMATION IN STEM CELLS

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KEYWORDS: stress fibers, differentiation, hMSC, cell shape, fluorescence microscopy.

Human mesenchymal stem cells (hMSC) can be directed to differentiate into various cell types by growing them on matrices of different elasticities *E*. In contrast to changes in lineage specific protein expression, which occur over a period of days to weeks, significantly different structures of stress fibres are observable within the first 24 hours of planting [1]. Further analysis of stress fibre structure, quantified by an order parameter *S*, can be used as an early morphological marker. It also dictates the morphology of the nucleus and therefore could directly affect gene transcription.
We use a massively parallel live-cell imaging set-up to record cells under physiological conditions (37 °C, 5 % CO_2) over a period of 24-48 hours. This way we obtain a large, statistically sufficient data set. To minimize the impact of the fluorescent marker, we use an optimized lifeact-TagRFP transfection of hMSCs to visualize the structure and formation of actin-myosin stress fibres. We aim for a full representation of filament processes over time and space, allowing for statistical analysis. The current understanding and classification of stress fibres (dorsal, ventral, arcs) is based on their location in the cell and function during migration. In contrast, we concentrate on an unbiased classification due to their temporal and spatial persistence that should also correlate with function. This might for example been represented by significantly different persistence in space and time and crosstalk with other cytoskeletal components. For this task we developed the 'Filament Sensor' [2, 3], a freely available tool for near real-time analysis of stress fibres. We present experimental data where we can distinguish the cytoskeletal structures of hMSCs on 1 kPa, 10 kPa and 30 kPa elastic substrates with 99 % confidence. We are working on single filament tracking, a sophisticated analysis of the structure in terms of orientation fields, and 3D filament tracing and tracking.

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INVESTIGATION OF THE CROSS-TALK BETWEEN DESMOSOMES AND TETRASPANIN-ENRICHED MICRODOMAINS

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KEYWORDS: cell-cell adhesion, desmosomes, tetraspanins.

Desmosomes are intercellular junctions that are responsible for providing strong adhesion between cells [1]. They are particularly important for maintaining the integrity of tissues that are subject to the mechanical stress such as the epidermis and myocardium. Desmosomes are able to adopt a strongly adhesive, or hyper-adhesive, state to facilitate cell-cell adhesion. They are able to adopt an alternative less adhesive state to facilitate wound healing in a process that is regulated by PKCα. Tetraspanins are membrane-spanning proteins that organise a network of interactions at the plasma membrane [2]. The tetraspanin CD82 regulates the function of adhesion receptors and signalling molecules such as PKCα. Our results show that CD82 strengthens cell-cell adhesion and specifically contributes to desmosomal adhesion. Using 3D-STORM we have shown that CD82 increases clustering of desmoplakin molecules in desmosomes. Cluster analysis was carried out using the Density-based spatial clustering of applications with noise (DBSCAN) algorithm for each 2 x 2-μm region of interest containing an individual desmosome. In addition, CD82 mediated clustering results in desmosomal plaque protein reorganisation. The CD82 mediated increase in cell-cell adhesion is likely to be PKCα dependent. These results suggest that CD82 plays a role in the assembly and maturation of desmosomes.

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AN AUTOMATED BAYESIAN PIPELINE FOR RAPID ANALYSIS OF SINGLE-MOLECULE BINDING DATA

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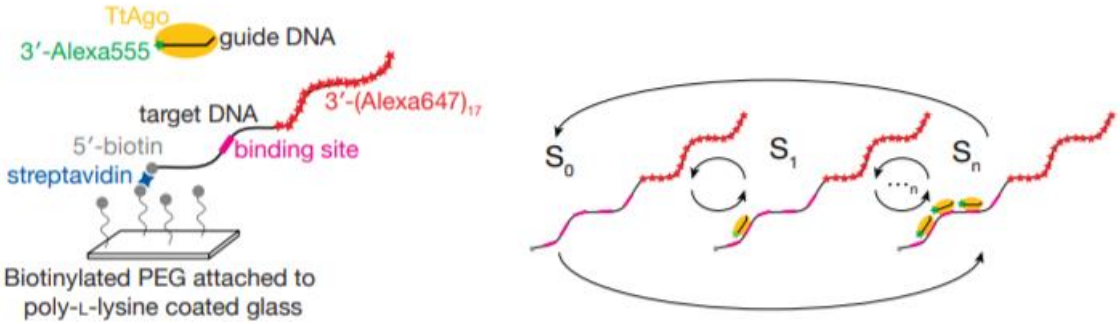
KEYWORDS: Co-localization Single-Molecule Spectroscopy (CoSMoS)

Single-molecule binding assays enable the study of how molecular machines assemble and function. Current algorithms can identify and locate individual molecules, but require tedious manual validation of each spot. Moreover, no solution for high-throughput analysis of single-molecule binding data exists. Here, we describe an automated pipeline to analyze single-molecule data over a wide range of experimental conditions. We benchmarked the pipeline by measuring the binding properties of the well-studied, DNA-guided DNA endonuclease, TtAgo, an Argonaute protein from the Eubacterium Thermus thermophilus. We also used the pipeline to extend our understanding of TtAgo by measuring the protein's binding kinetics at physiological temperatures and for target DNAs containing multiple, adjacent binding sites.



Imaging Complex Protein Machines by High-Throughput Localization Microscopy

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KEYWORDS: superresolution, automated microscopy, endocytosis

Single-molecule localization microscopy (SMLM) has become a popular tool in cell biology as it allows imaging cellular structures with a precision of tens of nanometers. However, acquiring superresolved images with SMLM is inherently slow, requiring up to hours for a single image and careful user intervention in between experiments. This effectively limits the throughput of SMLM to a handful of images of the structure of interest per study.

We overcame this limitation by developing a fully automated microscope for high-throughput superresolution (HT-SRM). This microscope is optimized for stability and capable of performing unsupervised SMLM imaging over the course of days. It is controlled by an open-source plugin for Micro-manager, allowing the design of complex experiments. In addition, it features a flat illumination system[1], providing homogeneous statistics across large fields of view. We used this microscope to automatically acquire 100000 sites of clathrin-mediated endocytosis in yeast[2]. This high statistical power allowed us to resolve fine details of the endocytic machinery. This application demonstrates that HT-SRM enables system-wide imaging of the structural organization of complex protein machines.

Our current efforts are aimed at improving the microscope automation by implementing feedback microscopy, using bright-field illumination or fluorescence images, to automatically detect region of interests and focus on the relevant structures.

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Abstract 35

Ultrastructure of Endo-Lysosomes during Cholesterol Efflux studied by Combined Fluorescence and Cryo-Soft X-ray Tomography

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Keywords: Soft X-ray tomography, fluorescence, image analysis, non-vesicular transport, cholesterol efflux

Cryo-Soft X-ray Tomography (Cryo-SXT) is an optical imaging technique which uses X-rays instead of visible light to visualize cellular ultrastructure in 3D tomograms. Cryo-SXT operates within the water window, defined as the X-ray absorption of the *K* edges of carbon (284 eV, 4.37 nm) and of oxygen (543 eV, 2.28 nm). Within this window, hydrated biological samples do not need to have any additional labeling, since a natural absorption contrast will occur from the carbon-rich biological structures [1]. Using Fresnel zone plates as optical elements and synchrotron radiation provided by the BESSY II at the Helmholtz Center Berlin, cellular ultrastructure can be imaged by SXT with a resolution down to 25 nm [1, 2]. By cryo freezing the biological sample, mammalian cells can be imaged in a near-native-state while being moderately protected from radiation damage, such that the sample can be exposed to multiple radiation doses enabling tilt series image collection. The tomograms are subsequently aligned and reconstructed, providing the additional advantage of not having to section the sample in order to obtain 3D information [3]. Since contrast is primarily created by absorption, X-rays can penetrate significantly into the sample allowing for gathering depth information in tomograms for up to 10 mm thickness [3].

Cellular cholesterol homeostasis depends on sterol efflux from late endosomes and lysosomes (LE/LYS), also called endo-lysosomes. By a combination of Cryo-SXT and quantitative fluorescent microscopy, we have studied Niemann Pick C2 (NPC2) protein mediated cholesterol export from LE/LYS. According to a well-established model, the small soluble sterol-binding NPC2 protein binds cholesterol inside endo-lysosomes and hands it over to the NPC1 protein, in the limiting membrane of LE/LYS, for exporting the cholesterol from endo-lysosomes to other cellular compartments [4].

We have used human skin fibroblasts from NPC2 lacking patients as a model system, to study efflux of plasma membrane derived sterol from LE/LYS, and the accompanying relocalization and morphological changes of endo-lysosomes. By quantitative fluorescence microscopy we showed that dehydroergosterol (DHE), a close fluorescent cholesterol analog [5], trapped in LE/LYSs can slowly efflux from NPC2 lacking fibroblasts, however this process was heavily accelerated upon internalization of bovine NPC2 protein. The efflux was accompanied by relocation of LE/LYS, which moved from the perinuclear region towards the cell periphery, and by extensive tubulation of these organelles. We characterized tubule formation on LE/LYS by both by fluorescent microscopy and Cryo-SXT. Using the exquisite 3D preservation of membranes, we were able to quantify the size of endo-lysosomes in dependence of the NPC2 and sterol content with high precision [6]. In addition, we observed shedding of micro-vesicles at the plasma membrane by SXT, which might represent a mechanism for cholesterol efflux.

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Advanced imaging flow cytometry

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Imaging flow cytometry (IFC) is a hybrid technology which extends conventional flow cytometry with additional high resolution morphological information. The objective of our work is to develop a microfluidic system for conventional and tomographic IFC.

Both, conventional and tomographic IFC is realized by advanced 3D hydrodynamic focusing which automatically aligns all particles as a sheet at a controllable z-position. Tomographic imaging Flow Cytometry extends conventional imaging flow cytometry for the image based measurement of 3D-geometrical features of cells. The required multidirectional views are generated by rotating all cells while passing the imaging window of the developed microfluidic chip. Rotation is implemented by guiding them at a shear flow position of the parabolic velocity profile. All cells pass the detection chamber as a two dimensional sheet under controlled rotation where each cell is imaged multiple times.

Experimental results show a strong focusing quality even under flow velocities below 1 mm/s. For the tomographic IFC, white blood cells with fluorescent stained nuclei are been recorded in parallel for the bright field and the fluorescence channel. Different subtypes of white blood cells can be distinguished by the shape of its nucleus. The experiments show that the multidirectional imaging enhances the identification of these subtypes compare to a single 2D view. Ongoing experiments are focusing on a label free classification of a mixed population of eight allergic pollen types using a convolutional neuronal network (CNN). The whole process requires a high effort in data-processing containing algorithms for object detection, particle tracking and mapping (multi-channel applications) and a CNN-model for the particle classification.

In our work we report on a microfluidic system and method for tomographic imaging flow cytometry, where the angular velocity of a rotating cell is controlled by its z-position in the parabolic velocity profile of a carrier fluid. We also show the need of advanced data-processing tools for image analysis.

We acknowledge the microsystem group and the cleanroom staff at the IPHT for the development and realization of the microfluidic units. The funding from WaterChip (EU Era-NET-DLR 01DQ16009A) is gratefully acknowledged.

IMAGE BASED MODELING OF LEAF DEVELOPMENT

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KEYWORDS: growth, fluorescent microscopy, gene expression pattern, organ shape.

We observe in nature a large variety of biological shapes. Plant leaf in particular is a remarkable example, with contours presenting varying overall silhouettes, with possible serrations at different scales that form teeth and/or lobes. However, during its development, the leaf first appears as a small group of cells with a simple, smooth shape called a primordium. Then it develops to become potentially extremely complex at the mature stage. We aim here at studying the morphogenetic determinants responsible for shape changes during development that lead to the observed diversity.

Modeling is a strategy adapted to the analysis of complex systems like development, and several models were proposed to explain leaf growth. However, none were able to precisely reproduce observed phenotypes. We currently develop a modeling strategy that integrates developmental factors, together with a simulation module that generates simulated growth trajectories, i.e. the shape of the organ during its expansion. To parameterize, test and validate the model, simulations must be compared to real data to quantitatively assess if real shapes may indeed be explained by the model. Because a mature organ is the result of processes that occur and follow each other during growth, analyzing only its final shape is not sufficient, but its evolution should be considered all along the development. Imaging techniques allow to digitize organs like leaves, sizing from a few tens of micrometers to tens of centimeters, and then to quantify shape parameters. It is also possible to compute the mean growth trajectory of the organ by averaging its shape at different times. Practically, a recently developed application, *MorphoLeaf* (Biot et al., 2016), allows the analysis in 2D of leaf shape parameters and the construction of leaf growth trajectories. Then, real and simulated trajectories can be compared to quantitatively evaluate the model of leaf development.

Additionally, gene reporters together with fluorescent microscopy allow the specific labeling and imaging of gene expression patterns over the organs. Resulting images are very informative but we aim to go beyond their illustrative aspect and to quantify the information they carry. Replaced in growth trajectories, gene expression maps imaged in different individuals and at different developmental times can be averaged to provide a statistical representation of the signal distribution over space and time. Moreover, such maps can be analyzed in relation with the shape changes of the growing organ. We will present here a general approach based on modeling and which integrates microscopy images of expression patterns. Then we will illustrate its potential to elucidate the complex developmental processes involved in the growth of *Arabidopsis thaliana* leaves.

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LIESS-FCS: Spatially Resolved Diffusion Modes Measured by STED Nanoscopy

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KEYWORDS: STED-FCS, diffusion modes, scanning FCS, plasma membrane heterogeneity

The cellular plasma membrane of living cells is a heterogeneous structure displaying a nano-scale interaction platform for a myriad of molecules [1]. Measuring diffusion dynamics of lipids and proteins provides versatile insights into the underlying organising principles of membrane heterogeneity and function.

Fluorescence correlation spectroscopy (FCS) is a popular method to assess diffusion dynamics in biomembranes. Varying the observation spot-size allows for the determination of diffusion modes [2], and combining this approach with super-resolution stimulated emission depletion (STED) microscopy enables the direct observation of the dynamics on the relevant length scales [3]. To date, uncovering these nano-scale diffusion modes has been challenging due to inter- and intra-cellular measurement variability, owing to the fact that conventional and super-resolved recordings could not be obtained at the same time at multiple locations. Herein, we present line interleaved excitation scanning STED-FCS (LIESS-FCS) [4], a technique based on fast beam scanning, where the observation spot is rapidly scanned along a line or circle, and confocal and STED measurements are acquired quasi-simultaneously yielding diffusion modes at multiple positions across one cell, effectively enabling diffusion mode imaging. We first validate LIESS-FCS using computer simulations and model membranes, and then apply it to determine the diffusion modes of fluorescently labelled lipids and to shed new light on the organisation of glycosylphosphatidylinositol-anchored proteins.

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High Content Cell Classification and Tracking Improvement by Morphology and Quantitative Phase Feature combination

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KEYWORDS: quantitative phase imaging, machine learning, high content imaging, apoptosis, cell cycle

High content screening consists in acquiring a large number of samples to obtain statistically significant information on cell populations and their changes.
Quantitative phase imaging (QPI) is used in microscopy for label-free imaging of semi-transparent samples [1]. Phase information is relevant as it allows classical morphological parameters determination (i.e. surface, perimeter, circularity…) but also quantitative measurements (i.e. density, mass, mass distribution…) on segmented cells. Machine Learning techniques [2] are powerful tools for mass data classification. However the performance of such algorithms strongly relies on features selection.

The contribution of this paper is twofold. First, we show that adding quantitative phase features to standard morphology features greatly improves the performance of cell classification algorithms. Second, we study the evolution of these features for long time periods at the individual cell level and deduce new cell features.

We use QuadriWave Lateral Shearing Interferometry as the QPI technique, available as a commercial product by PHASICS (SID4Bio, Phasics SA, Palaiseau, France). We built an imaging platform able to keep cells in growing conditions for several days. The principle of the experiment is to acquire a statistically large amount of data by screening different cell populations for more than 48h. After cell segmentation, we use Machine Learning techniques to classify them.

We describe the use of this method as a diagnostic tool to differentiate between different cells populations or cells populations in different experimental conditions. Results on different populations treated or not with Staurosporine (STS), an apoptose inducer, will be shown. We will also apply this strategy to automated determination of cell position within its cycle (from G1 to mitosis).

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SEGMENTATION OF ADJACENT CELLS IN A C. ELEGANS EMBRYO USING A MULTI-COMPARTMENT ACTIVE CONTOUR

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KEYWORDS: Active contour, C. elegans, light sheet fluorescence microscopy, cell membrane segmentation.

Active contours - a.k.a snakes - are popular models for the segmentation of biological structures. They consist in an initial closed curve that evolves towards the boundary of the object of interest. The evolution is driven by the minimization of an application-dependent energy term.

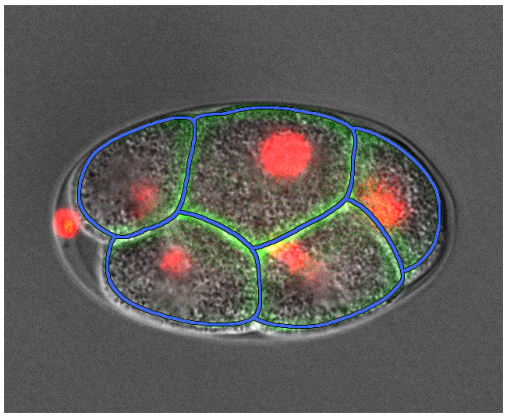

Figure 1: A C. elegans embryo in a light sheet fluorescence microscopy image. Red: nuclei; Green: membranes; Blue: snake.

We present a framework to automatically segment a compact cluster of cells in a C. elegans embryo in 2D light sheet fluorescence microscopy images. For such a purpose, we designed a multi-compartment active contour to fit a structure composed of several adjacent cell membranes. We take advantage of subdivision schemes: for each compartment we recursively apply a refinement process to an initial set of few points to produce a continuous limit curve. The final multi-compartment curve is then deformed in a global manner using a suitable ridge-based energy attracted by the fluorescent membranes. We automatize the initialization of the snake by constructing a Voronoi diagram around the labeled nuclei. This framework has several advantages: 1) the coherent structure of the multi-compartment curve is maintained even on membranes with low or missing fluorescent information. It avoids the leaking problem which afflicts classical image-processing methods; 2) it requires few parameters (i.e., control points), which results in faster optimization and better robustness; 3) the user can intuitively interact with the curve by modifying some control points.
We validated our framework both on synthetic and real fluorescence microscopy images showing that our algorithm is robust to dim staining and to high levels of noise.

We implemented this framework as a user-friendly plugin for the bioimaging software package Icy.

UNRAVELING MOLECULAR ARRANGEMENT OF SYNAPTIC PROTEINS WITH MULTIPLE COLOR SUPER RESOLUTION MICROSCOPY AND STATISTICAL OBJECT DISTANCE ANALYSE WITH ICY SODA.

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KEYWORDS: Statistical Image Analysis, Super-resolution microscopy (SIM, STED, 3D STORM), synapse, SNARE.

During development and plasticity, synaptic molecules are transported to the synapse via vesicular and endosomal carriers in the range of 50-100 nm. Fusion of these carriers with the synaptic zone is achieved by SNARE proteins. We aim to unravel the specificity and contribution of two neuronal v-SNAREs VAMP2 and VAMP7 in the construction and morphogenesis of the synapse at both pre and post-synaptic sites. Elucidating molecular organization during synapse construction requires to precisely localize single or aggregated molecules and to analyze quantitatively their spatial distributions.

We investigated the distribution of synaptic proteins with multi-color Super resolution microscopy (SIM, STED and STORM) on primary hippocampal neurons and brain slices. To unravel fine distance and molecular arrangement we developed a new user friendly plugin called SODA for Statistical Object Distance Analysis. SODA uses micro- and nano-scopy to significantly improve standard colocalisation analysis and is freely available in ICY [1]. Based on Ripley's function our method considers both the geometry of the cell and the densities of molecules to provide colored maps of isolated and statistically coupled molecules.

We used SODA with three-color Structured-Illumination Microscopy (SIM) images of hippocampal neurons, and statistically characterized spatial organization of thousands of synapses. We show that presynaptic synapsin is arranged in asymmetric triangle with the 2 post-synaptic markers homer and PSD95 indicating a deeper localization of homer. As a proof of concept, we then imaged presynaptic glutamatergic terminals with 3D-STORM microscopy and analysed the coupling between more than 180,000 localizations of vesicular Glutamate Transporter (VGLUT) and Synapsin molecules inside synaptic boutons. We evaluated with SODA that each Synapsin or VGLUT localization is at a mean distance of 52 ± 0,04 nm.

These results demonstrate that SODA is a versatile and effective tool to statistically map large data sets of multi-color molecular assemblies with high spatial resolution. Here, we present our latest results on the subcellular localization of VAMP2 AND VAMP7 in the presynaptic terminal and the postsynaptic dendritic spine. Using quantitative analysis of STED and STORM microscopy, we investigate if these v-SNAREs are present in the same synaptic vesicle or if they are present in different synaptic vesicles pools.

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3D Motion Estimation in 3D Light Microscopy Image Sequences: Application to Cell Migration

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Keywords: *Optical flow, Census Transform, Patch Match, Fluorescence Microscopy, Cell Migration*

Three dimensional (3D) motion estimation for light-sheet microscopy is of paramount importance to the quantification of the mechanisms underlying cellular behavior. However, a complete estimation of the 3D motion field is challenged by the heterogeneous scales and nature of intracellular dynamics [2, 3, 4]. As typical examples in cell imaging, blebbing of a cell has smaller motion magnitude while cell migration may show large displacement between frames. To tackle this problem, we present a coarse-to-fine 3D optical flow method extending the two- dimensional PatchMatch [1, 2] paradigm to 3D data. We compare several similarity measures to match voxels between two successive volumes. We exploit multiple spatial scales to explore the possible range of intracellular motions. Our findings show that the metric based on Census transform [6] is more robust to noise present in the images and to intensity variation between time steps. Since this approach estimates only discrete displacements, we additionally consider a 3D variational optical flow computation stage to obtain a refined sub-voxelic flow map. The variational approach still involves a data fidelity term based on the Census transform. The combination of the PatchMatch and the variational stage is able to capture both large and small displacement.

We assessed the performance of our method on data acquired with two different light sheet microscopes [7, 8] and compared it with a couple of other methods. The dataset depicts blebbing and migration of MV3 melanoma cells, and collagen network displacement induced by cell motility. As seen in Fig. 1, our method is able to estimate various range of motion during cell migration and blebbing.

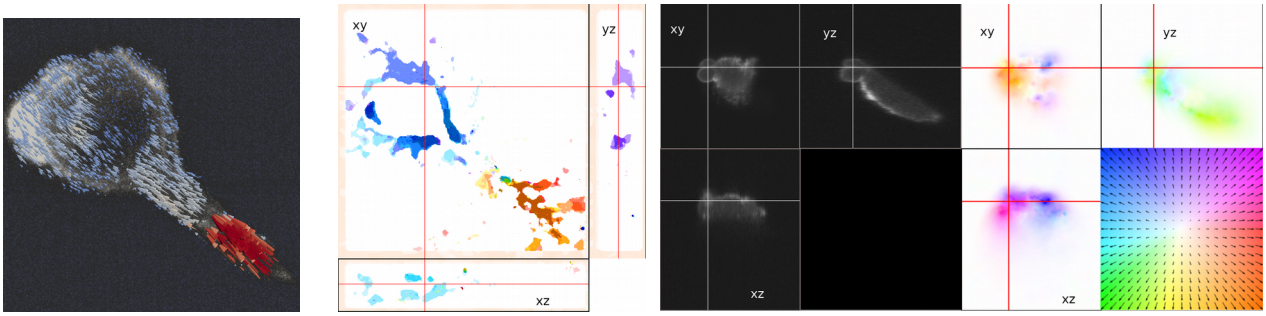

Figure 1: (a) Migration of MV3 melanoma cell in collagen; (a.1.1) Depicting 3D flow field in a slice of cell data with glyphs. Larger motion magnitude is coded in warm colors and smaller motion in cold colors. (a.1.2) Depicting motion map of collagen channel in 3 orthogonal planes. (b) Blebbing of MV3 cell in a cover-slip; (b.1.1) Depicting 3 orthogonal planes of cell data. (b.1.2) Depicting motion map of the cell data in 3 orthogonal planes. The motion map is color coded as shown in lower-right corner of b.1.2.

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Using time-lapse microscopy to unravel the stochastic nature of *Listeria monocytogenes* infection

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KEYWORDS: Time-lapse microscopy, Listeria, Infection, PrfA, macrophages

Listeria monocytogenes is a human pathogen capable of causing serious disease and complications in immunocompromised and pregnant individuals. This pathogen replicates inside infected cells, then hijacks the host cytoskeleton to spread into neighbouring cells. While a lot is known about the interaction between host and *L. monocytogenes* during infection, it is not known how stochastic differences in gene expression in the host cells and the pathogen impact the likelihood of a successful infection at the single cell level.

We have discovered that only a fraction of *L. monocytogenes* that invade macrophages successfully replicate and spread, resulting in a successful island of infection. We predict that stochastic differences in gene and protein expression in host and bacterial cells determine if *L. monocytogenes* is successful.

We have *L. monocytogenes* with fluorescent reporters for virulence gene expression and macrophage cell lines with fluorescently tagged host immune response regulators. We are currently using time lapse confocal microscopy to visualise the impact of the stochastic expression of these factors on infection outcome at the single cell level.

Improving spatiotemporal resolution of 3D localization microscopy

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KEYWORDS: localization microscopy, fluorescence microscopy, high density localization, PSF engineering

Super resolution imaging based on stochastic single molecule localization microscopy has developed into a powerful tool to study biological systems at nanometer scale. However, it still difficult to apply to live cell microscopy, due to the limited spatiotemporal resolution. Temporal resolution is severely limited, as many frames are needed to construct a single super resolved image. Increasing the fluorophore density can improve time resolution, but requires localization methods that allow fitting of overlapping point-spread-functions. Methods such as CSSTORM, FALCON, MFA and others have demonstrated effective simultaneous localization of multiple emitters with overlapping PSFs, but are typically limited to Gaussian PSF approximations that are often inaccurate. We have investigated the use of alternative PSF models applied to 2D and 3D multi-emitter localization, and show how these impact the ability to distinguish multiple overlapping emitters from each other.

POLYGON-BASED COLOCALIZATION ANALYSIS FOR MULTICOLOR SINGLE-MOLECULE LOCALIZATION MICROSCOPY DATA

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KEY WORDS: single-molecule localization microscopy, colocalization, Voronoï tessellation.

Over the last decade, single-molecule localization microscopy (SMLM) has revolutionized cell biology, making it possible to monitor molecular organization and dynamics with spatial resolution of a few nanometers. When used with multiple colors, it enables the investigation of potential interactions between subcellular components at the nanoscale. However, while colocalization analysis has been thoroughly used on pixel-based images, most of these techniques are not adapted to the pointillistic nature of SMLM data. More generally, quantification of SMLM data has proven to be particularly complex due to several experimental parameters influencing localization densities (e.g. fluorophore photophysics, labeling density, acquisition time, localization errors, etc.).

We recently developed a segmentation and quantification method for 2D SMLM data, distributed as an open-source software called SR-Tesseler [1]. In this framework, localization coordinates are directly used to compute a Voronoï tessellation, partitioning the image space in polygons of various sizes centered on each molecule. Here, we propose to extend the intrinsic multiscale capabilities of the Voronoï tessellation with a new metric in order to perform normalized colocalization analysis of multicolor SMLM data. Using simulation and experimental data, we will demonstrate that such a method can achieve robust analysis of multicolor SMLM data with different relative molecular densities.

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TWO DYNAMIC BEHAVIOURS OF THE MICROTUBULES AT THE CELL CORTEX REVEAL THE PULLING AND PUSHING FORCES THAT POSITION THE MITOTIC SPINDLE IN C. ELEGANS EMBRYOS.

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In the *Caenorhabditis elegans* zygote, the mitotic spindle assembles in the cell-centre and is maintained there during metaphase by the so-called centring forces. Then, after the anaphase onset, cortical pulling forces displace the spindle posteriorly. It is achieved by astral microtubules likely pushing against and being pulled from the periphery, respectively. These forces are essential to position the mitotic spindle, and in turn to properly distribute daughter cell fate determinants.

We developed an assay to measure the spatial distribution of the residence times of the microtubules at the cortex. We viewed labelled microtubules by spinning disk fluorescence microscopy along the mitosis, denoised the images and tracked the spots corresponding to the microtubule contacts. We computed the per–embryo histogram of the track durations (residence times) and performed a maximum likelihood fit over all embryos, sharing model parameters. By a Bayesian analysis comparing models, we found that a double exponential offered the best fit, suggesting the presence of two dynamic behaviours. Because the two corresponding characteristic times are close, typically 0.5 s and 1.5 s, and because fitting a mixture of exponentials is a known difficult problem, we validated our image analysis pipeline by processing *in-silico*-fabricated images. We also used different algorithms for denoising and tracking. We challenged the statistical analysis through the generation of *in-silico*-track duration. Overall, it suggests that our approach is robust and precise and enabled us to highlight the minimal number of tracks and embryos to ensure reliable results.

Modelling of anaphase oscillations suggested that single cortical pulling event lasted less than 1 s [1], and dynein was found to reside at the cortex for about 0.6 s [2]. Both durations are consistent with the one of the short-lived population. To support that this population reflects the pulling force events, we observed that it is polarised and that its distribution depends on the proteins involved in generating pulling forces and their regulators. Conversely, we genetically depleted proteins targeting microtubule dynamics and altered mostly the long-lived population, indicating that this latter corresponded to microtubules growing against the cortex. Overall, we suggest that different functions of microtubules at the cell periphery correlate with distinct dynamics, which in turn offers a readout of the distribution of the forces regulating spindle positioning in space and time. It opens a novel avenue to explore the pushing- and pulling-force mechanisms and how they co-exist over the course of the mitosis.

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MEASURING LIGAND 2D/SURFACE DENSITY ON VARIOUS SUBSTRATES USING FLUORESCENCE FLUCTUATION MICROSCOPY

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KEYWORDS: ligands, ligands surface density, surface functionalization, biomaterials, confocal imaging, fluorescence fluctuation microscopy, image correlation spectroscopy, fluorescence correlation spectroscopy, photobleaching.

Micro-fabrication and surface functionalization imply to know the equilibrium surface density of ligands. In particular, these techniques are useful for cellular biology which studies isolated cells in synthetic environments [1]. It was found that the surface density of extra-cellular matrix protein has a huge impact on cells behavior (motility, spreading, and fate). Paradoxically, this crucial parameter is often poorly controlled and even less quantified [2].

We have used a technique belonging to the family of fluorescence fluctuation microscopy, namely Image Correlation Spectroscopy (ICS) [3,4], to measure the absolute surface density of various ligands (fibrinogen, fibronectin, and laminin) adsorbed on glass and PDMS substrates. As these ligands are immobile, the autocorrelation of the confocal image obtained by scanning the sample has the same width as the confocal Point Spread Function. However, the amplitude of the autocorrelation is directly related to the average number of ligands simultaneously illuminated by the laser beam and therefore to their surface density. We have studied the surface density of ligands versus the initial concentration of these molecules in the solution which has been deposited on the surface. The estimation of this relation can be biased for several reasons: the concentration of ligands in solution is difficult to control; the measurement of the surface density of adsorbed molecules can be strongly underestimated if the surface coverage or the molecular brightness is not uniform. We propose to combine ICS with photobleaching to detect these artifacts and estimate the actual surface density, together with control parameters. Globally, fluorescence fluctuation microscopy is a powerful set of techniques that makes it possible to quantify the surface density of ligands in absolute values and at the micrometer scale.

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SEMI-BLIND SPATIALLY-VARIANT DECONVOLUTION AND FOCUS ESTIMATION IN OPTICAL MICROSCOPY BY USE OF CONVOLUTIONAL NEURAL NETWORKS

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Optical microscopy allows researchers and physicians to acquire qualitative and quantitative data about cellular function, organ development, or diseases. However, light traveling through any imaging system undergoes diffraction, which leads to image blur. This represents the determining factor for the resolution of an optical instrument, and thus limits visual access to details. Image formation can be modeled as the convolution of the original object with a point spread function (PSF), which also characterizes the optical aberrations. Knowledge of the PSF corresponding to the blur can be used to restore details in the image through deconvolution. Measurement or estimation of the PSF can be cumbersome and difficult in practice, in particular when the PSF varies in space.

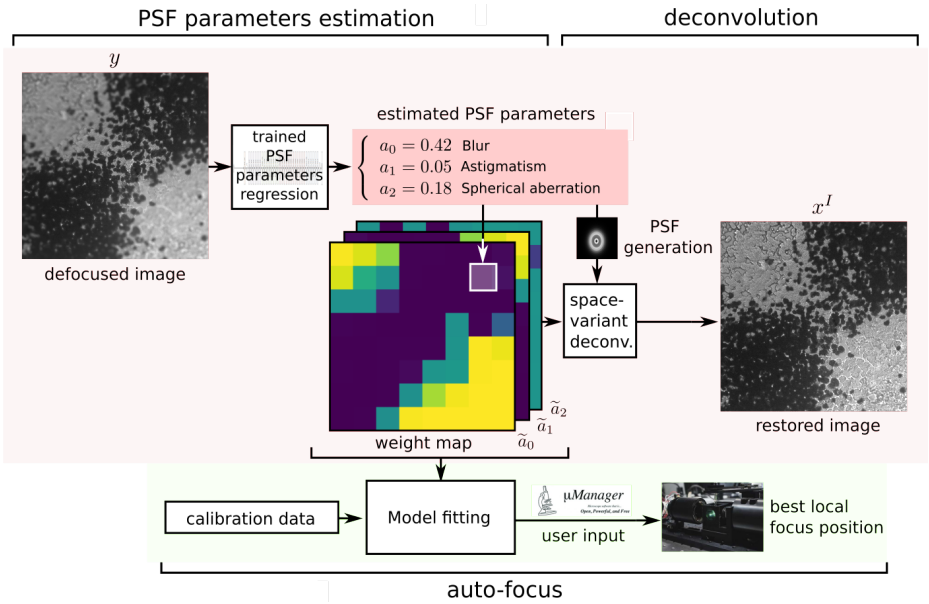

Fig. 1 *Method to estimate a map of local PSFs that degraded an image that can be fed into a spatially variant deconvolution algorithm or an auto-focus algorithm.*

We recently developed a semi-blind, spatially-variant deconvolution technique aimed at optical microscopy that combines a local estimation step of the PSF and a deconvolution step based on a spatially-variant, regularized Richardson-Lucy algorithm (Fig. 1) [1]. To find the local PSF map in a computationally tractable way, we rely on a convolutional neural network (CNN) that performs a regression of a parametric model of the PSF. We train the CNN by synthetically blurring a library of image patches.
This approach has the following features: (1) It does not require the experimental measurement of a PSF, only synthetic training data is necessary; (2) compared to non-parametric blind deconvolution techniques, the problem complexity remains low and therefore is more easily amenable to optimization; (3) parameters with a physical meaning (Zernike polynomials) are inferred from the image itself; (4) the algorithm is computationally efficient, resulting in a near real-time kernel regression and mapping.

Here, we describe our recent efforts to optimize a deconvolution tool and adapt it to allow for PSF model parameter mapping in real-time, implemented as a µManager auto-focus plugin. The latter allows estimating the best local focus depth using only a few acquisition points.

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COMBINING ANNOTATION OF MOVIES AND LEARNING FOR FATE PREDICTION FROM EARLY EMBRYO DEVELOPMENT OBSERVATION

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KEYWORDS: developmental biology, classification, annotation, GUI, quantitative microscopy

Predicting the fate of bovine embryos from early morphokinetics observations obtained in vitro remains an open issue in diffraction-limited microscopy and raises new challenges with the emergence of new learning methodology. The objective of this study is to develop a methodology to read and predict different *in vitro* developmental potential of bovine embryos by combinating morphokinetical parameters.We recently presented a methodology based on a standard annotation and a complex mathematical decision allowing the combination of parameters observable at the very first embryonic cycles to predict 6 major morphokinetical profiles of development of bovine embryos produced in vitro (IVP) (Reis et al. 2018). The aim of the present work was to develop a ready to use software (EasyPickAndPredict) to predict the in vitro developmental potential of the IVP embryos based on the this learning process and allowing the extension of this methodology to other embryologists.

Material and methods : the production of a new software of high portability containing two main functions : «manual annotation» and «automatic prediction», embedded with the classifier presented by Reis et al. (2018) and a guidelines for standard annotation was developed. JAVA language was employed to improve the users experience. The embedded classifier was built under R language. It is based on the randomforest and the VSURF R packages. It handles quantitative as well as qualitative observations. Missing data are imputed. The different variables corresponding to the observations are tagged with a number fields allowing the recognition of the type of action necessary for the annotation of the variable and its positionning in the submenus of the graphical interface.

Results : The predictive software is easy to handle, and fast to load and has high portability. The function «manual annotation» is based on click actions to annotate the discriminant parameters within the 4 first embryonic cycles. The function «prediction» calls the embedded classifier. An additional function called «report» creates customised reports including the embryo classification, the summary of the measures and the accuracy of the prediction (vote system). Time lapse pictures were taken every 15 minutes throughout the culture period of 172 embryos (672 pictures/embryo ; Primovision™) used for training. The software can handle a bench of several embryos observed through a single movie. In this case several movies are automatically created from this movie and the user can navigate through the collection of movies for the annotation.

Conclusion : The predictive software is easy to manipulate and the graphical interface is automatically built from the standardised discriminant parameters for prediction. The two main functions: «manual annotation» and «automatic prediction» and two complementary fonctions: «help» and «report» supply embryologists with a standardised approach to predict and analyse morphokinetical profiles of the embryos produced in their laboratories. This approach is necessary to improve the capacity of comparison of morphokinetical works in different laboratories and enhance knowledge about the IVP bovine embryo. The software architecture is developed in such a way it could be easily extended for the the annotation, learning and prediction in others contexts than embryology.

Reis, A.P. et al, AETE scientific meeting, Nantes, 2018.

MEASURING LIGAND SURFACE DENSITY ON VARIOUS SUBSTRATES USING FLUORESCENCE FLUCTUATION MICROSCOPY

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KEYWORDS: ligands, ligands surface density, surface functionalization, biomaterials, confocal imaging, fluorescence fluctuation microscopy, image correlation spectroscopy, fluorescence correlation spectroscopy, photobleaching.

Micro-fabrication and surface functionalization imply to know the equilibrium surface density of ligands. In particular, these techniques are useful for cellular biology that studies isolated cells in synthetic environments [1]. It was found that the surface density of extra-cellular matrix protein has a huge impact on cells behavior (motility, spreading, and fate). Paradoxically, this crucial parameter is often poorly controlled and even less quantified [2]. We have used a technique belonging to the family of fluorescence fluctuation microscopy, namely Image Correlation Spectroscopy (ICS) [3,4,5], to measure the absolute surface density of various ligands (e.g. fibrinogen, fibronectin, and laminin) adsorbed on glass, gels and PDMS substrates. As these ligands are immobile, the autocorrelation of the confocal image obtained by scanning the sample has the same width as the confocal Point Spread Function. However, the amplitude of the autocorrelation is directly related to the average number of ligands simultaneously illuminated by the laser beam, which therefore gives their surface density. We have studied the surface density of ligands versus the initial concentration of these molecules in the solution which has been deposited on the surface. The estimation of this relation can be biased for several reasons: the concentration of ligands in solution is difficult to control; the measurement of the surface density of adsorbed molecules can be strongly underestimated if the surface coverage or the molecular brightness is not uniform. We propose to combine ICS with photobleaching to detect these artifacts and estimate the actual surface density, together with control parameters. Globally, fluorescence fluctuation microscopy is a powerful set of techniques that makes it possible to quantify the surface density of ligands in absolute values and at the micrometer scale.

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Modelling the spatial distribution of the type 6 secretion system in Pseudonomas aeruginosa

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Keywords: Morphology, wavelets, watershed, spatial point patterns

Work to date has examined the structural and genomic make up of the type 6 secretion system (T6SS) [1] within Pseudomonas aeruginosa (P. aeruginosa) but a quantitative description is yet to be explored. By analysing time-lapse, multimodal microscopy videos, our work aims to develop an understanding of the spatial behaviour exhibited by the T6SS on P. aeruginosa.

From the time-lapse microscopy videos we extract shape descriptors and fluorophore locations using the à trous wavelet transform [2] whilst correcting for non-linear drift using a method we developed based on morphological skeletonisation [3]. To register all P. aeruginosa onto a unified coordinate system we model their shape as an ellipse, where the major and minor axis lengths are parameters with added truncated Gaussian noise. Maximum likelihood estimators are available analytically for the model when the variance can be considered small defining our unified coordinate system to which all P. aeruginosa and T6SS can be mapped too.

Finally, we will discuss some preliminary statistical analysis on the data extracted. In particular, we examine if there exists a relationship between size of the bacteria and the occurrence of the T6SS and whether the secretion system exhibits spatial preference on the cell membrane of P. aeruginosa.

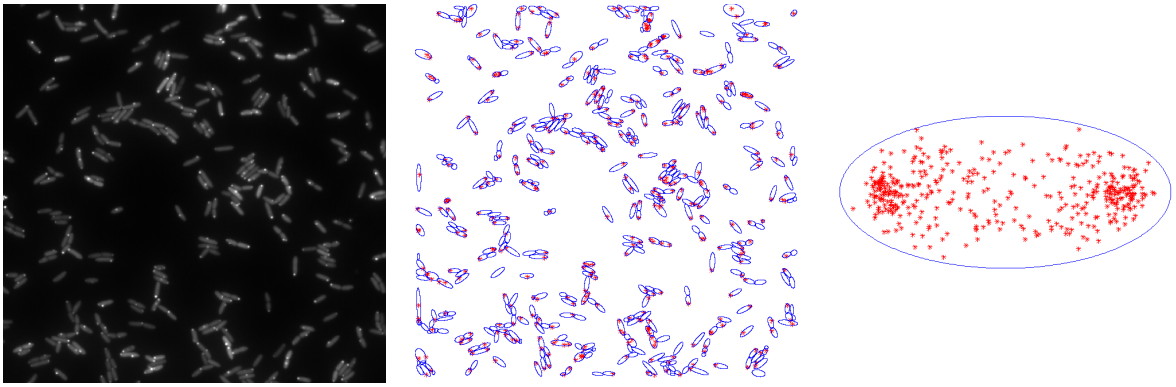

Figure 1: Example of our image processing pipeline. *Left:* Raw YFP image, *middle:* extracted bacteria (indicated as ellipses) and fluorophores (indicated as asterisks), *right:* fluorophores registered onto the unified coordinate system.

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Spatiotemporal quantification of 3D cellular parameters during leaf morphogenesis

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Keywords: Morphogenesis, 3D Cell Morphology, 3D registration, Image Analysis Tools

In plants, cell division and growth are the major cellular mechanisms that drive morphogenesis. Leaves, which are the main source of biomass in plants, are initiated from a few cells that subsequently undergo several rounds of cell division and expansion to reach the final organ shape and size with adaxial (upper side) and abaxial (lower side) cells separated by the leaf margin. Leaf morphogenesis is characterized by global changes (such as the onset of blade curvature) and local changes (such as the formation of teeth at the margin). How cellular mechanisms are integrated at the organ scale to regulate these shape changes remains largely unknown. A fundamental bottleneck is the current lack of image analysis techniques to systematically quantify the spatiotemporal evolution of 3D cell parameters during leaf development. Another challenge is the inter-individual variability that hinders the interpretation and analysis of cellular patterns.

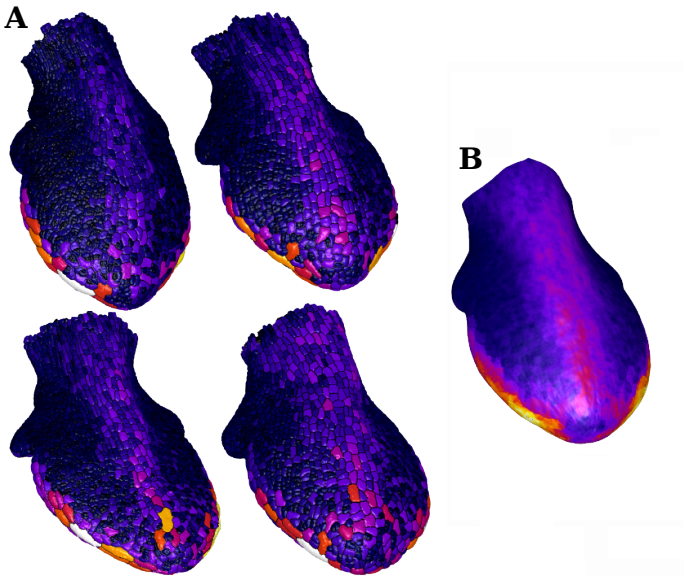

Figure 1. Surface rendering of parametric maps: cell volume. **A**: Color-coded rendering of cell measurements in individual leaves. **B**: Average leaf shape with average values.

To address these issues, we develop a comprehensive framework for automatic analysis and statistical quantification of epidermal tissue organization and changes in 3D [1]. We first introduced new descriptors and methods to quantify epidermal cell thickness and orientation. Based on these new features, we then proposed an automatic method to separate cells from the adaxial and the abaxial sides and obtain an horizontal leaf axis. Comparing with manual leaf side annotations of four biologist experts validated this method. We also describe how to compute a second, orthogonal axis, thus establishing a leaf-centered coordinate frame. Based on the resulting parameterization of the leaf surface, we finally show how parametric maps from different leaves at a given developmental stage can be registered and averaged [2] to generate a prototypical shape with average parameter distribution (Fig.1). Using landmarks at the leaf margin, the registration accuracy was estimated to be about 10 µm, corresponding to ~2.5% of leaf length.

We applied our pipeline to watershed-segmented 3D confocal images of fixed leaves at various developmental stages. We revealed a differential evolution of cell growth between the two sides of the leaf, which could contribute to the evolution of the global leaf shape and the apparition of the blade curvature. In addition, the average parametric maps revealed the existence of distinct cell populations that could be related to local shape changes. This framework will allow to compare maps between different developmental stages and to reconstruct in 3D the dynamics of average developmental trajectories, thus providing an integrative view of the evolution of the cell morphology over time and space in relation with leaf shape changes.

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4Pi single molecule localization with an experimental PSF model that achieves theoretical minimal uncertainty

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KEYWORDS: iPALM, 4Pi-SMS, experimental PSF, Cramér-Rao lower bound

Interferometric photoactivated localization microscopy (iPALM, 4Pi-SMS) uses multiphase interferometry to localize single molecules and currently achieves the highest axial resolution of all 3D superresolution approaches[1][2]. In theory, 3D sub-10 nm resolution can be achieved with only 250 photons collected in each objective for an individual molecule[3]. However, the resolution achievable with the current image analysis workflow is substantially worse than the theoretical limit. Here, we developed an experimental PSF fitting method for the interference 4Pi-PSF. As the interference phase is not fixed with respect to the shape of the PSF, we developed a new 4Pi-PSF model, which decouples the phase term from the shape of the PSF. Using a spline-interpolated experimental PSF model[4] and by fitting all 3 or 4 phase images globally, we showed on simulated data that we can achieve the theoretical limit of 3D resolution, the , Cramér-Rao lower bound (CRLB), also for 4Pi microscope.

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MICRO-FLUCTUATIONS OF THE LENGTH OF THE MITOTIC SPINDLE REVEAL ITS MECHANICS AND ITS DYNAMICS DURING CELL DIVISION

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KEYWORDS: time-frequency analysis, short-time Fourier transform, cell mechanics, cell division.

The mitotic spindle correctly segregates the sister chromatids to maintain ploidy in daughter cells and ensures faithful divisions. The spindle comprises microtubules constantly alternating polymerisation and depolymerisation (dynamic instability), various molecular motors generating forces, crosslinkers in particular coupling microtubules emanating from opposite poles and regulators. Although its components and structure are well known, the link to its functions remains elusive. We propose an image processing based, non-invasive method combined with a data model to reveal the mechanics of the mitotic spindle along time.

We fluorescently labelled the spindle poles and tracked them at high temporal (30 Hz) and spatial resolutions (20 nm) to measure the fluctuations of its length, *in vivo*. We computed their power density spectrum using short-time Fourier transform—a blueprint of spindle mechanics. We then fitted this spectrum with a Kelvin-Voigt with inertia model (composed of a spring, a damper and an inertial element in parallel). We validated this method by recovering the model parameters from simulated lengths.

Using this method, we characterised the mechanics of the mitotic spindle of the one-cell embryo of the nematode *C. elegans*. The metaphase appeared dominated by damping and inertial elements, consistent with the slow elongation observed, but in contrast with the common thought that a mechanism maintains the spindle length. At anaphase onset, all three parameters collapsed, before increasing about 130 s later to reach a regime where damping and inertia dominated again, suggesting the overlapping spindle microtubules may play a minor role in early anaphase spindle elongation.

This method paves the way not only towards understanding the fundamentals of spindle mechanics but also towards accounting for spindle functional resistance to defects such as poly- or aneuploidy.

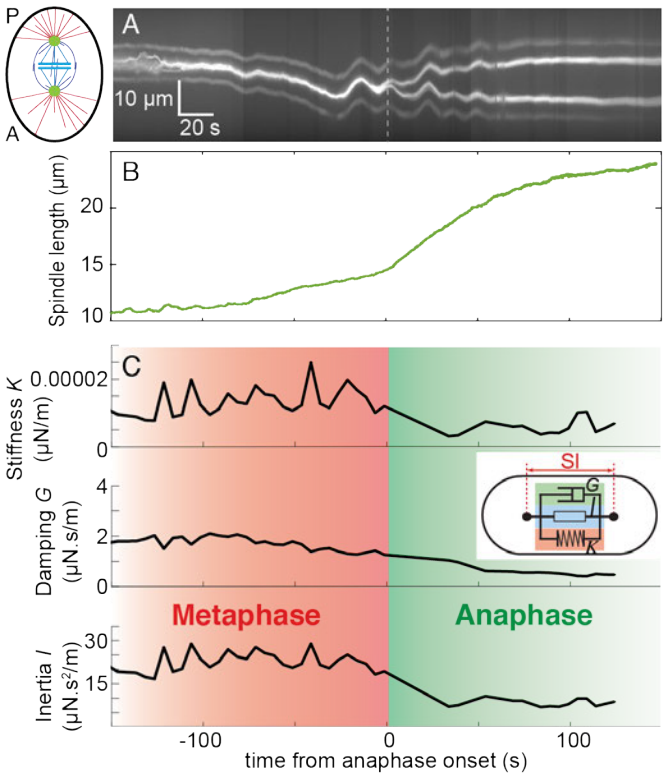

Figure 1: (**A**) (left)Schematic of a *C. elegans* one-cell embryo, with posterior side oriented towards the top, and (right) kymograph of the spindle poles (outer tracks) and the chromosomes (inner tracks) in a γ-tubulin::GFP; H2B::GFP labelled strain (right). (**B**) Length of the spindle over time, and (**C**) Temporal evolution of its mechanical characteristics as measured by the proposed assay. Inset, schematic of the Kelvin-Voigt with inertia model within an embryo.

Local Maximum Likelihood estimation for time-varying Single Particle Tracking models

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The tracking of small particles is a very important area of research. Usually, the models describing how small molecules, proteins, and other organic material move are considered to be time-invariant, that is, the particles undergo only one mode of motion. Although, this assumption is valid in most of the cases, there are other cases where this is not the most appropriate to describe particle motion. It is well-known, for example, that particles can undergo different modes of motion depending on the local environment and on the biochemistry of the process they are involved. Thus, to reflect this variability, time-varying parameters may be considered.

Maximum likelihood (ML) estimation has been utilized in previous works in single particle tracking (SPT) to estimate key parameters arising in motion models. These works, although successful, pose the limitation that the parameters are considered to be time-invariant.

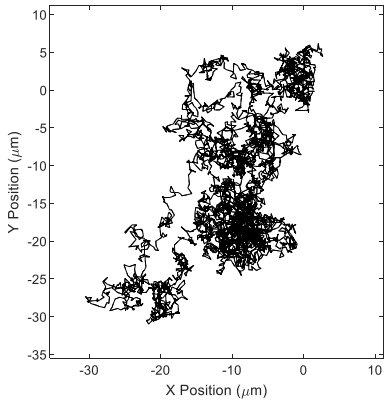

Figure 1. Example Brownian motion (BM) trajectory

Here, we develop a local time-varying ML estimation algorithm to track time-varying parameters arising in the single particle tracking models. In particular, these parameters refer to the noise variances of a random walk model with noisy observations.

One way to optimize this time-varying likelihood function is by using a local (or windowed) likelihood centered at time t. This is carried out by introducing a weight $K((u(i)-t)/h)$, where $u(i)$ is the data inside the window, h is the window size, and t is a chosen point inside the window (usually the centre). The idea is then to use this window (of nominated span h and time point t) to estimate the parameters that maximize the log-likelihood function within the window. The estimation algorithm continues when the time points are increased by one unit and terminates when the last data point is included in the window.

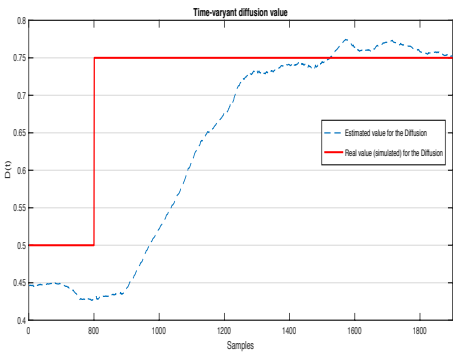

Figure 2. Example of a time-varying diffusion value for a simulated random walk with noisy observations.

Results of the estimation of a random walk considering time-varying diffusion are shown in Fig. 2. We can observe that our estimation algorithm is capable of producing a time-varying parameter for the diffusion, which is in accordance with the real values of the generated data.

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Use of X-ray micro computed tomography imaging to analyze the morphology of wheat grain through its development

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KEYWORDS: X-ray micro computed tomography, image analysis, wheat grain, plant development

Wheat is one of the most important staple source in the world for human consumption, animal feed and industrial raw materials. To deal with the global and increasing population demand, enhancing crop yield by increasing the final weight of individual grain is considered as a feasible solution. Morphometric analysis of wheat grain plays an important role in tracking and managing developmental processes by assessing potential impacts on grains properties, size and shape that are major determinants of final grain weight. X-ray micro computed tomography (μCT) is a very powerful non-destructive imaging tool that is able to acquire 3D model of an individual grain, and it has increasingly enabled the morphology of wheat grain to be analyzed [1, 2]. However, these methods focused on extracting morphometric data of mature grains. Therefore, it is essential to develop a robust method in order to quantify changes of morphology during growth stages of wheat grain from μCT images.

We developed an image-processing pipeline, including global thresholding, region selection and morphological filtering, to identify the wheat grain within μCT images (see Figure 1). An active contour model was employed to partition between wheat outer and inner tissues. We have extracted several basic morphometric measures of individual wheat grains such as grain's dimension (length, width and thickness), grain's volume In addition, we analyzed and quantified new morphometric features such as volume of void within wheat grain and distribution of the crease depth. The evolution of voids within wheat grain reflects lysis of outer tissues and growth of inner tissues. A deep crease is a feature of the wheat grain that is not found in other cereal grains such as barley and rye, and has impact in disease management and in the milling industry. Our quantitative study of the crease shape and its evolution during grain development helps us understand the genesis of the wheat shape.

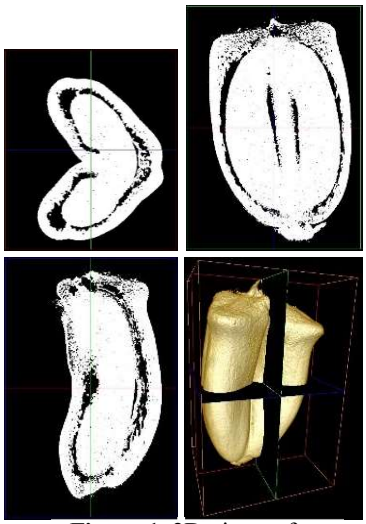

Figure 1. 2D views of a 120-ºDAA sample.

This work shows that μCT acquisitions and image processing methodologies are powerful tools to extract morphometric parameters of developing wheat grain. The results of quantitative analysis revealed remarkable features of wheat grain growth. This quantitative description of morphology will help build a model of wheat grain growth.

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3D ConvNets improve macromolecule localization in 3D cellular cryo-electron tomograms

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KEYWORDS: cryo-electron tomography, deep learning, 3D analysis

Cryo-electron tomography (cryo-ET) allows one to capture 3D images of cells in a close to native state, at sub-nanometer resolution. However, noise and artifact levels are such that heavy computational processing is needed to access the image content [1]. We propose a deep learning framework to accurately and jointly localize multiple types and states of macromolecules in cellular cryo-electron tomograms. We compare this framework to the commonly-used template matching method on both synthetic and experimental data. On synthetic image data, we show that our framework is very fast and produces superior detection results. On experimental data, the detection results obtained by our method correspond to an overlap rate of 86% with the expert annotations. In addition, we show that our method can be combined to template matching procedures to reliably increase the number of expected detections. In our experiments, this strategy was able to find additional 24.3% membrane-bound ribosomes that were missed or discarded during manual annotation.

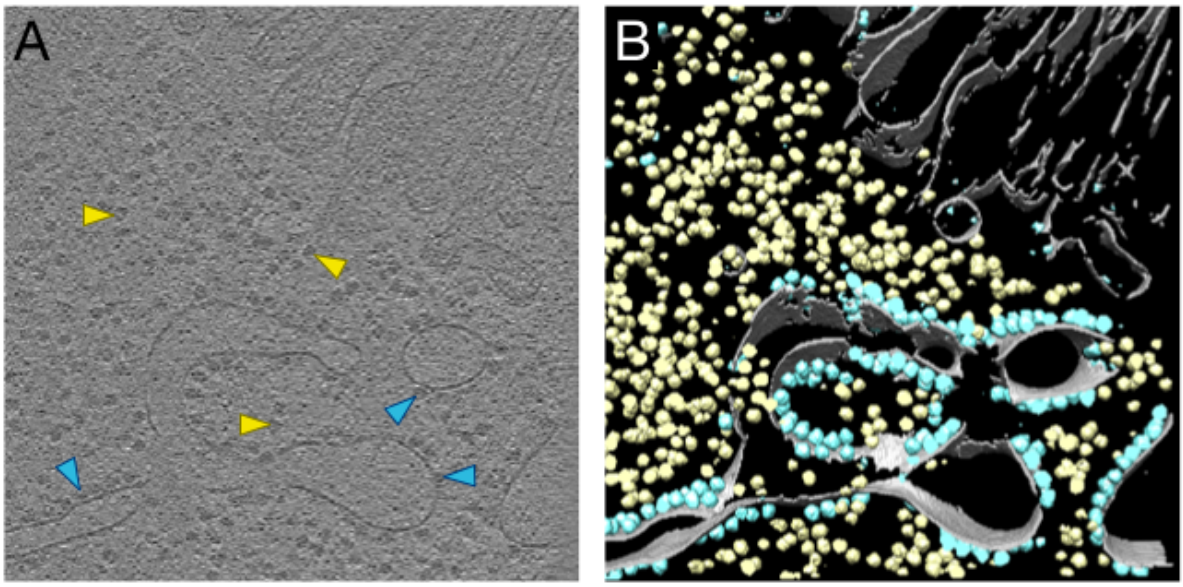

Figure 1. Chlamydomonas cell. (A) Tomogram slice; blue arrows indicate membrane-bound ribosomes; yellow arrows indicate cytoplasmic ribosomes. (B) Corresponding voxelwise classification obtained by our 3D CNN, performed for 3 classes: mb-ribos (blue), ct-ribos (yellow) and membrane (gray).

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PROTEIN COUNTING IN LOCALISATION MICROSCOPY

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KEYWORDS: 3D chromatin modeling, 3D Single Molecule Localization Microscopy, Applications in cell biology, Protein Counting, Simulation, Superresolution microscopy

One of the major advantages of fluorescent microscopy is its specificity, which gives the opportunity to detect only the labeled parts (typically specific proteins) of the sample with very high contrast. Localisation techniques provide a tool to determine the coordinates of these markers with a precision of few tens of nanometers. However, due to the labeling stoichiometry, the data acquisition and the photodynamics of the applied fluorescent molecules, a single labeled protein results in a cluster of localized positions. If the lateral distance between the proteins is smaller than the size of this cluster then the visual separation becames impossible, but with visualization, we lose information, which could be extracted from the raw data.

I determined the bleaching rate of the used fluorophores from the measured data and reduced the number of extra localisations caused by acquisition artifact with trajectory fitting. I deduced the possible stochiometric options with the help of biological considerations and information from the dye manufacturer. By modeling the photodynamics [1] of the labeling molecules, the probability density function of the number of blinks and the expected value of localisations belonging to a single protein could be determined. In this way the clusters could be color coded to signal their protein numbers as can be seen in Figure 1.

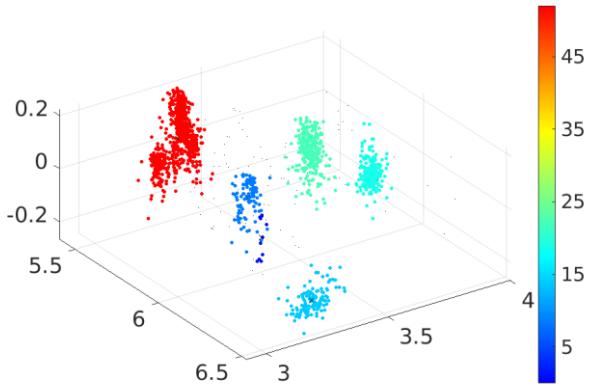

Figure 1. 3D localisations of labeled proteins (γH2AX). Axes signal position in μm and the color signals protein number in the cluster.

Furthermore, I compared the evaluations of 2D and 3D measurements and simulations and investigated the limits of them. I used rainSTORM [2] for localisation, trajectory fitting and visualization, and TestSTORM [3] for simulations.

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Quantitative phase imaging of adherent mammalian cells: a comparison of three different techniques

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KEYWORDS: Microscopy, Quantitative phase imaging, Cell culture quantitative analysis.

The quantitative phase imaging methods have several advantages when it comes to monitoring cultures of mammalian cells. Because of their low photo-toxicity, they can follow cells in a minimally invasive way, over a long period of time. In addition, the ability to measure optical path differences allows the measurement of the cell dry mass, an important metric for studying e.g. cell cycle kinetics. Here we present an analysis of the comparison between three different quantitative phase imaging techniques: digital holographic microscopy (DHM), lens-free microscopy (LFM) and quadriwave lateral sheering interferometry (LSI). The experimental design consists in the comparisons of optical path difference measurements performed over several tens of adherent cells. To ensure consistency, we performed the measurements on a fixed cell culture the same day, on the same location with the different instruments. The statistical analysis of these measurements allowed us to estimate the precision of the cell measurements without any reference material. We found a good agreement between the different quantitative phase imaging methods when measuring cell optical path differences. We also highlight the necessity to finely tune the post-processing algorithms (baseline subtraction, cell segmentation) in order to yield the best achievable precisions.

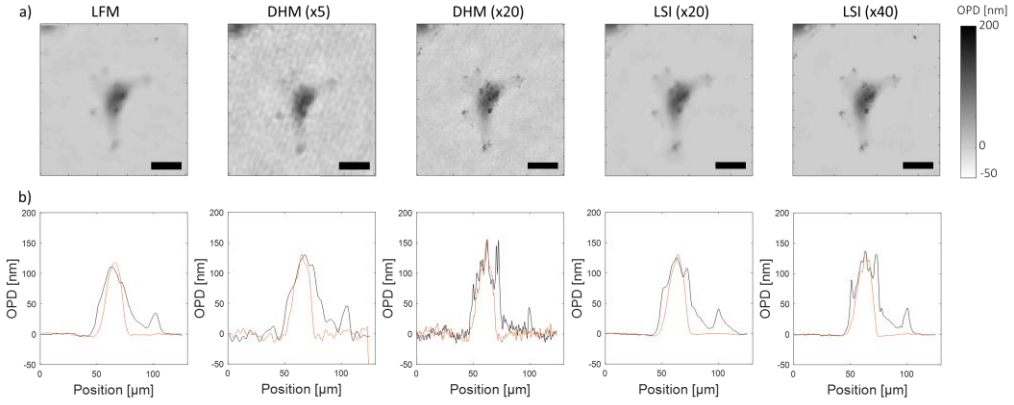

Figure 1: Optical path difference (OPD) maps of fixed COS-7 cells obtained with the different QPI techniques. Scale bar is 25 µm. (b) OPD profiles measured through the cell center (black: vertical, orange: horizontal).

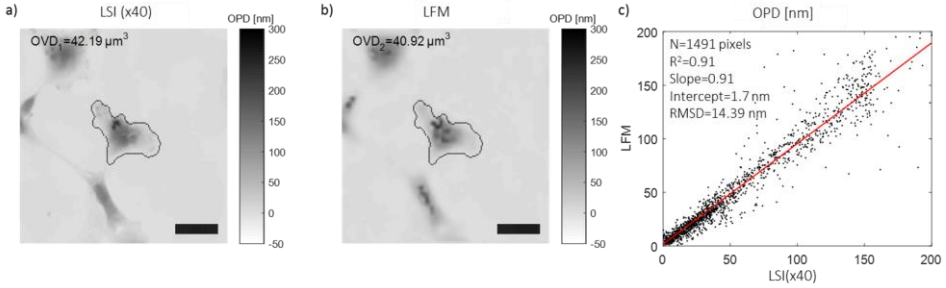

Figure 2: Comparison between LSI(x40) and LFM. Optical path difference (OPD) maps of a fixed COS-7 cell obtained with (a) LSI(x40) and (b) LFM. Scale bar is 25 µm. The contour of the ground truth cell segmentation area is shown in black. (b) OPD map obtained with LFM. (c) Pixel to pixel comparison between the OPD maps shown in (a) and (b). Only pixels of the segmented cell area are considered. The results of the linear regression fitting are indicated (slope intercept, R^2, RMSD).

MAPPING AND COMPARING SPATIAL DISTRIBUTIONS IN BIOLOGICAL IMAGING

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KEYWORDS: point process, density map, nearest neighbor distance, intensity estimator

Because organization in space and function are closely related in biology, analyzing the spatial distribution of biological objects is key in the study of many biological systems. Imaging techniques combined with fluorescent labeling are able to reveal the spatial organization of structures in 3D. For instance, using confocal microscopy, it is possible to image the 3D distribution of a specific protein labeled by a fluorescent marker within the cells of a tissue. Furthermore, because experiments are most often repeated, they yield ensembles of images so that appropriate statistical methods are required for their integrated analysis, including the comparison of groups and the localization of potential differences.

We address here the organization of structures that can be assimilated to sets of points, such as neurons in the brain or endomembrane compartments within a cell. Image analysis allows the extraction of the 3D positions of the structures of interest from the images [1], so that a data sample becomes a set of point patterns. Because of their punctual nature, addressing the spatial analysis of such objects is not straightforward. It is indeed difficult to discern and compare organization rules from discrete (and repeated) data, especially in 3D. To address these problems, readable, meaningful representations are required. For this, in most proposed strategies, repeated punctual data are pooled, so that the problem comes down to the simpler one of analyzing single patterns. However, the specificity of individual patterns is lost and this prevents, e.g., for statistical comparisons. We will present an alternative, more adequate strategy to deal with and compare the spatial distribution of punctual structures from pattern samples. First, statistical density maps are built to provide a synthetic representation of the distribution of the points. For this we introduced a statistical estimator of local point density [2]. Next, a method for the statistical comparison of two density maps will be presented, based on the comparison of local point density [3]. Biological data from different systems at different scales will be used to illustrate the strategy.

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Automatic detection of tumoral tissue in hepatocellular carcinoma digital slides using deep convolutional networks

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KEYWORDS: Deep learning, Hepatocellular Carcinoma (HCC), digital slides, H&E.

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Background and Objectives.
Histological diagnosis of hepatocellular carcinoma, the most frequent primary liver cancer, can be challenging as well-differentiated tumors may highly resemble non-tumoral liver tissue. The aim of our study was thus to build a deep learning classifier able to automatically detect tumor areas on digital slides of hepatocellular carcinoma (HCC).

Methods.
We used 728 digital slides from H&E stained tissues slices of HCC cases acquired at 40x (resolution 0.228 µm/pixel) with a Hamamatsu Nanozoomer slide scanner. All the digital slides where manually annotated by a pathologist specialized in liver disease with Hamamatsu software NDPI Viewer. Two classes of annotations where drawn: tumoral tissue (positive class) and non tumoral liver tissue (negative class). The data set was randomly split in a training set containing 583 slides (80% of the data set) and a test set containing the remaining 145 slides. Digital slides and annotations were then imported in Visiopharm VIS software. Using R scripts, 512x512 labeled tiles (at 5x magnification) were exported from Visiopharm VIS. They were thereafter split as 64x64 sub-tiles to match the input requirements of the Convolutional Neural Network (CNN) used for classification. For this purpose, we used Keras for R, with Tensorflow as backend, installed on a Windows7 computer equipped with a NVIDIA GTX1080 (8Gb) GPU. Test slides were exported as 512x512 tiles which were thereafter split as 64x64 sub-tiles as the training set. These sub-tiles were then classified with the model and the predicted labels imported in Visiopharm VIS. The tumor detection performance was evaluated in Visiopharm VIS by comparing the area of tissue predicted as tumoral by the model to the ground truth annotation area.

Results and conclusion.
The bests results were obtained with a VGG16 CNN pre-trained on the ImageNet data set. With this network, the performance achieved was 89.6% for the overall cross-validation accuracy after 40 training epochs.
As an example of result, the tumor detection performance computed on the test slide shown on figure 1 was: precision = 0.90, recall = 0.93, F1-Score = 0.92. However, those values can vary from one slide to another depending on the complexity of the case.

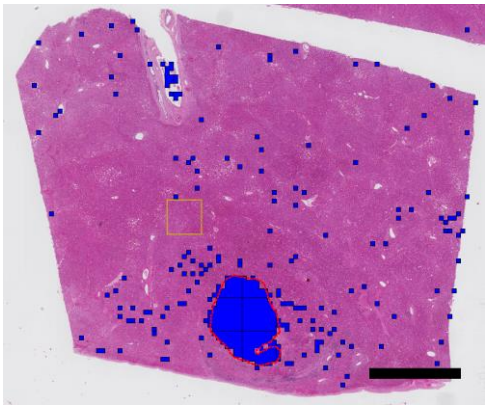

Figure 1. Example of tumoral tissue detection on a test slide (scale bar = 2.5 mm)

After those preliminary results we are currently investigating the impact of image resolution and context area on the classification performance, using different training sets with 64x64, 128x128, 256x256 tiles exported at 10x and 20x. We expect this method to be used in the future as an automatized pre-screening tool applied before careful examination by a pathologist.

Artefact free high density localisation microscopy analysis.

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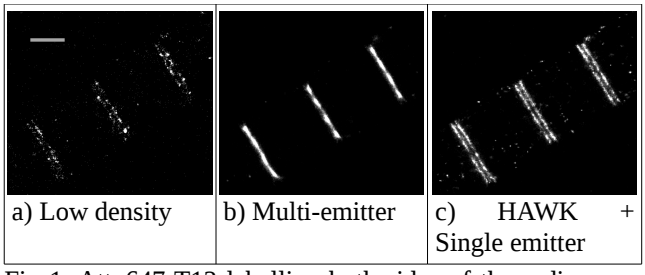
Conventional localisation microscopy (PALM & STORM) relies on sparse activation of flurophores so that individual emitter point spread functions (PSFs) can be accurately fitted, necessitating acquisition times far too long for most live cell experiments. To Solve this problem, a number of algorithms have been developed to cope with high emitter density and overlapping PSFs. Although an improvement, these still produce significant image artefacts as the density is increased. Worse still, these 'sharpening' artefacts are easily mistaken for high resolution when using metrics such as FRC.

Here we demonstrate that these artefacts can be avoided by pre-processing (filtering) the image sequence with Haar wavelet kernels (HAWK). Our technique shows substantially improved resolving power over other high density methods, especially when the emitter density is very high. A significant additional advantage is that when HAWK fails, it produces a blurred lower resolution image of the true structure rather than an artificially sharpened one, avoiding the false indication of high resolution.

The performance of HAWK is tested on simulated, experimental and live cell data and compared with other high density methods. Comparison of simulations with ground truth show not only the superior resolving power of HAWK at very high density but the absence of the substantial sharpening and artificial clustering artefacts produced by other methods. Results on experimental data are validated by use of samples where the underlying structure is well know.

For live cell experiments the true structure is not know in advance. To validate our results we performed the novel technique of simultaneous two colour imaging of the fluorescent proteins in the photo-converted and unconverted states. This gives both high and low density data on the same structures. The results of the high density methods can therefore be cross validated with the artefact free low density image.

In all the above cases HAWK produces best correlation with the real structure despite often appearing to have lower resolution. The other high density methods often totally fail to resolve features in the 100-200nm range yet produce very sharp images. The method can achieve 60-70nm of resolution in a few seconds of imaging in live cells and significantly better than this in prototypical biological samples under conditions where other methods become dominated by image artefacts.



a) Low density	b) Multi-emitter	c) HAWK + Single emitter

Fig 1: Atto647-T12 labelling both sides of the z disc on muscle sarcomers. Low density imaging with single emitter fitting (a) reveals two lines separated by 160nm as expected from electron microscopy [1,2] . When imaging at very high emitter density other methods such as multi-emitter fitting (b) cannot resolve the spacing producing a greatly sharpened image. HAWK processing followed by single emitter fitting produces a clear image with the correct spacing. Scale bar 1μm.

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MULTI-COLOR CRYO-FLUORESCENCE MICROSCOPY

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KEYWORDS: fluorescence microscopy, super-resolution, single molecule, nanoscopy, multi-color, mirror-based, PSF-engineering, achromatic fluorescence

Wide-field microscopy can reach 10–50nm localization precision at room temperature by estimating of the center location of diffraction-limited spots. At cryogenic temperatures, localization precisions well below 1nm can be reached thanks to increased photostability of the fluorophores [1]. This improvement in localization precision effectively eliminates optics as the bottleneck of resolving power for static phenomena with sparse emitters – for a single color channel. As refractive optics are wavelength-dependent, chromatic aberrations are likely to pose the next big challenge to multi-color imaging at the nanometer scale.

Here, we present a mirror-based cryo-fluorescence microscope that promises multi-color wide-field imaging of sparse emitters with nanometer localization precision. By making use of a reflective, mirror-based objective and parabolic mirrors, dispersion by refractive elements of the microscope is eliminated, removing the main source of chromatic aberrations. In addition to the resolution improvement, fixation of the dipole moment of the fluorophore allows novel avenues of counting individual fluorophores, as well as determining distance, orientation, and stoichiometry. The ability to interrogate molecules with nanometer precision opens up a new range of macro-molecules (such as DNA, RNA and protein complexes) for optical investigation and has the potential to solve countless outstanding questions in cell and molecular biology.

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Non-equilibrium forces govern anomalous dynamics of subcellular domains in mammalian cells

Key words: Single particle tracking, subcellular dynamics, non-equilibrium dynamics, anomalous diffusion

Diffusion is the basic mode of motion for subcellular domains, often with a subdiffusive scaling of the anomaly exponent $\alpha < 1$. Our recent work reveals that subdiffusive motion in cells counterintuitively is governed by non-equilibrium forces and significantly depends on a functional microtubule cytoskeleton.
In a first approach we have investigated the random walk properties of telomeres, structure conserving protein motifs at the terminal end of chromosomes in eukaryotic cells. Single particle tracking analysis revealed anomaly exponents scaling ~ 0.5 and a significant reduction after drug induced disruption of microtubules. This testifies a reduced effective temperature in the stochastic forces governing the motion of telomeres [1].
In a sequel we have adressed the challenge to characterize the dynamic behaviour of exit sites (ERES) on endoplasmic reticulum (ER) membranes. The ER is a highly interconnected network, ubiquitous in mammalian cells and the central site of lipid synthesis and protein translation. ERES are mobile hotspots in the ER membrane, at which cargo filled vesicles bud off. Our data reveal anomalous dynamics of ER-junctions with a significant dependence on intact microtubules [2]. The motion of ERES is rather described as a subdiffusive subordinated random walk on ER-tubules between ER-junctions than by being actively transported along microtubules [3].

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[3] L. Stadler, K. Speckner, and M. Weiss, Biophys J, submitted (2018)

Overcoming Blinking Artifacts in Nanocluster Detection with Two-Color STORM

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KEYWORDS: super-resolution microscopy, two-color STORM, over-counting, protein nanoclustering

Observations using single molecule localization microscopy have led to the belief that the majority of tested membrane proteins are organized in clusters at sizes below the diffraction limit. These nanoclusters are thought to play an important role in cellular signaling. However, concerns about the existence of nanoclusters have been fueled by the notion that virtually all fluorescent probes show complex blinking behavior including long-lived dark states. This results in localization clusters due to the repeated observation of single molecules. Existing post-processing approaches commonly struggle to reliably distinguish real molecular clustering from such blinking artifacts.

Here, we present a novel analytical method using information from two-color STORM experiments for overcoming the erroneous detection of clustering due to fluorophore blinking. Targeting the same protein species with differently labeled antibodies allows for the calculation of distance distributions between localizations from both color channels. Molecular clusters exhibit a characteristic bias towards shorter distances. Applying toroidal shifts to the data breaks correlations between the two color channels, thus providing realizations of the null hypothesis of independence (randomly distributed molecules). This allows for statistical significance tests without the necessity of additional calibration. Monte Carlo simulations showed the reliability and robustness of the proposed method over a wide range of simulation parameters. Moreover, the method was validated with experiments on both clustered and randomly distributed membrane proteins.

3D stochastic process simulation for better interpretation of molecular dynamics related to cell wall biogenesis observed with TIRF microscopy

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Keyword: super-resolution, TIRF microscopy, classification, spatial and temporal analysis, simulator

The mechanism of the construction of cell wall is an essential subject for the biologists. With the recent development of super-resolution fluorescence microscopy, we can have better inspection into the role of some key proteins and their interactions in live cells during the cell wall extension. Knowing that MreB is one of the key proteins responsible for cell wall construction, we aim to analyze the dynamics of MreB in rod-shaped bacteria *Bacillus*.

Previous work concerning the dynamics of MreB started from the classification of the trajectories[1], constructed by single particle tracking (SPT). Trajectories are classified into three categories, directed motion, Brownian motion and confined motion. The images used are acquired by TIRFM which records only the events near the observation plan. So the images obtained are actually the projection of the dynamics on the cell membrane near the observation plan. The classification is simply done with 2D images ignoring the curvature of the rod-shaped cell wall and the thickness of TIRFM plan. To evaluate the effect of these approximations, we have developed a simulator to generate the trajectories on the surface of the cylinder. The processes for simulating the three kinds of motion mentioned above are Brownian with drift, Brownian motion and Uhlenbeck-Ornstein process[2].

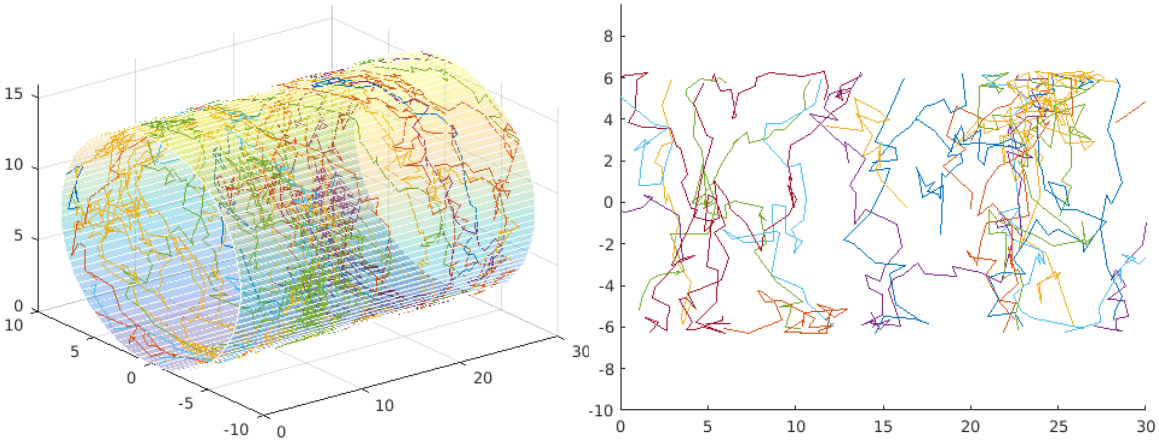

Figure 1 trajectories generated on the surface of the cylinder (left) and the view of TIRF microscope (right) which is the projection of the dynamics on the cylinder near the support surface. The unity in the two figures is in pixel and in our TIRF images 1pixel ≈ 64 nm. As the theoretical thickness of TIRF is 200nm, the trajectories whose *z* coordinate is under 3.125 pixels are projected into *x-y* plan. Colors for different trajectories are random.

Currently our work concerns spatial distribution exploration. For particles doing directed motion, we are interested in exploring its spatial distribution and the number of times a particle circles the cell wall before disappearing from the TIRF observation field. Furthermore, we model motions by Langevin Stochastic Equation $\dot{\boldsymbol{X}} = \boldsymbol{b}(\boldsymbol{X}) + \sigma(\boldsymbol{X})\dot{\boldsymbol{w}}$, and thereafter the velocity field and diffusion field are calculated using local information in each point. We aim to reconstruct the dynamics on the surface of the cylinder for the part that can't be seen by the microscope.

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SCALABLE ANALYSIS OF ULTRA-TERABYTE BRAIN IMAGES WITH DEEP LEARNING

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Deciphering brain architecture at a system level requires the ability to quantitatively map its structure with cellular and subcellular resolution. Besides posing significant challenges to current optical microscopy methods, this ambitious goal require the development of a new generation of tools to make sense of the huge amount of raw images generated, which can easily exceeds several TeraBytes for a single sample. We present an integrated pipeline allowing the image transformation from a collection of voxel gray levels to a semantic representation of the sample.

As a first step, the hundreds of adjacent tiles produced by the microscope needs to be aligned and fuse together. To this aim, we developed ZetaStitcher [1], a software for image stitching that computes global optimal alignment of imaging datasets as big as 8 TB in less than an hour. The fused volume is then generated virtually, without the need to create a physical copy of the dataset, by means of a dedicated API.

The virtually fused volume is then processed to extract meaningful information. We demonstrate two complementary approaches based on deep convolutional networks. In one case, a 3D conv-net is used to 'semantically deconvolve' the image [2], allowing accurate localization of neuronal bodies with standard clustering algorithms (e.g. mean shift). The scalability of this approach is demonstrated by mapping whole-brain spatial distribution of different neuronal populations with single-cell resolution.

To go beyond simple localization, we exploited a 2D conv-net estimating for each pixel the probability of being part of a neuron [3]. The output of the net is then processed with a contour finding, obtaining reliable segmentation of cell morphology. This information can be used to classify neurons, expanding the potential of chemical labeling strategies.

[1] https://github.com/lens-biophotonics/ZetaStitcher

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3D-EM-ISH – a novel tool to visualize specific chromatin regions

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Organization of chromatin in the interphase nucleus still remains elusive. Understanding of the way in which a two-meter strand DNA is packaged in ~ 10 micrometers-size nucleus will allow us to better understand not only organization of the basic structural units but also shed light on the functional aspects like regulation of gene expression and causes of genetic diseases. However, the resolution of light microscopy is not enough to study chromatin fibers with a diameter about 5-30 nm. Recent superresolution methods such as localization microscopy achieve lateral resolution as low as 10–30 nm. As an alternative for them we developed a novel method based on the three dimensional electron microscopy with lateral resolution down to 5 nm. Our high resolution approach -3D-EM-ISH combines three dimensional electron microscopy (3D-EM) and DNA *in situ* hybridization (ISH). We used 3D-EM-ISH to probe the structure of chromatin domains revealed by ChIA-PET (Chromatin Interaction Analysis by Paired-End Tag Sequencing) – the technique to study spatial chromatin interactions. We propose our method as a tool to study how these domains - basic structural and functional units of chromatin are organized in single cells, and present their structure and cell to cell variability.We discusse reconstruction and quantification of the 3D structures of topologically associating domains (TADs) in electron microscopy images.

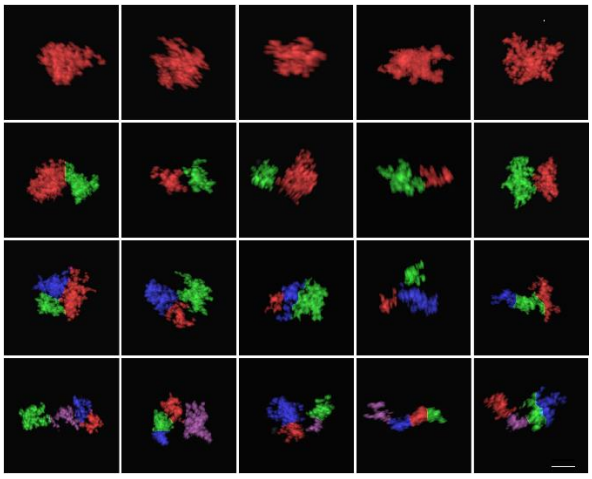

Fig. 1. Chromatin topologically associating domains (TADs) reconstruced from the electron microscopic images. Pseudocolor: the structure was segmented into multiple domains by detecting the local density centers and performing diffusion process from the centers, using the image density as a diffusion coefficient. Scale bar: 500 nm.

PROTEIN COPY NUMBER ESTIMATION IN BIOLOGICAL SAMPLES WITH HIGH FLUORESCENT BACKGROUND

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KEYWORDS: protein counting, photon statistics, quantitative scanning nanoscopy

A key goal in quantitative microscopy is to access the number of participating molecules in biological structures as directly as possible. A technique to accomplish this over a large range of copy numbers in protein complexes is counting by photon statistics (CoPS) [1]. The application of CoPS to intracellular samples, however, strongly depends on the properties of specific samples and fluorophores. Modeling the background fluorescence as a single weak emitter is problematic for samples with high cluster density or high fluorescent background.

To gauge the impact of background fluorescence on the CoPS number estimate and to explore different methods of background treatment, we present simulations of single photon data with different origins of background fluorescence. Experimental validation can be accomplished by experiments with standard samples with known copy numbers such as prokaryotic enzyme complexes [2]. Since CoPS effectively estimates the number of fluorescent labels, this needs to be accompanied by a robust estimation of the degree of labeling [3]. The combination of experiments *in silico* and with well-characterised biological samples should further develop CoPS towards a quantitative imaging technique for complex intracellular systems.

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Intracellular dry-mass density measurements of bacteria using quantitative phase microscopy

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Optical microscopy is routinely employed to study microbial growth on the level of single cells. However, typically only shape and volume are monitored to measure growth. This follows the assumption of a tight volume regulation keeping intracellular dry-mass density strictly constant. In order to evaluate this assumption, we implemented quantitative phase imaging, namely Spatial Light Interference Microscopy (SLIM) [1], to measure the optical path difference of single bacteria. This in turn can be used to infer cellular dry mass [2]. Additionally, we deduce volume estimates from the shape of the cells. Together the ratio of these quantities gives us the intracellular dry-mass density.

However, SLIM inherently suffers from halo and shade-off artefacts, affecting the measured phase in a manner dependent on the shape and size of the phase object [3]. In order to account for these effects, we characterized our optical system, enabling us to accurately simulate halo-affected phase images. Similarly, we use forward convolutions with the measured point-spread function of our setup to account for the effect of diffraction on the shape of cells.

It is has been reported that SLIM dry-mass measurements cannot compare in relative precision to recent buoyant-mass measurements via suspended microchannel resonators (SMR) [4], but our implementation shows precision similar to SMR. Additionally, we multiplex our approach with the full functionality of a light microscope including fluorescence measurements, and the integration of microfluidics. Thus, we can now follow mass and volume of a population of micron-sized objects of sub-picogram weights. Using this technique, we uncover remarkable aspects of bacterial volume regulation during steady-state growth behaviour and upon sudden perturbation in volume expansion or mass-growth.

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u-track 3D: A tracking framework to quantify, observe and contextualize intracellular dynamics in three dimensions.

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KEYWORDS: 3D fluorescence microscopy, multiple particle tracking, intracellular process, error propagation, 3D visualization.

Light-sheet fluorescence microscopy (LSM) achieves three-dimensional (3D) imaging of the entire cellular volume with reduced phototoxicity, fast sampling and near-isotropic resolution. Those recent advances provide new opportunities for a deeper understanding of dynamic intracellular processes *in toto*. However, while computer vision techniques for 2D microscopy have proven powerful in unbiasedly interrogating biological processes, we show that the heterogeneity and visual overlapping of dynamics measured in 3D require computational tools to even comprehend local measurements, let alone formulating biological hypothesis. In the u-track 3D platform, we tackle this fundamental challenge with three algorithmic strategies:

- Measurement validation: The size of the datasets and the molecular clutter hinders the visual control of tracking errors to parameterize the algorithm and refining the acquisition conditions. We propose an entirely new metric to evaluate trackability across the entire dataset and adjust parameterization accordingly.
- Changing frame of reference: The additional degree of freedom provided by LSM data necessitates multiple physical frame of reference to integrate and compare different instances of a same process inside the cell. By automatically detecting cellular fiducials through non-supervised estimation of nucleation site or membrane location, we are able to integrate data from thousands of processes to obtain quantitative insight on kinetochore fiber formation timing and late endocytic event maturation on a moving membrane.
- Data-driven hypothesis formulation: taken together, molecular clutter and structural complexity typically hinder the comprehension of the data and the formulation of hypothesis. Dynamic region-of-interest (ROI) size are thus defined to follow the targeted biology. This tools enabled the discovery of transient but important mechanisms involved in microtubule bundle assembly and a representative mapping of the visual heterogeneity in bleb formation.

With those combined strategies, u-track 3D proposes a systematic framework for the unbiased study of heterogeneity of molecular process fluorescently labelled in the whole cell.

CLUSTER ANALYSIS ON THE CELL MEMBRANE

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KEYWORDS: 3d super-resolution microscopy, spatial statistics, Ripley's K function.

Three dimensional super-resolution techniques are becoming robust tools in fluorescence microscopy imaging. In the case of STORM and PALM, several approaches can be used to obtain 3d information, such as astigmatism based 3d imaging, double helix point spread functions (1), interferometry information (2) etc. State of the art techniques can achieve the imaging of several micrometers in depth (2). However, the analysis of the point pattern and the software necessary to perform this analysis is not yet fully developed.

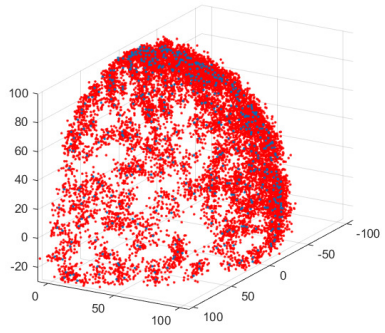

Figure 1. Clustering on a lower dimensional manifold

In this work we will focus on the study of the clustering of localisations obtained via 3d super resolution light microscopy in the special case of membrane processes. The 3d point coordinates are lying on a manifold of lower dimension and the natural distance to consider in this case is the geodesic distance. To our knowledge, currently all the analysis is performed using the Euclidean distance (either in 2d or in 3d).

The data poses several challenges: typically, 3d datasets consists of a huge number of data points, they are often corrupted by outliers and noise and the sampling rate can be very heterogeneous in space. We discuss pre-processing steps to construct a mesh from the given 3d point-cloud and the algorithm used for the subsequent computation of the geodesic versions of summary statistics, such as K-function, k-nearest neighbor, point correlation function etc.
We explore the differences with the Euclidean based case at different scales, and present results computed both on simulations and on real data.

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The topography of biological membranes artefactually creates the appearance of anomalous diffusion: a remedy

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KEYWORDS: topography, anomalous diffusion, SPT, plasma membrane

Single particle tracks (SPTs) on biological membranes are generally analysed using the mean squared deviation (MSD) using Euclidean distance measurements and report that diffusion is anomalous. Using Euclidean distances would be fine except that it requires that biological surfaces like the plasma membrane are both flat and aligned with the imaging plane. There is no evidence that these requirements are met with live cells. On the contrary the available evidence shows that the plasma membrane is not even locally flat. MSD-based analyses and the associated membrane models are therefore of doubtful value.

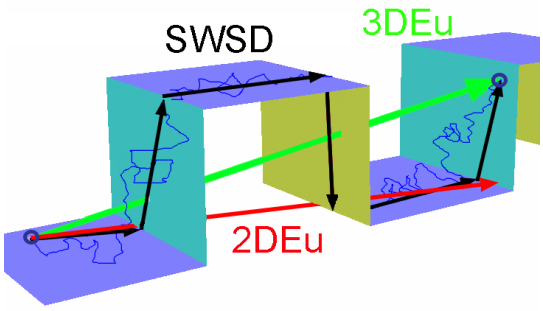

Figure 1. Different distance measurements on a non flat surface

Since the plasma membrane is not flat, 2D Euclidean distances measurement require that the tracked molecules leave and then rejoin the surface, which is rather unlikely. To establish the magnitude of the problem we compared 2D and 3D Euclidean measurements with a new measure, which dictates that membrane molecules remain in the membrane, by measuring the shortest within surface distance (SWSD).

Findings

(1) On surfaces that can be created by folding a flat sheet, 2D and 3D Euclidean measures underestimate the rate of diffusion and falsely flag anomalous diffusion. By contrast the SWSD effectively unfolds the surface and accurately reports the movement.
(2) On more complex surfaces, which would require differentially stretching a sheet, both Euclidean measures are even less satisfactory and surprisingly, the SWSD, while outperforming the Euclidean measures, still underestimates the rate and pattern of movement.
(3) Topography itself can cause both sub- and superdiffusion.

Our major conclusion is that topography prevents SPT, FCS and FRAP, accurately measuring the rate and pattern of movement on non-flat surfaces.

The solution requires (i) imaging the topography of live cells, (ii) using simulations to obtain the pattern of movement expected with Brownian motion and (iii) comparing this pattern with experimentally observed movement. The aim is to differentiate true anomalous movement from artefactual topography-dependent anomalous diffusion and to identify real interactions between surface associated molecules and biological membranes and to generate well founded models of biological membranes.

MULTISCALE COMPUTATIONAL MODELING OF CHROMATIN THREE-DIMENSIONAL STRUCTURE USING IPALM MICROSCOPIC HIGH-RESOLUTION IMAGES

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KEYWORDS: Applications in cell biology, fluorescent microscopy, Methods development - Image analysis, Methods development - Software development, superresolution microscopy, 3D chromatin modeling

The three-dimensional structure of chromatin is of fundamental importance for gene regulation and cellular function. It determines genome compaction and activity in the nucleus. Chromatin fibers form many different loops and a variety of dynamic conformations to achieve structural high packing density. However, the spatial organization of chromatin fiber have been difficult to observe using conventional microscopy. Recent developments in high-resolution microscopy allow us to study genome conformation and chromatin contacts using not only high-throughput genomic data (Hi-C, ChIA-PET) but also chromatin imaging data at different resolutions. .

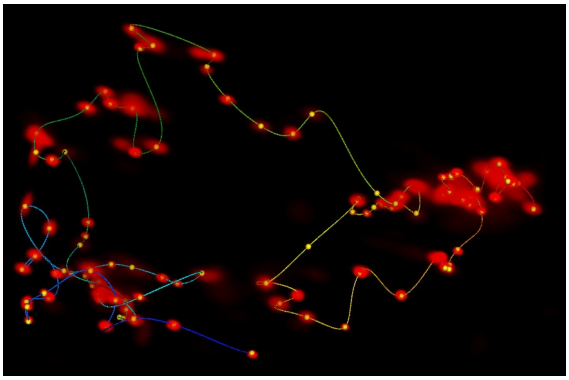

Figure 1. Chromatin loop 3D structure reconstructed form iPALM image.

We present single chromatin loop modeling based on super-resolution microscopy (iPALM - interferometric PhotoActivated Localization Microscopy). Our aim is to process 3D images from super-resolution microscopy to identify all attached oligo probes used in FiSH (Fluorescence in Situ Hybridization) staining. We present an algorithm based on connected component analysis at different image brightness level which identifies all possible markers in 3D space. Our goal is to compute image driven models that can be compared to genomic data driven models, and to build a bridge between population data and single cell imaging to obtain the most probable model of 3D chromatin structure. Here we present examples of single chromatin loop image- based modeling and application to multi domain region.

A QUANTITATIVE LOOK AT CELLULAR OLIGONUCLEOTIDE DELIVERY

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KEYWORDS: fluorescence correlation spectroscopy, fluorescence lifetime imaging, dSTORM superresolution, nanomedicine

Oligonucleotides are a highly promising class of novel therapeutics, but efficient cellular uptake and biological activity depend on delivery vectors that induce cellular uptake and endosomal release into the cytosol Cationic polymers and lipids package the oligonucleotides into nanoparticles of about 100 nm in diameter. In which way delivery vectors that show activity differ from those that do not is only poorly understood. Here, we used dSTORM (direct stochastic optical reconstruction microscopy) superresolution microscopy and combined lifetime imaging and fluorescence correlation spectroscopy to investigate the composition of peptide-oligonucleotide polyplexes and their trafficking inside cells. For nanoparticles formed from the polycationic peptide nona-arginine and messenger RNA, dual-channel STORM was employed to investigate to which degree the packaging of mRNA into nanoparticles depended on the excess of peptide. Furthermore, we were able to quantitatively determine the number of peptides and mRNA molecules per nanoparticle. Such information is crucial to understand how efficiently mRNA-based therapeutics reach cells and are translated into therapeutic proteins.
Using fluorescence correlation spectroscopy, for nanoparticles consisting of Cy5-labeled antisense oligonucleotides and a cationic peptide we determined nuclear oligonucleotide concentrations at which therapeutic effects were observed, thus providing for the first time a molecular understanding of dose-response functions. Remarkably, some of the Cy5-labeled oligonucleotides also gave mitochondrial staining. Using a beta setup of the Leica SP8 FALCON (Fast Lifetime CONtrast) system that gave us the possibility to combine fluorescence correlation spectroscopy (FCS) and fluorescence lifetime imaging (FLIM), we unambiguously assigned mitochondria bound and cytosolic fluorescence to free and oligonucleotide-bound dye, respectively.
In summary, our data show that advanced imaging provides for quantitative information in nanomedicine that is highly valuable for the understanding and optimization of formulations and for providing a molecular and cellular understanding of factors that govern cellular activity.

WIDE DEPTH REPRODUCIBLE MULTICOLOR BIOIMAGING THROUGH A COMBINATION OF 3D SINGLE MOLECULE LOCALIZATION STRATEGIES

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KEYWORDS: 3D SMLM, supercritical angle fluorescence, depth imaging

The use of 3D Single Molecule Localization Microscopy (SMLM) in structural and dynamic biological studies currently remains limited because of its lack of versatility, but a great deal of effort is being made to render it both more precise, quantitative and reproducible. This requires to address several instrumental problems that may considerably limit the relevance and reliability of obtained data. In particular, the anisotropy of the localization between the lateral and axial precisions as well as the strong dependence of these precisions on the axial position may restrict the potential applications of a microscope. Besides, axial drifts, chromatic aberrations and field aberrations (especially spherical aberrations) often hamper Point Spread Function (PSF) shaping based experiments, inducing dramatic losses of resolution and axial biases [1].

We propose a detection scheme that addresses these issues to provide more reliable results for biological applications. To minimize the loss of lateral information when improving the axial resolution, we developed a dual-view optical setup that decouples lateral and axial detections. Moreover, we combined strong astigmatism PSF shaping with supercritical angle fluorescence (SAF) detection [2]: as SAF information is obtained from an intensity measurement of the fluorophores' near-field emission coupled into propagative waves at the sample/coverslip interface, it yields a complementary absolute axial measurement that gives a reference to the astigmatism approach. This technique, called depth astigmatic imaging with SAF yield (DAISY), provides 3D absolute information over a 1.2 µm capture range above the glass coverslip and an axial localization precision down to 15 nm with minimal loss of lateral resolution and little sensitivity to field aberrations. Furthermore, it exhibits an almost isotropic resolution that slowly varies with the depth, which will be illustrated on living *E. coli* bacteria (Fig. 1 a,b) in the framework of the study of new click chemistry labelling techniques.

We will discuss the implementation and the optimal merging of the axial information sources and the performances of DAISY in terms of localization precisions and intrinsic biases corrections. We will show how the SAF absolute information helps retrieving drift-free and chromatism-insensitive data, and how the nominal 1.2 µm depth capture range can be furthermore extended over several µm depth by stacking the results of sequential acquisitions performed at known, unbiased heights [3]. Dual-color images on cytoskeletal networks (Fig. 1c) and endocytosis structures will illustrate the improved resolution over several µm depth

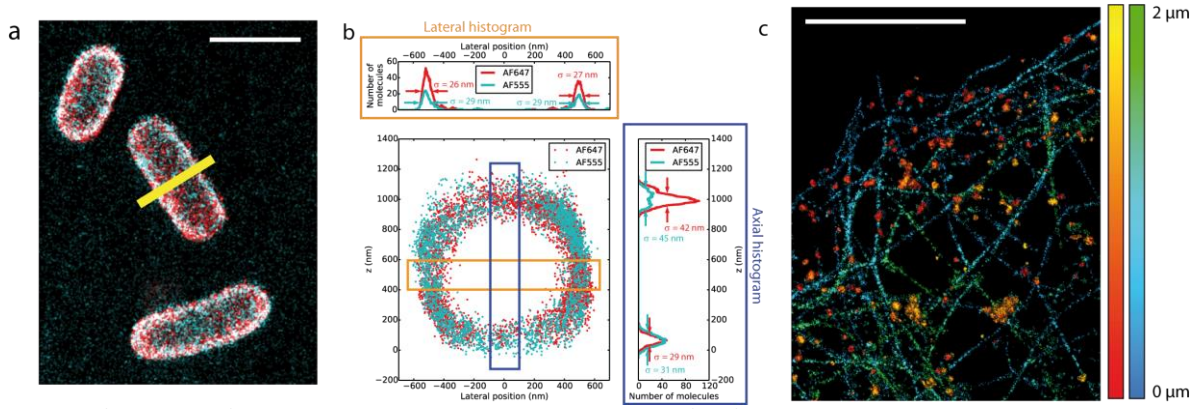

Figure 1. (left) 2D image of *E. coli* bacteria labelled with AF647. (center) 3D profile of a bacterium along the displayed blue line. (right) Dual color 3D image of the cytoskeleton of a COS-7 cell (blue-green: tubulin, yellow-red: clathrin).

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STRUCTURED ILLUMINATION FOR SINGLE MOLECULE LOCALIZATION MICROSCOPY AT DEPTH

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KEYWORDS: 3D SMLM, depth imaging, iso-localization

Most quantitative biological studies require both specificity and high resolution imaging. Super resolution optical imaging achieves these performances through Single Molecule Localization Microscopy (SMLM). However, 3D SMLM at important depths remains limited as few methods such as iPALM or Lattice Light Sheet can obtain high performance over a few microns at the cost of a certain complexity that limits their use. Here, we propose a straightforward optical setup that exhibits an isotropic resolution and little sensitivity to aberrations inherent to deep imaging.

This method relies on the coupling of centroid technique for the transverse molecular localization and modulation of a structured illumination pattern for the axial localization. The sample is illuminated with a fringe pattern. Recording the fluorescence image on the camera for different displacements of the illumination pattern enables the calculation of the phase of each molecule, which is then converted into a z position. This technique thus produces 3D images of (d)STORM or (DNA-)PAINT labelled samples (**Fig. 1**) featuring an isotropic 3D localization precision around 10 nm. This localization precision remains constant over the whole capture range. Besides, as this technique is based on intensity measurements, it exhibits little sensitivity to sample- or index-mismatch-induced aberrations and can thus be used to image biological samples at important depths inaccessible with most 3D SMLM techniques.

After discussing the implementation and the characterization of the performances of the system, we will show results obtained on biological samples at different depth ranges to highlight the usefulness of the method.

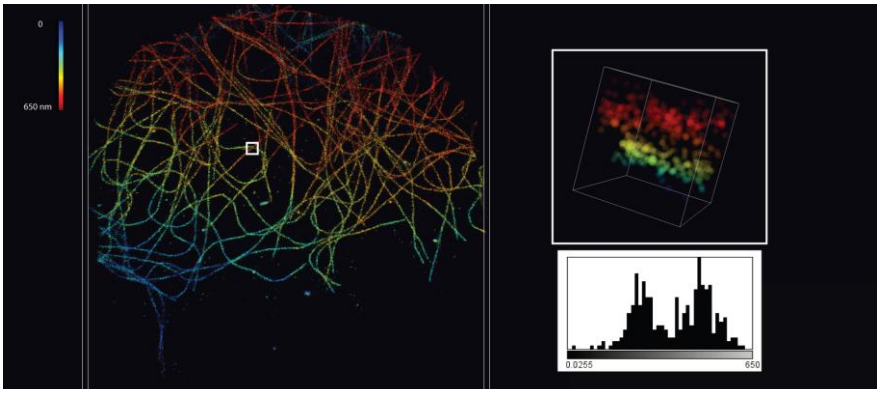

Fig. 1: 3D image of tubulin labelled AF-647

Single-shot fluorescence holography for 3D tracking in live cells

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Holography has emerged as a powerful tool for studying 3D systems and their dynamical evolution based on single-exposure "2D-images" but has thus far mainly been applied in the context of coherent light. For fluorescent emitters, holography based 3D particle tracking has thus far not been realised albeit its great potential due to its intrinsically background-free nature and the availability of site-specific labeling strategies.

I will present our recent advances in establishing digital holography based approaches in the context of imaging with incoherent light such as fluorescence or spontaneous Raman microscopy. Our technique enables the measurement of the complex electric field of fluorescent light in a single-shot thus making it suitable for imaging of dynamically evolving systems. By relying on this additional information we are able to use basic Fourier-optics principles to, for example, computational refocus out-of-focus images or quantify sample-induced image aberrations. Ultimately, we are able to track fluorescent particles over a large field-of-view in an aberration free manner in all three dimensions by relying on a single image.

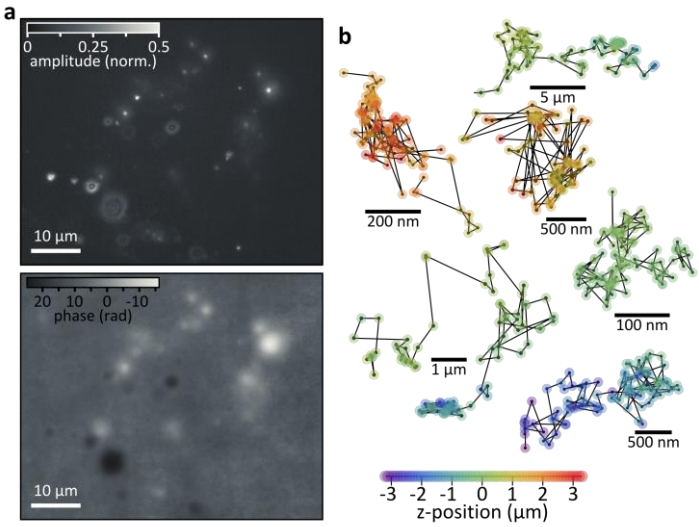

Figure 1. a) Widefield fluorescence image of freely diffusing particles inside a living green monkey kidney cell (top) alongside the fluorescence's phase (bootom). b) Trajectories of individual particles inside the cell reconstructed from 100 frames recorded at an integration time of 100 ms.

I will present a detailed description of the technical implementation followed by brief proof-of-concept experiments on particles emitting incoherent light, e.g. fluorescence or Raman, as well as single molecules. We employ our technique to observe and follow the endocytosis of fluorescently labeled nanoparticles (10x10x40 nm) into freely moving live cells (Figure 1) as well as the interaction of extracellular vesicles, e.g. exosomes, with live cells.

CNN based semi-supervised FIB-SEM image segmentation via propagation of learned information from a small subset of a single image stack

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KEYWORDS: CNN, Electron Microscopy, semi-supervised segmentation, cancer

The use of deep neural networks and more especially convolutional neural networks (CNNs) to segment biological features of interest in focused ion beam scanning electron microscopy (FIB-SEM) images has grown rapidly in recent years [1]. Although most of the developments in this area have focused on neural tissue and connectomics [2, 3], our group has begun to use auto-encoder CNNs to segment patient-derived tumors in order to gain a better understanding of the 3D ultrastructure of cancer cells. The main drawback to the use of CNNs is that neural networks require large amounts of hand annotated training data to produce a generalizable model [4]. Acquiring this data is costly; hand annotation can take several months and requires specialized training [5]. To overcome these challenges, we have adopted a semi-supervised approach for pixel-level segmentation by training a CNN on a small subset of 2D manually segmented images in the acquisition plane and propagating the learned features to segment the remaining images in the volume. We applied this method to three different tissue samples derived from human tumors and one breast cancer cell line to segment nuclei and nucleoli. The models were trained for 5000 weight update iterations which took roughly 3 hours to complete and predictions on the remaining images required minutes. The automatically predicted segmentation attained on average 97% accuracy and 92% intersection over union (IoU) using 10% of the data, 70 – 130 images dependent upon dataset size, to train on. Qualitatively, the predicted segmentation required only minor manual correction, reducing the overall segmentation time on average 85-90%. Our findings indicate that information within 2D slices is redundant; thus, only a small fraction of the total images is needed to train a model to segment the same features across the entire sample. We also observed that accurate models perform poorly, a decrease in accuracy and IoU of 20-30% on average, when applied to sister datasets from the same source. Moreover, this method allows for faster generation of annotated data that can be used in the future to build general segmentation models.

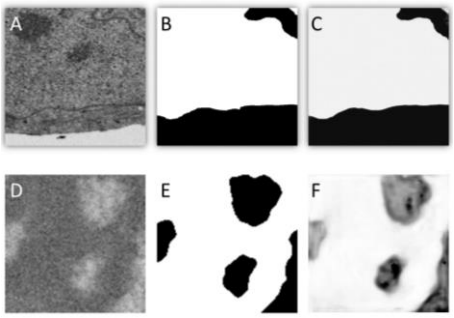

Figure 1: (A-C) Segmentation results from the breast cancer cell line after training on 10% of the sample volume. This sample contained 1395 images total. Image A is an input image not used for training the model, B is the ground truth annotation, and C is the predicted segmentation. (D-F) Nucleolar segmentation results from patient-derived xenograft from pancreatic tumor tissue. The model used to make these predictions was trained on a human breast tissue image volume. Panels D, E, and F correspond to the input image, ground truth, and network prediction, respectively.

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Mapping fluid and single-cell dynamics in a controlled thermal landscape

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Systems characterised as being away from thermal equilibrium are central to many key phenomena in nature, from the motion of an ensemble of particles in the presence of thermal gradients to the complex temperature sensing machinery that triggers changes in our immune cell behaviour. Although an impressive number of techniques have been developed to deliver heat to a sample, a distinct challenge, however, remains regarding our ability to map the resulting temperature profiles as well as the effects it has on its surrounding in a label-free arrangement.

To bridge this gap, we developed an all-optical platform based on a digital holographic microscope that allows us to: simultaneously shape the local heat source, measure the resulting temperature profile, and follow the dynamics of objects in three dimensions. I will specifically show how plasmonic nanostructures in combination with resonant excitation, can be used to engineer and control the thermal landscape at the macroscale. Furthermore, I will demonstrate that the heat transferred into the sample by the plasmonic structures can be quantitatively determined in a label-free manner by measuring the phase difference caused by the thermally-induced refractive index variations of the aqueous medium. Using this methodology, we achieved to measure differences in temperature from a diffraction-limited heat source as low as 0.3 K and follow the heating and cooling dynamics of the system with a temporal resolution of 0.040 ms.

To illustrate the potential of our optical platform to probe the dynamics of systems away from thermal equilibrium, I will present studies on neutrophils within a flow-cell system. Specifically I will show quantitative maps of the fluid dynamics together with the response of the neutrophils in the presence of well-controlled and localised temperature gradients.

THE TCR IS RANDOMLY DISTRIBUTED IN THE PLASMA MEMBRANE OF RESTING T CELLS

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KEYWORDS: label titration SMLM, TCR, nanocluster, T cell, PALM, STORM, STED

Over the last decade single molecule localization microscopy has been intensively used to study protein nanoclusters at the T cell plasma membrane. In particular, the T cell receptor (TCR) complex was suggested to be non-randomly organized in resting T cells [1, 2]. This finding has led to a variety of models to explain highly specific and sensitive antigen recognition, and T cell activation. However, repeated detection of fluorescent labels and resulting overcounting of labelled proteins may be easily misinterpreted as molecular clusters. In an attempt to circumvent this problem, we previously developed a method to discriminate between true protein clusters and apparent localization clusters[3].

Here, we applied this method to study the organization of the TCR complex in the plasma membrane of resting and activated T cells. While there was clear evidence for the formation of clusters upon activation, we found no indication of a non-random distribution under resting conditions. In independent experiments, we confirmed our observations with STED microscopy. We conclude that the TCR is randomly distributed in non-activated T cells. We suggest that a random TCR distribution reflects an evolutionary adaptation to maximize scanning speed in the initial phase of antigen recognition.[4]

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PartSeg - GUI for image processing algorithm in Python

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There are many methods in bio-imaging that can be parametrized. This gives more flexibility to the user as long as tools provide easy support for tuning parameters. On the other hand, the datasets of interest constantly grow which creates the need to process them in bulk. Again, this requires proper tool support, if biologist is going to be able to organize such bulk processing in an ad-hoc manner without the help of a programmer. Finally, new image analysis algorithms are being constantly created and updated. Yet, lots of work is necessary to extend a prototype implementation into product for the users. Therefore, there is a growing need for software with a graphical user interface (GUI) that makes the process of image analysis easier to perform and at the same time allows for high throughput analysis of raw data using batch processing and novel algorithms. Main program in this area are written in Java, but Python grow in bioinformatics and will be nice to allow easy wrap algorithm written in this language.
Here we present PartSeg, a comprehensive software package implementing several image processing algorithms that can be used for analysis of microscopic 3D images. Its user interface has been crafted to speed up workflow of processing datasets in bulk and to allow for easy modification of algorithm's parameters. In PartSeg we also include the first public implementation of Multi-scale Opening algorithm descibed in [1]. PartSeg allows for segmentation in 3D based on finding connected components. The segmentation results can be corrected manually to adjust for high noise in the data. Then, it is possible to calculate some standard statistics like volume, mass, diameter and their user-defined combinations for the results of the segmentation. Finally, it is possible to superimpose segmented structures using weighted PCA method. Conclusions: PartSeg is a comprehensive and flexible software dedicated to help biologists in processing, segmentation, visualization and the analysis of the large microscopic 3D image data. PartSeg provides well established algorithms in an easy-to-use, intuitive, user-friendly toolbox without sacrificing their power and flexibility.

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NOISE CONTROLLED IMAGE RECONSTRUCTION FOR STRUCTURED ILLUMINATION MICROSCOPY

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Structured Illumination Microscopy (SIM) offers better lateral resolution and optical sectioning compared to microscopes with uniform illumination [1]. The current standard in image reconstruction, so-called generalized Wiener-filtering [2], depends on ad-hoc tunable parameters and may result in a variety of artefacts [3], such as halo and edge ringing, structured noise, or a honeycomb imprint. The confusion between genuine resolution improvement and deconvolution artefact in SIM is intensely debated in the literature [4][5]. Here, we show how an explicit noise model, based on a physical analysis of noise propagation through the image reconstruction chain, enables a solution to standing problems in SIM image reconstruction concerning noise artefacts and true signal content in the final SIM image. We demonstrate the best possible linear reconstruction of the fluorescent object given the available signal-to-noise ratio (SNR), based on the Wiener criterion. A key ingredient of this true Wiener-filtered reconstruction is the combination of the noise model with a spatial frequency averaging approach for establishing a self-consistent estimate of the spectral SNR from the data itself. Next, we demonstrate how SIM reconstructions suffer from a trade-off between contrast and noise enhancement at intermediate length scales. We propose a companion image reconstruction method that overcomes this structured noise artefact while maintaining resolving power. This flat-noise SIM reconstruction can be used to identify possible noise enhancement artefacts in high-contrast state-of-the-art or true Wiener reconstructions, and as proper noise normalized input for subsequent non-linear deconvolution methods, such as Richardson-Lucy deconvolution. Another advantage of these noise controlled SIM reconstructions is that they eliminate ad-hoc user-controlled parameters, in particular the regularization parameter. The newly proposed methods are demonstrated on images of GFP-labeled zyxin, a building block of focal adhesions, of nano-fabricated test structures and of the synaptonemal complex.

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3D TRACKING OF ENDOCYTIC EVENTS USING LATTICE LIGHT SHEET MICROSCOPY

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KEYWORDS: light sheet microscopy, 3D tracking

Live cell imaging has been helpful in discovering various phenomenon and mechanisms that a static image cannot. Nevertheless, live cell imaging has been restricted by the microscopy techniques available to mainly two dimensions or limited by the speed. The study of the whole cell dynamics of endocytic events has proven difficult until recently, because of lack of sensitivity, limited speed, photobleaching and phototoxicity associated with conventional imaging modalities. The Lattice Light Sheet Microscopy (LLSM) allows overcoming these difficulties. This microscope uses an ultra-thin structured laser light sheet to image successive planes of the specimen [1]. The benefits in relation to previous techniques are low phototoxicity and photobleaching during image acquisition. In addition, high resolution images (~250 nm in xy and ~700 nm in z) can be acquired at high speed (~200 planes per second) [1]. This allows 3D images to be captured over longer time, and enables the study of signaling, transport, and stochastic self-assembly in complex environments with single molecule sensitivity. However, facing the amount of information provided by LLSM, cutting-edge image processing algorithms need to be investigated, at a time regime compatible with live cell imaging.

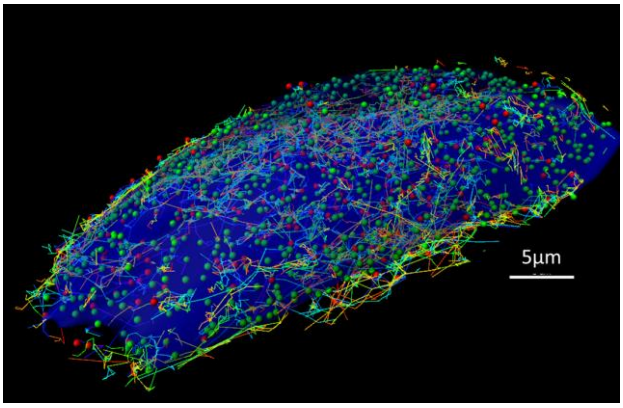

Figure 1. 3D tracking of Galectin-3 (red) vs AP2-eGFP (green) in SUM159 cell.

We present a quantitative analysis of endocytosis dynamics of AP2 adaptor complex, Galectin-3 (Figure 1) and Transferrin using two colour single particle tracking analysis of 3D+time data. These examples demonstrate the advantage of lattice light sheet microscopy for imaging endocytic events in single cells.

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3D FLUORESCENCE WHOLE SLIDE IMAGING using CONFOCAL MULTI-LINE SCANNING

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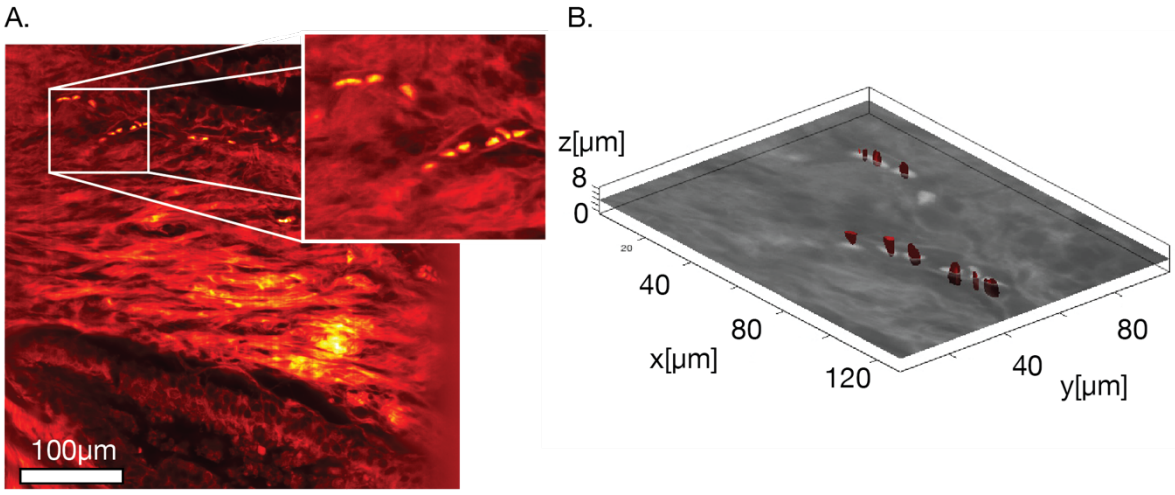
KEY WORDS: Whole Slide Imaging (WSI), Digital Pathology, Fluorescence Microscopy, SLM, Laser, Confocal, Line scanning

In the field of pathology there is an ongoing transition to the use of Whole Slide Imaging (WSI) systems which scan tissue slides at intermediate resolution (~0.25 µm) and high throughput (15mm^2/min) to digital image files [1]. Most scanners currently on the market are line-sensor based push broom scanners for three-color (RGB) brightfield imaging [2]. Adding the ability of fluorescence imaging would open up a wide range of possibilities to the field, in particular the use of specific molecular (proteins, genes) imaging techniques. In recent work, we showed that multi-color fluorescence scanning can be implemented by using multi-LED color sequential illumination [3]. This is an uncomplicated and low-cost solution, but it is intrinsically slow because of the limited illumination power in the objective etendue.

The limitations of LED illumination can be overcome by the use of laser illumination, which is not etendue limited. The laser is focused into a single line inside the sample, optically conjugate to the line image sensor to have confocal illumination. This has the advantage of a very high efficiency as well as low bleaching compared to wide field illumination. Line rates in the order of 100 klines/s are anticipated. Additionally, the confocal illumination ensures a high signal-to-background contrast. This might be especially beneficial for scanning thicker slides (e.g. 20 µm).

We present design details of our optical architecture. In particular, we will discuss the development towards multi-line scanning, using a novel diffractive structure for focusing the individual lines at different depths. This technique can be used for continuous auto-focus and highly efficient z-stack scanning with intrinsic alignment of the layers. We will present results of our system on clinical samples.



A. Multi-line scan result for simultaneous multi-focal scanning showing the center layer of a z-stack of a 20µm thick tissue section. The inset shows two capillaries with red blood cells.
B. A 3D segmentation was applied to find the red blood cells in the capillaries. A difference in z-position can be observed.

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Membrane rolling by annexins inferred from combined fluorescence and AFM imaging

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KEYWORDS: Fluorescence, AFM, Annexin, protein-membrane interactions, membrane dynamics

Maintaining integrity of the plasma membrane is essential for cell life. Thus, efficient cell membrane repair mechanisms are crucial for handling membrane disruptions resulting from external perturbations of eukaryotic cells. Cancer cells experience enhanced membrane stress when navigating through the dense extracellular matrix, which increases the frequency of membrane injuries. Yet the underlying molecular details of plasma membrane repair are not well understood. Plasma membrane injury followed by Ca^{2+} influx, activates the recruitment of Annexins to membrane wound edges.

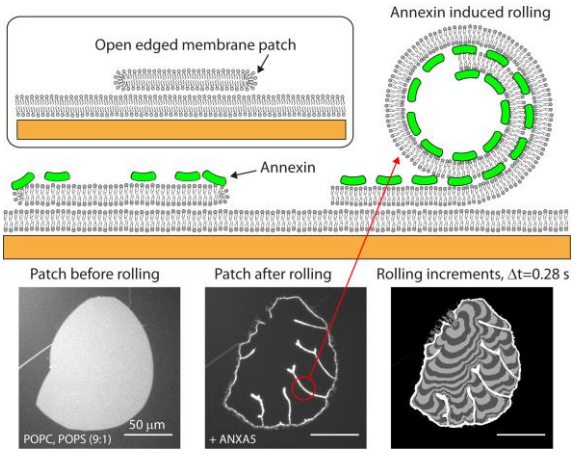

Figure 1 Annexin-induced rolling of a double supported membrane patch measured by time-lapse fluorescence.

In cancer cells subjected to localized laser damage, we find that binding of several annexin members promotes the repair of lesions. In a planar model membrane system, we show that curvature stress induced by annexin-binding leads to roll-up of the membrane as initiated from free membrane edges. The rolling process is deduced by analysis of time-lapse fluorescence images, combined with the morphology of rolls as measured by AFM on the same sample region. In a complementary approach, the rolling process is modeled theoretically, taking into account curvature stress and adhesion to the underlying surface [1].

The observation of rolling identifies membrane curvature near hole-edges as a potential key event in the plasma membrane repair (PMR) process. In the geometry of a membrane hole we propose that spontaneous curvature leads to the formation of a characteristic neck structure around the hole as the first step in the repair process[2]. Formation of a neck geometry is further supported by theoretical modeling.

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Abstract 97

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Medical image analysis: Brain tumor grade classification.

Abstract: The recent segmentation and tumor grade classification techniques of brain Magnetic Resonance (MR) Images is the objective of this study. In Magnetic Resonance Imaging (MRI), the tumor might appear clear but physicians need quantification of the tumor area for further treatment This is where the digital image processing methodologies along with machine learning aid further diagnosis, treatment, prior and post-surgical procedures, synergizing between the radiologist and computer. These hybrid techniques provide a second opinion and assistance to radiologists in understanding medical images hence improving diagnostic accuracy. This study aims to retrospect the current trends in segmentation and classification relevant to tumor infected human brain MR images with a target on gliomas which include astrocytoma.

Keywords: Gliomas, Medical Image Processing, MRI, Brain Tumor, Classification

Combining Deep Learning and Active Contours Opens The Way to Robust, Automated Analysis of Brain Cytoarchitectonics.

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KEYWORDS: histology, image segmentation, cell detection, deep learning, active contours

Systematic studies of the cortical cytoarchitecture are indispensable to understand the functional organization of the human brain. Classical works based on qualitative description of cell counts and shapes in physical 2D sections of the human cortex revealed functional areas and segregation in the brain [1]. These brain parcellations are currently updated and refined using automated image analysis. Even 3D imaging of post mortem brain tissue at microstructural resolution are within reach using recent light sheet fluorescence microscopy (LSFM) [2] and tissue clearing protocols [3]. Combined with advanced image analysis, these techniques enable studying cortical cellular organisation in the human brain with unsurpassed precision. To reach this goal we need robust computational analysis relying on minimal manual annotations. Deep learning has thoroughly changed the field of image analysis yielding impressive results whenever enough annotated data can be gathered. While partial annotation can be very fast, manual segmentation of 3D biological structures is tedious and error-prone. Additionally, high-level shape concepts such as topology or boundary smoothness are hard if not impossible to encode in Feedforward Neural Networks. Here we present a modular strategy for the accurate segmentation of neural cell bodies from light-sheet microscopy combining mixed-scale convolutional neural networks and topology-preserving geometric deformable models. We show that the network can be trained efficiently from simple cell centroid annotations, and that the final segmentation provides accurate cell detection and smooth segmentations that do not introduce further cell splitting or merging.

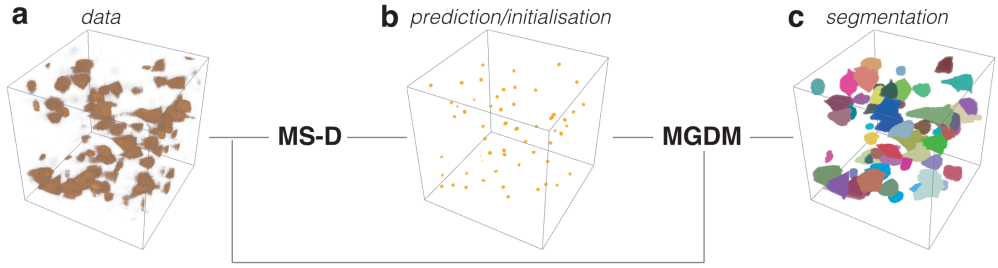

Figure 1: Schematic overview of method in 3D. We train a mixed-scale dense neural network [4] from sparsely annotated cell centroids. The predicted cell positions are used as initialization and topology prior for the multi-object geometric deformable model [5].

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MULTIMODAL MICROSCOPY REVEALS STIFFNESS-DEPENDENT NANOSCALE REMODELING OF ACTIN MODULES AT CELL PROTRUSIONS

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Tissue stiffness sensing is essential for many cellular processes during development and tissue homeostasis and is thought to be chiefly regulated by focal adhesions (FAs) in cells that anchor and migrate through interstitial matrix and by actin-based protrusions (invadosomes) in cells that degrade and cross basement membranes. While the molecular machinery that controls stiffness sensing in FAs has been well described, it is still largely unknown how invadosomes respond to changes in substrate stiffness. Here, we used a multimodal imaging approach to investigate the mechanisms that control invadosome stiffness sensing.

First, we exploited conventional microscopy, STORM nanoscopy and correlative multi-colour super-resolution and electron microscopy (SR-CLEM) [1] to demonstrate that the actin filaments that radiate from and interconnect individual invadosomes shorten on soft substrates. This is accompanied by a condensation of vinculin and myosin, but not talin, to the direct invadosome core vicinity. Next, we used AiryScan super-resolution live cell imaging, followed by spatiotemporal image correlation spectroscopy (STICS) analysis to demonstrate that the average actin and vinculin velocity at invadosomes is higher on stiff substrates, while peak velocity is higher on soft substrates. These findings together with the visualization of distinct nanoscale architecture of actin isoforms at individual invadosomes provide a molecular and structural basis to understand invadosome mediated stiffness sensing and suggest that invadosomes use their radiating actin filaments to actively scan and probe stiff substrates and focally protrude their branched actin core into soft substrates.

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CHARACTERISATION OF GFP AND DERIVATIVES FOR SUPER-RESOLUTION CRYOGENIC SINGLE MOLECULE LOCALISATION MICROSCOPY

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KEYWORDS: cryogenic super-resolution microscopy, single molecule localisation microscopy, photophysics.

The application of single molecule localisation microscopy (SMLM) at cryogenic temperatures is limited by the number of available fluorophores which retain their photoswitchable and photoactivatable properties at low temperature. Although important photophysical properties of both fluorescent dyes [1] and fluorescent proteins [2] are well-characterised at room temperature, no such comprehensive study currently exists for cryogenic temperature. We quantify the photoblinking properties, such as the average photon budget and on-time per blink, for several GFP-derivatives, including EGFP, mEmerald, and Clover. High photon budgets help to improve localisation precision in SMLM [3], whilst shorter blink times can reduce the chance of localisation errors due to overlapping fluorophores being detected simultaneously. We therefore aim to screen green fluorescent proteins to determine that which gives (on average), the highest photon budget and shortest blink time, both at the lowest laser power possible.

We show that the ability to photoblink at cryogenic temperatures is preserved by tested members of the GFP-family. Initial results suggest that mEmerald outperforms EGFP, with an average blinking on-time 2.3-fold shorter, but with a similar photon budget. mEmerald also begins to blink at approximately 50% of the power required for EGFP, making it more gentle on cryogenically-fixed samples.

By measuring the photophysics of more fluorescent proteins, we hope to provide a solid reference for the community of cryo-SMLM researchers, allowing them to choose the optimum fluorescent probe for their studies.

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AUTOMATIC REGISTRATION FOR MULTIDIMENSIONAL CORRELATIVE MICROSCOPIES WITH ERROR ASSESSMENT AND DETECTIONS OF DEFORMATIONS

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KEYWORDS: Error analysis, Correlative microscopy, Image and volume registration

Correlative microscopy allows combining different scales of observations and different contents, functional and morphological, based on the large panel of microscopy technologies available for life or material sciences. Data fusion methods have to be considered when developing an imaging workflow, from the sample preparation to imaging acquisition. The purpose is to accurately follow a region of interest across modalities and scales of imaging, for guiding and targeting the specimen preparation procedure needed for another modality, to guide the acquisition at the second microscope and to adjust the field of view of the microscope to this feature. In a last, and most accurate registration step, the acquired data from both modalities are mapped.
We present a point-based registration paradigm (figure 1), which presents several advantages.
The only step of our registration workflow which differs between workflows, whatever the dimensionality of data, is the feature extraction step, which can be learned on uncorrelated images. We also present a statistical framework of error estimation on the registration error allowing to answer the question of the confidence of the matching of structures, and to select the model of deformation between rigid and non rigid.

We have made this work available through the ICY image analysis platform [1] in a set of plugins gathered in EC-Clem [2].

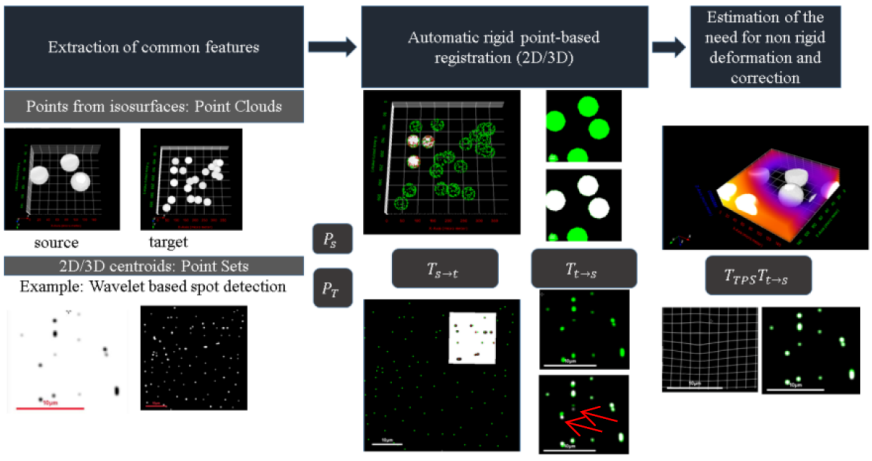

Figure 1: A general framework of data fusion: after the extraction of common features, our method generated and registered point set and point clouds. We simulated these datasets (2D and 3D) to have a ground truth. The 2D dataset was locally non-rigidly deformed (red arrows). Our error estimation framework detected it and corrected it here by thin plate spline interpolation.

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EXPERIMENTALLY-GENERATED GROUND TRUTH FOR TRACKING LYMPHOCYTE POPULATIONS IN IMAGE-BASED IMMUNOTHERAPY SCREENS

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KEYWORDS: CAR, deep learning

Chimeric antigen receptor (CAR) is a form of immunotherapy whereby T lymphocytes are engineered to selectively attack cancer cells. Therapies based on CAR show promise for improving the prognosis of cancers such as acute lymphoblastic leukemia, the most common and fatal form of cancer in children in the United States [1]. As a result, CAR therapies are currently of great interest. However, among the existing inventory of technologies that have been developed to measure CAR activity in vitro—image-based or otherwise—various shortcomings exist. In particular, image-based screens of CAR-T cells, combining phase contrast and fluorescence microscopy to distinguish cell types, suffer from the gradual quenching of the fluorescent signal, making the reliable tracking of cell type populations (T cells and target cells) across time-lapse imagery difficult. In addition, the fluorescent marking of cells is expensive, time-consuming, and potentially invasive to the experiment.

We propose to leverage the available fluorescent markers as an experimentally-generated ground truth. With some simple image processing, we are able to segment and assign cell type classes automatically. This ground truth is sufficient to train deep convolutional neural networks (CNN) to classify cell type from the phase contrast signal alone, ultimately eliminating the need for the cumbersome fluorescent markers. In addition, through some simple simulation of closely clustered and overlapping cells, we are able to bootstrap our dataset to cover a range of cases broad enough to create a robust object detection system, capable of tracking cell population level over time from phase contrast images alone. This will underpin the development of cheap and robust microscope-based protocols to quantify CAR-T activity against tumor cell in vitro.

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COMBINING 3D IMAGE ANALYSIS AND COMPUTER MODELLING TO UNDERSTAND CELL DIVISION PATTERNS IN PLANT EARLY EMBRYOGENESIS

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KEYWORDS: 3D imaging, cell shape, division plane, cell lineage, stochastic model.

During development, several cellular mechanisms orchestrate the progressive transition from the zygotic cell to a large number of cells organized in tissues and organs with specific shapes. In plants, cells are surrounded by a pecto-cellulosic wall that prevents cell migrations. As a consequence, cell division and oriented growth are the major cellular determinants of tissular organizations and developmental shape transitions. A major challenge in plant developmental biology is thus to understand how the position and the orientation of division planes are selected. Since the XIXth century, several phenomenological rules have been proposed to relate division plane selection and cell geometry. It is generally considered that symmetric cell divisions, which contribute to cell proliferation, correspond to a default mechanism driven by physical constraints. In particular, the minimum plane area principle embedded in Errera's rule has been shown to explain a number of experimental observations. However, most research efforts to date have concentrated on symmetric divisions and specific systems where cell shapes can be assimilated to 2D geometries. Therefore, to what extent 3D cellular geometry may influence symmetric and asymmetric divisions and consequently cell division patterns remains an unsolved issue. Here, we show how we combined 3D confocal imaging, automated image analysis, cell lineage reconstruction, and stochastic computational modelling to analyze cell division patterns during early embryogenesis in the model plant *Arabidopsis thaliana*. Our results highlight invariant principles relating mother cell shape and division plane positioning. In addition, we demonstrate the existence of a new geometrical rule allowing to predict the selection of the division plane in both symmetric and asymmetric divisions. Our results further suggested that this rule could be interpreted as surface minimization constrained by passing through the cell nucleus, which was validated using live imaging data of cell divisions in the leaf stomatal lineage. An important consequence of our findings is that the apparently complex cell organization of the *Arabidopsis* early embryo could be interpreted as a self-organized structure emerging from a geometrical feedback loop between cell geometry and division plane positioning.

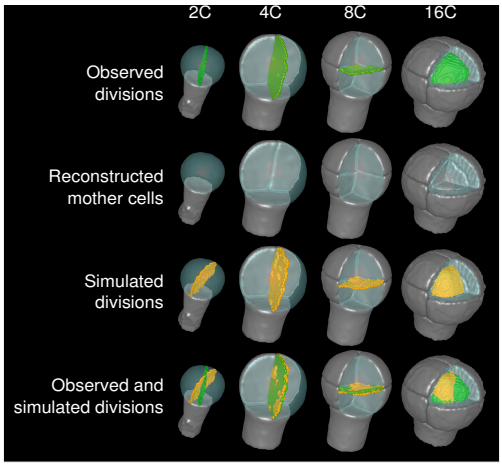

Figure 1. Image-based computational modeling of 3D cell divisions: observed and predicted patterns.

Deep learning based detection of cells in 3D Light sheet fluorescence microscopy

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We consider the detection of cells in light sheet fluorescence microscopy with illustration on 3D tumor spheroids. This informational task is the first step of image processing analysis of such tissue [1]. Cell detection has mainly been addressed with handcrafted or shallow machine learning approaches in the literature and only recently tested with deep learning [2]. In this communication we further explore a deep learning approach for cell detection. We specifically discuss the interest of data augmentation, the gain of transfer learning techniques [3] with simulated cells [4] and simulated quality of images in LSFM [5]. We also explore the question of the necessity of deconvolution when using convolutional neural networks.

Authors acknowledge support from the European PROCHIP FET project.

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Neural stem-cells behavior in the adult zebrafish brain: investigating the spatio-temporal patterning of neural stem cell activation events

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KEYWORDS: neural stem cells, spatio-temporal patterning

In adult vertebrate brains, the majority of adult Neural Stem Cells (NSC) are quiescent but, they can be activated (*i.e.* re-entering into the cell cycle to divide). NSC quiescence and activation need to be carefully regulated in time and space to accommodate both NSC maintenance and neuronal production during months/years.
Due to the very low frequency of these activation events and the difficulty of imaging, virtually nothing is known on how this tight equilibrium is coordinated in time and space at the level of the NSC population. To address this question, our model is the dorsal telencephalon of the adult zebrafish, which displays a large pool of mostly quiescent neural stem cells (qNSC).
In this study, we analyzed the nearest neighbors 3D distribution of the NSC activation events, either relative to each other or to the committed neural progenitors (aNP) generated from NSC. This distance distribution was compared to a simulated counterpart using a simple model varying the strength and the range of their influence on one another. We could show various types of influences, ranging from uncorrelated to strong and anti-correlation depending on the tested source and target populations of the effect. This study revealed a spatial short-range (~1-2 cell diameters) inhibition of the NSC activation events from the aNP.
Finally, to understand how the pattern of NSC division events evolves over time, we conducted a spatial point pattern analysis on the positioning of these events relative to each other at different time intervals. Our preliminary results suggest a spatial inhibition over time at specific time intervals.

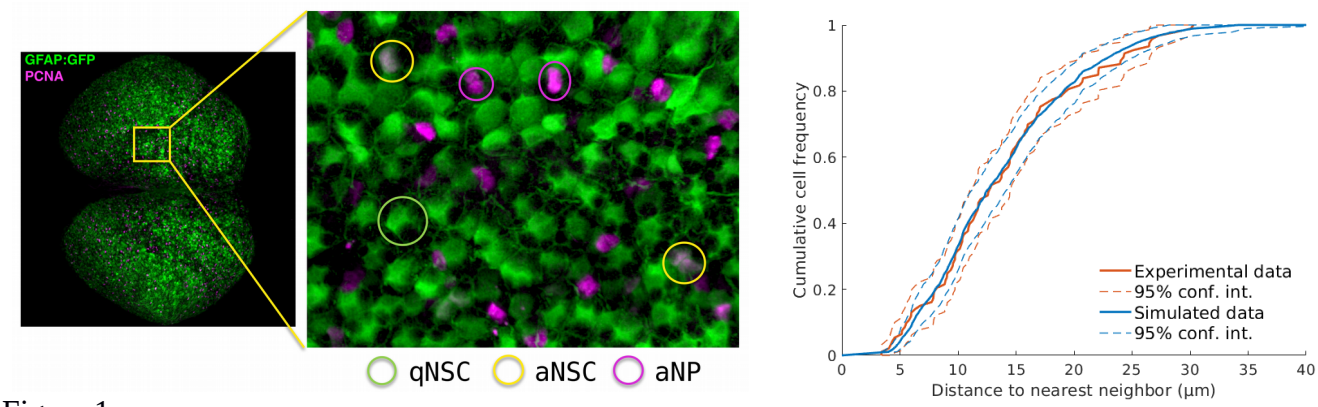

Figure 1:
Left panel) Typical fluorescence image of a zebrafish telencephalon. Right panel) Overlay of experimental and simulated cell to nearest neighbor distributions for a single pair of parameters with confidence intervals.

SIMPLE AND COMPACT MICROSCOPE FOR TIME-LAPSE PHASE AND FLUORESCENCE IMAGING BASED ON CHROMATIC ABERRATION

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KEYWORDS: phase imaging, fluorescence microscopy, time-lapse cell imaging

We propose a simple and compact microscope combining phase imaging with fluorescence. This compact setup can be easily inserted in a standard biological incubator and allows observation of cellular cultures over several days. Phase image of the sample is reconstructed from a single, ~50 µm defocused image taken under semi-coherent illumination [1]. Fluorescence in-focus image is recorded in epi-fluorescence geometry. The phase and fluorescence images are taken sequentially using a single CMOS camera. No mechanical movement of neither sample nor objective is required to change the imaging modality. The only change is the wavelength of illumination and excitation light for phase and fluorescence imaging, respectively. The slight defocus needed for phase imaging is achieved due to specifically introduced chromatic aberration in the imaging system.
We present dual modality time-lapse movies of cellular cultures observed over several days in physiological conditions inside an incubator. A field-of-view of 3 mm^2 allows observation up to thousands of cells with micro-meter spatial resolution in quasi-simultaneous phase and fluorescence mode. We believe that the simplicity, small dimensions, ease-of-use and low cost of the system make it a useful tool for biological research.

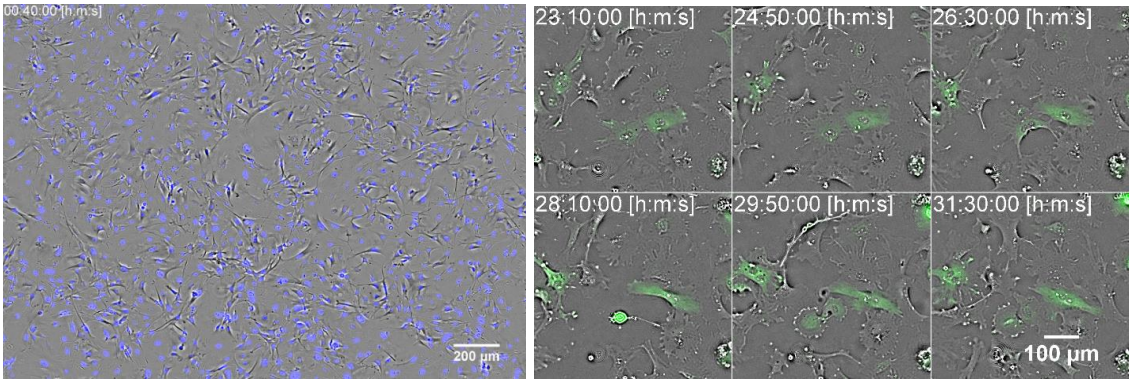

Figure 1: Mouse embryonic fibroblasts (MEF) stained with Hoechst (left) and with a subpopulation producing GFP (right, ROI of a full field of view). Merge of reconstructed phase and fluorescence images. Both experiments were performed directly in a standard incubator. The cells were placed in a petri dish with nutritive media and observed over 72 hours with recording every 10 mins.

[1] Allier, C. et al., "Imaging of dense cell cultures by multiwavelength lens-free video microscopy," Cytometry Part A 91(5), 433 (2017).

Establishing the theoretical density/SNR limits for SMLM analysis leads to the design of UNLOC, a parameter-free and fast computing ImageJ plugin

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KEYWORDS: SMLM, high density, SNR

Resolving structures at an unpreceded scale is now available by using single-molecule localization microscopy (SMLM). The initial prerequisites of such technique was to image and localize spatially isolated particles, i.e. at low density (LD) but recent applications aim at working at high density (HD) of particles per frame.

The motivation of this work[1] was (1) to provide a more general understanding on the localization accuracy by establishing a theoretical study without prior on the intensity α, (2) to thoroughly and systematically validate the results on synthetic data, and (3) to implement a new algorithm integrating the results and conclusions of the theoretical study.

At first, we show that only a very informative prior on the intensity approaches, at best, the Cramér-Rao bounds (CRB) established at a known α value (Fig. 1). If knowing α has no incidence under LD conditions[2], this leads to an overestimation of the localization precision under HD conditions. In its whole, our theoretical study highlights the fact that the precision evaluation requires to take into account the local density, background and signal, i.e. the SNR (dB). Thus, we identify the physical limits requiring a dedicated algorithm for HD data as well as the limits from which particles are no longer resolvable (NR), i.e. no detectable and therefore uncountable (Fig. 2).

Therefore, we developed UNLOC - UNsupervised particle LOCalization, a parameter-free ImageJ plugin, for reliable particle localization at variable local density with minimal computational cost. UNLOC is also designed to achieve the best performance. As demonstrated on synthetic or experimental data, UNLOC efficiently challenges the variability of background, SNR, or density.

Further developments are currently implemented to extend the UNLOC application for 3D imaging and real-time SMLM analysis.

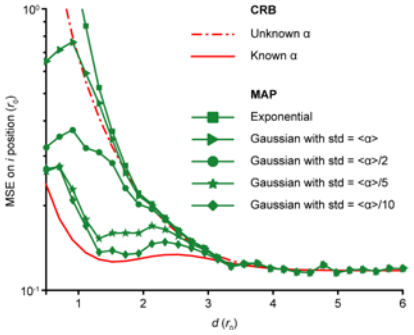

Figure 1. CRB and maximum a-posteriori probability (MAP) at unknown intensity decreases the theoretical achievable resolution.

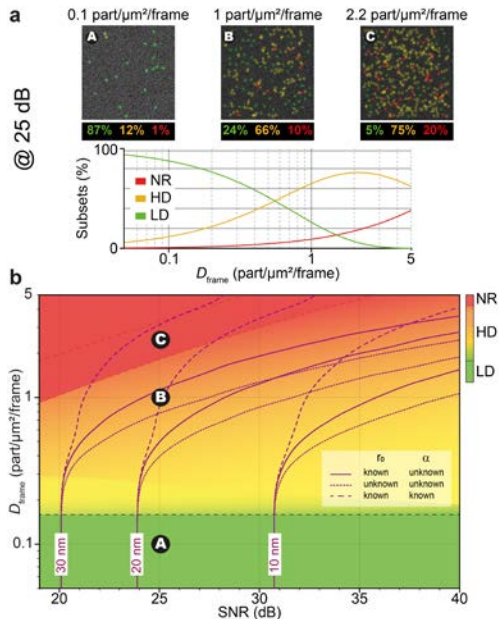

Fig. 2 (a) Definition of LD, HD and NR subsets; **(b)** Impact on the localization precision of α, density, SNR and PSF size.

UNLOC is freely available for academic and nonprofit use at http://ciml-e12.univmrs.fr/App.Net/mtt/.

1. Mailfert, S., et al., *Biophysical Journal* **2018,** *115*, 565-576.
2. Ober, R. J., et al., *Biophysical Journal* **2004,** *86*, 1185-1200.

Adaptive optics allows 3D STED-FCS measurements in the cytoplasm of living cells

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Stimulated Emission Depletion Fluorescence Correlation Spectroscopy (STED-FCS) is a powerful tool for investigation of molecular diffusion properties in living cells. STED-FCS has found many applications in 2 dimensional systems like cellular membranes, but was more rarely used in 3-dimensional systems. Besides, most STED-FCS measurements in 3D systems rely on constraining only the lateral resolution, using the underlying assumption that there is a structural invariance along the optical axis. Potential uses in crowded heterogeneous environments, such as cellular cytoplasm, are thus constrained.

The main reason why the 3D STED depletion pattern, which constrains also the axial dimension of the observation volume, has not been widely used in STED-FCS, is because it is extremely sensitive to optical aberrations. While these are often tolerated in imaging, they can be detrimental for FCS. This issue can be addressed using Adaptive Optics (AO). AO has previously been successfully used to correct for aberrations in 3D STED [1] and in FCS [2], but was never employed in STED-FCS.

We present here a new sensorless AO method for STED-FCS. Using a Spatial Light Modulator (SLM) as the adaptive optical element, we correct the aberrations affecting the depletion beam. We demonstrate the efficiency of this method by performing 3D STED-FCS in the cytoplasm of living cells using an oil immersion objective. While the aberrations resulting from the index mismatch preclude STED-FCS measurements even at moderate STED powers, our aberration correction results in improved signal levels as well as smaller focal volumes and thus promises further applications to probe diffusion properties of complex samples such as cellular cytoplasm.

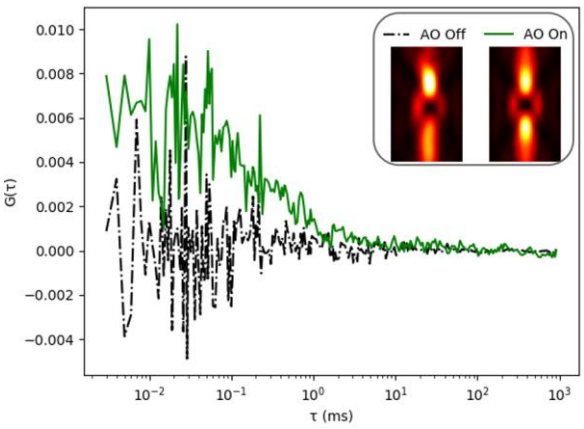

Figure 1. Effect of Adaptive Optics on 3D STED-FCS curves acquired in living cells. The corresponding depletion patterns (legend) are simulated using the measured wavefront

[1] B. R. Patton, D. Burke, D. Owald, T. J. Gould, J. Bewersdorf, and M. J. Booth, "Three-dimensional STED microscopy of aberrating tissue using dual adaptive optics," *Opt. Express*, vol. 24, no. 8, p. 8862, 2016.

[2] C.-E. Leroux, I. Wang, J. Derouard, and A. Delon, "Adaptive Optics for fluorescence correlation spectroscopy," *Opt. Express*, vol. 19, no. January, 2011.

ANALYSING AND QUANTIFYING INTRACELLULAR PARTICLES MOVEMENTS IN 3D LLSM DATA

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KEYWORDS: Lattice Light Sheet Microscopy, particle diffusion process, motion type change

Thanks to modern techniques such as Lattice Light Sheet Microscopy (LLSM) or asymetrical multiangle TIRF microscopy, it is now possible to observe in detail live cell mechanisms in 3D and over long periods of time. In cell biology, analysing and quantifying intracellular dynamics with such cutting-edge microscopy set-ups is of paramount interest to understand cell mechanisms. Several approaches have been then investigated to address these issues : i/ optical flow or motion computation algorithms that allow to visualise the overall inside movements of a cell, ii/ tracking-based algorithms focusing on the motion of particles.

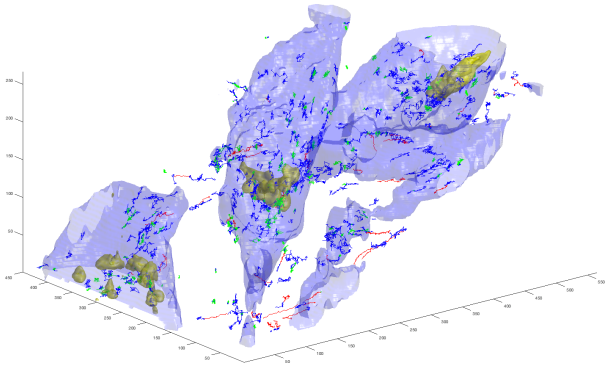

Figure 1. Movements of galectin-3 proteins in a few HeLa cells. The cell membranes are represented in blue, the golgis in yellow. Green, red and blue trajectories respectively correspond to confined, directed and free diffusion processes estimated along individual protein tracks.

In the sequel, we focus on particle tracks and assume that the motions of particles follow a certain class of random process : the diffusion process. Traditionally, confined, directed and free diffusion (namely brownian motion) are the three main types of particle motion that can be observed on cells. However, when we observe a long trajectory (longer than 100 points), it is possible that the particle switches between different motion modes over time depending on the cell environment. Consequently, fitting a single model to the trajectory, as described in [1], can be misleading. Nevertheless, trajectory type classification and change point detection methods can be combined to produce a finer particle movements analysis. We will present a statistical framework based on [1] to solve the problem of particles motion changes. The approach described in [2] is demonstrated on real LLSM images depicting moving particles/ proteins in the cell in interaction with Golgi apparatus.

[1] Briane et al., A statistical analysis of particle trajectories in living cells, Physical Review E, 2018

[2] Briane et al., A Sequential Algorithm to Detect Diffusion Switching along Intracellular Particle Trajectories, arXiv:1804.04977v1, 2018

A TILTED LIGHT SHEET FOR SINGLE-MOLECULE SUPER-RESOLUTION IMAGING IN THICK CELLS

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KEYWORDS: super-resolution light sheet microscopy, point spread function engineering

Point spread function (PSF) engineering enables the extension of single-molecule localization microscopy into three dimensions. By phase-modulating the collected light in the Fourier plane of the microscope using a chosen phase mask, one can encode the axial position of each emitter into the shape of its image on the detector. The depth-of-field for high-NA single-molecule localization can thus be extended from a few hundred nanometers to several microns with high 3D localization precision. Such an imaging system is a powerful tool for the study of thick samples (e.g. mammalian cells) at the single-molecule level. However, in epifluorescence widefield illumination, thick samples often emit high fluorescent background due to out of focus emitters, impurities and autofluorescence from the large illuminated volume.

We demonstrate a simple microscope design termed TILT3D [1] in which background reduction is achieved via light sheet illumination, introduced side-on into the sample, in a way that is specifically tailored to utilize the advantages of PSF engineering. The light sheet thickness is matched to the axial range of the double-helix PSF (~2 µm), enabling introduction of the beam through a low-NA, long-working distance objective. A ~10^{o} tilt of the excitation beam gives easy access to the coverslip surface without incurring aberrations due to refractive index mismatch at the glass-medium interface. Meanwhile, emitted light is collected by a high-NA objective as usual. Live drift correction is performed using fiducial beads imaged with a tetrapod point spread function with a 6 µm axial range, and a phase-retrieved model of its PSF [2] yields ~3 nm lateral and ~7 nm axial localization precision over this range. We achieve single-molecule localization precision down to ~15 nm laterally and ~25 nm axially throughout the 5 µm thickness of a HeLa cell, enabling imaging of microtubules, mitochondria, and the full nuclear lamina.

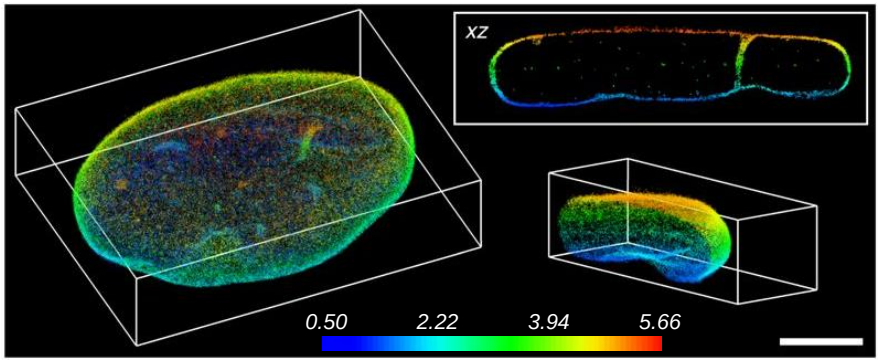

Figure 1. 3D super-resolution reconstruction of the entire nuclear lamina (lamin B1) in a HeLa cell. The *xz* view shows a slice through the cell featuring a lamin meshwork which envelops an intranuclear channel. The lower right shows the right cap of the reconstruction. Scale bar is 5 µm; color bar is *z* position in microns.

[1] A.-K. Gustavsson, P. N. Petrov, M. Y. Lee, Y. Shechtman, W. E. Moerner, "Single-molecule super-resolution imaging with a tilted light sheet," *Nat. Commun.* **9**(1), 123 (2018).

[2] P. N. Petrov, Y. Shechtman, W. E. Moerner, "Measurement-based estimation of global pupil functions in 3D localization microscopy," *Opt. Express* **25**(7), 7945-7959 (2017).

Speed OPIOM (Out-of-Phase Imaging after Optical Modulation) for quantitative multiplexed fluorescence imaging against autofluorescence under ambient light

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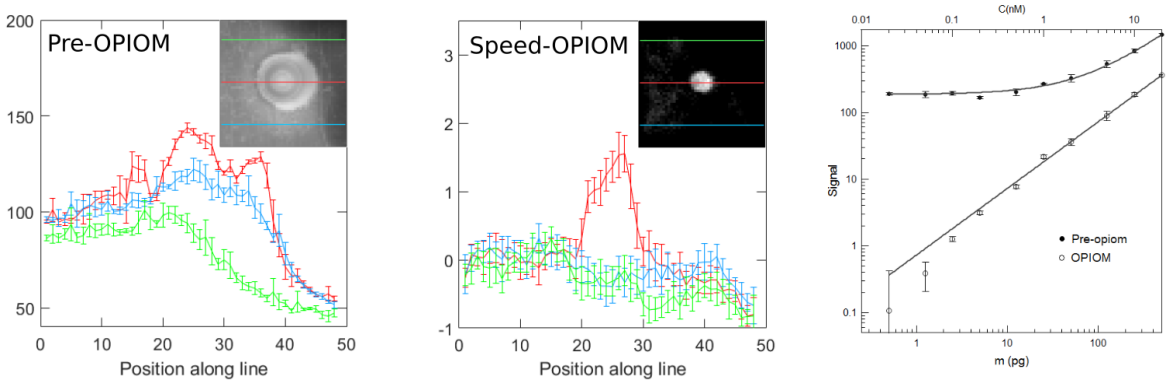
Fluorescence imaging is increasingly used to observe biological samples. However it may suffer from spectral interferences originating from ambient light, or autofluorescence of the sample or its support. We have built a simple and cheap fluorescence macroscope which has been used to demonstrate the relevance of the Speed OPIOM - reference-free dynamic contrast protocol to selectively and quantitatively image reversibly photoswitchable fluorophores as labels against detrimental autofluorescence and ambient light [1,2]. By tuning the intensity and radial frequency of modulated illumination to Speed OPIOM resonance and by adopting a phase sensitive detection scheme which secures noise rejection, we first enhanced sensitivity and signal-to-noise ratio for fluorescence detection in blot assays by a factor of 50 and 10 respectively, over direct fluorescence observation under constant illumination (see Figure). We then overcame the strong autofluorescence of growth media currently used in microbiology and achieved multiplexed fluorescence observation of colonies of spectrally similar fluorescent bacteria with a unique configuration of excitation and emission wavelengths. Finally we easily discriminated fluorescent labels from the autofluorescent and reflective background in labeled leaves, even under interference of incident light at intensities comparable to sunlight. Fully compatible with micro and macroscale imaging, the Speed OPIOM protocol is expected to find multiple applications in fluorescence imaging ranging from biological assays to outdoor observations.



Speed OPIOM selectively retrieves an RSFP signal in the presence of interfering light. Conventional (Pre-OPIOM) and Speed OPIOM images of 2.5 pg of Dronpa-2 deposited on a nitrocellulose membrane within 400 μm-diameter blot. Analyses of both Pre-OPIOM and Speed OPIOM images were performed along three lines: one crosses the middle of the blot (displayed in red) whereas the two others (shown in green and blue) are located out of it. Resulting linear calibration curves for PreOPIOM and Speed OPIOM imaging modalities.

[1] J. Quérard, R Zhang, Z. Kelemen, M.-A. Plamont, X. Xie, R. Chouket, I. Roemgens, Y. Koperina, S. Albright, E. Ipendey, M. Volovitch, H. L. Sladitschek, P. Neveu, L. Gissot, A. Gautier, J.-D. Faure, V. Croquette, T. Le Saux, L. Jullien, *Nat. Commun.*, **2018**, 969 ; [2] R. Zhang, R. Chouket, M.-A. Plamont, Z. Kelemen, A. Espagne, A. G. Tebo, A. Gautier, L. Gissot, J.-D. Faure, L. Jullien, V. Croquette, T. Le Saux, *Light Sci. Appl., under revision.*

Biophysical characterization of the interactions between Influenza A virus matrix protein M1 and host plasma membrane

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KEYWORDS: virus assembly, protein-protein interaction, fluorescence correlation spectroscopy, SPR, image correlation spectroscopy, MD

Influenza is a prominent cause of mortality in modern society and a major burden on health systems globally. The Matrix Protein 1 (M1) is an essential component involved in the structural stability of the Influenza A virus (IAV) and in the budding of new virions from infected cells. During virus assembly, M1 is recruited to the host plasma membrane (PM) where it interacts with specific lipids and other viral proteins. The structure of M1 is only partially characterized and the molecular mechanisms determining how the protein interacts with the PM, as well as those governing protein-protein interaction and multimerization, have not been yet clarified. We quantitatively investigated M1 multimerization and its interaction with lipids, both in model membranes and in living cells. To this aim, we used a combination of biophysical techniques including FRET, confocal microscopy imaging, raster image correlation spectroscopy, CD spectroscopy, surface plasmon resonance and Number and Brightness (N&B) analysis. Our results show that M1 forms multimers upon interaction with phosphatidylserine (PS)-rich domains in the PM. Protein-lipid interactions are mediated by specific residues in the N-terminal domain of M1 and cause alterations in protein structure and intra-molecular dynamics. Our experimental findings are supported by molecular dynamics simulations as monomer in solution or bound to a negatively-charged lipid bilayer. Taken together, our results provide novel quantitative information regarding the molecular interactions between IAV and host cellular membranes.

Large scale feature extraction and image classification for high content screening microscopy

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High-content screening microscopy or other automated imaging techniques used in large-scale biomedical studies usually generate vast image datasets that need to be profiled using automated image processing techniques and advanced statistical analyses. While sample preparation and data acquisition can usually be sufficiently handled in most laboratories, the automated extraction and processing of quantitative features from a large number of images remains a major challenge. Even though dedicated solutions exist, they require sophisticated input/output or file architectures like databases that are not familiar to unexperienced users. The goal of this project is thus to provide an intuitive image-driven toolbox accessible to a wide range of biomedical researchers for the scoring and classification of biological samples. For this purpose, we use the data analytics software KNIME that provides several tools for feature extraction and data classification (including several state-of-the-art classifiers like clustering, random forest and neural network). Moreover, this modular platform also offers advanced image-processing and visualization capabilities based on the same library than the standard ImageJ software. On top of the technical solution, we will provide guidelines that will help researchers to rapidly proceed to their analysis without the need to dive into complex programming documentation. The toolbox will be benchmarked on toxicological studies from the zebrafish animal model and should help developing standard protocols and workflows for the scoring of morphological features.

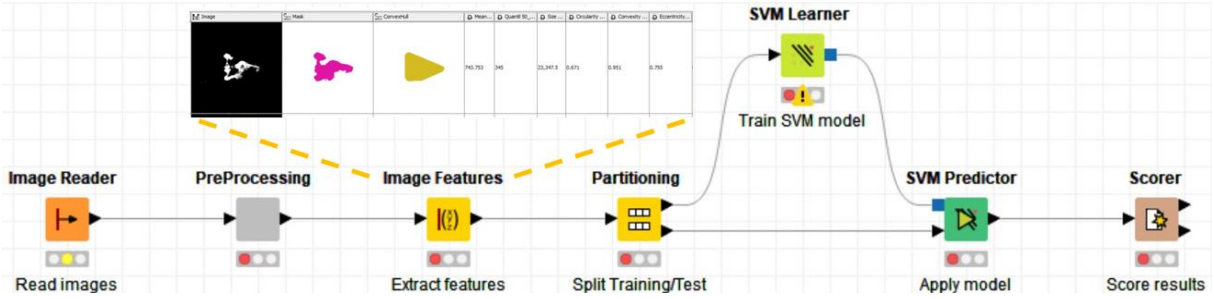

Figure 1. A typical image classification workflow in KNIME
Here the features are extracted from the images of a fluorescent Zebrafish kidney after preprocessing, and classified using a SVM classifier.

QUANTITATIVE EVALUTATION OF A NEXT GENERATION SCIENTIFIC CMOS (sCMOS) CAMERA FOR HIGH FIDELITY IMAGING

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KEYWORDS: scientific camera, sCMOS, quantitative imaging, read noise distribution, fixed pattern noise, photoresponse non-uniformity (PRNU), dark signal non-uniformity (DSNU), linearity, quantum efficiency map, individual pixel variance, individual pixel mean

Since the release of "Scientific" CMOS (sCMOS) camera, sCMOS camera has become the standard imaging technology for microscopy and advanced imaging. However the read noise characteristics, non-uniformity of pixel read noise and issues related to linearity stemming from two level digitization architecture[1] prevented use in some applications.

Therefore we designed the new sCMOS sensor chip and developed the next generation sCMOS camera. The sensor chip used in this camera has extremely low read noise, CCD-like homogeneous noise uniformity, reliable linearity at all light levels, more pixels and faster pixel throughput. To evaluate the next generation sCMOS camera, we measured the *distributions* and *spatial maps* of individual pixel offset, dark current, read noise, linearity, photoresponse non-uniformity (PRNU) and variance distributions of individual camera pixels. Measurements are taken with highly uniform and controlled illumination over low light conditions from dark conditions and at multiple low light levels.

In this presentation we report that the next generation sCMOS camera using the new sensor has extremely low read noise and high uniformity of read noise distribution. The higher SNR by extremely low read noise and high read noise uniformity at low light level makes it possible to shorten the exposure time or reduce the excitation light power. The former increases the frame rates and the later extends the observation time by reducing photo bleaching and photo toxicity.

Additionally the high uniformity of read noise not only increases the image quality at low light level but also makes the image correction for irregular noise unnecessary providing the signal which is more close to the true data.

[1] S.Watanabe, T. Takahashi and K. Bennett, *Proceedings of the SPIE*, Volume 10071, id. 100710Z 8 pp. (2017).

SEGMENTATION OF 3D IMAGES OF PLANT TISSUES AT MULTIPLE SCALES USING THE LEVEL SET METHOD

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Background: Developmental biology has made great strides in recent years towards the quantification of cellular properties during development. This requires tissues to be imaged and segmented to generate computerised versions that can be easily analysed. In this context, one of the principal technical challenges remains the faithful detection of cellular contours, principally due to variations in image intensity throughout the tissue. Watershed segmentation methods are especially vulnerable to these variations, generating multiple errors due notably to the incorrect detection of the outer surface of the tissue.

Results: We use the level set method (LSM) to improve the accuracy of the watershed segmentation in different ways. First, we detect the outer surface of the tissue, reducing the impact of low and variable contrast at the surface during imaging. Second, we demonstrate a new edge function for a level set, based on second order derivatives of the image, to segment individual cells. Finally, we also show that the LSM can be used to segment nuclei within the tissue.

Conclusion: The watershed segmentation of the outer cell layer is demonstrably improved when coupled with the LSM-based surface detection step. The tool can also be used to improve watershed segmentation at cell-scale, as well as to segment nuclei within a tissue. The improved segmentation increases the quality of analysis, and the surface detected by our algorithm may be used to calculate local curvature or adapted for other uses, such as mathematical simulations.

[1] A.Kiss, T.Moreau, V. Mirabet, C. I.Calugaru, A. Boudaoud, P. Das *"Segmentation of 3D images of plant tissues at multiple scales using the level set method"*. **Plant Methods** 13:114 (2017) Collection "Plants in Computer Vision", https://doi.org/10.1186/s13007-017-0264-5.

Measuring the Absolute Degree of Labeling for Self-Labeling Protein Tags

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KEYWORDS: Degree of labeling, Calibration, Protein counting

Knowledge about the composition and stoichiometry of molecular assemblies is key for a mechanistic understanding of many biological processes. Several fluorescence microscopy techniques such as quantitative localization microscopy or counting by photon statistics enable in situ measurements of protein copy numbers in complexes or subcellular structures. To translate the measured fluorophore numbers to target protein copy numbers, it is essential to determine the degree of labeling (DOL) which is the ratio of fluorescently labeled to non-labeled targets. Although most fluorescent labeling schemes achieve sub-stoichiometric labeling efficiencies and/or attach more than one fluorophore per target binding site, relative DOL measures and not the absolute fluorophore to target ratio are usually being reported.

To overcome this limitation, we developed a staining efficiency probe that allows for measuring the absolute DOL for genetically encoded protein tags in situ on a single-cell level. We demonstrate the use of our probe by determining absolute DOLs for two widely used self-labeling protein-tags, SNAP-tag and HaloTag, across a wide range of labeling conditions, in different cell lines and with different fluorophores. Our concept allows to calibrate apparent fluorophore quantification to actual target protein numbers at non-stoichiometric ratio between target and label. We found that for both tags, the absolute DOL for intracellular labelling is substantially lower than previously reported values obtained from studies with purified proteins. Furthermore, the choice of fluorescent substrates may critically influence the achievable degree of labeling.

Our approach is readily expandable to other protein tags and can also be used to determine the maturation efficiencies of fluorescent proteins allowing for quantitative applications with a wide range of tags and fluorophores in both live and fixed cells.

Single molecule nucleocytoplasmic transport dynamics in live cells

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KEYWORDS: single molecular tracking, HILO, TIRF, dextran, nucleocytoplasmic

Recent studies taking advantage of single molecule dynamics have been highlighted in nucleocytoplasmic transport. Studies have investigated transport dynamics using super-resolution microscopes, such as TIRF (Total internal reflection fluorescence microscope), STORM (Stochastic Optical Reconstruction Microscopy) and PALM (Photoactivated localization microscopy). Most of the recent work has been done with digitonin permeabilized cells to all the delivery of labelled cargo at an appropriate level for single molecule imaging. We set out to study nucleocytoplasmic transport dynamics in the human cell line SH-SY5Y cells at the single molecular level in intact cells, using fluorescently-tagged dextran by highly inclined and laminated optical sheet (HILO) microscopy (near-TIRF).

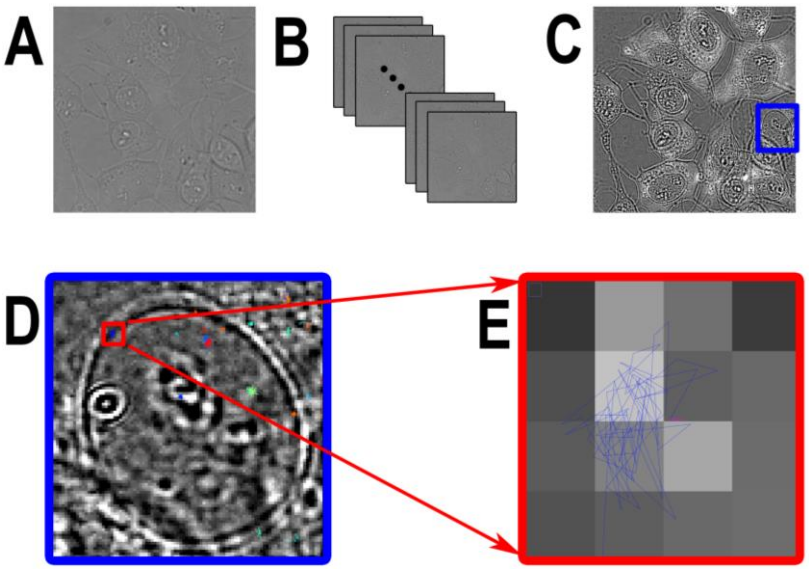

Figure 1.
Data acquisition and processing workflow in live cells; (A) 1000 bright filed images are firstly taken (18ms, ≤20 sec). (B) Bright field images are processed into a single image (Convolve and Z Project). (C) The result after image processing from the 1000 bright filed images showing a clear nuclear envelope (bold black circle). (D) overlaying single dextran tracking on the bright filed image. (E) Magnification of single dextran tracking on nuclear envelope.

Our results suggest that HILO microscopy (Beam angle: ≤60 degree) can allow single molecular tracking at 2 µm depth in cells, using 53fps (images per 18 ms, Andor iXon EMCCD) in a Nikon TIRF microscope as shown in Figure 1. We are monitoring how dextran cargoes are transported across the nuclear envelope and whether it is successfully transport into nucleus. We also find that dextran size variation influences the transport success rate.

We are now developing our technique using HILO microscopy to further analyse the transport dynamics of single molecule cargo/receptors through the inner and outer selection barriers of individual nuclear pores and the impact of disease-associated proteins in neurodegeneration.

MEMBRANE CURVATURE ESTIMATION METHOD FOR CRYO-ELECTRON TOMOGRAPHY

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KEYWORDS: biological membranes, curvature estimation, cryo-electron tomography, tensor voting

Curvature is an important quantitative morphological descriptor of cellular membranes. Current curvature estimation methods cannot be applied directly on membrane segmentations (set of voxels) in cryo-electron tomography (cryo-ET). Additionally, a reliable estimation requires to cope with quantization noise. Therefore, we developed and implemented a method for membrane curvature estimation from tomogram segmentations.

From a membrane segmentation, a signed surface (triangle mesh) is first extracted. The triangle mesh is then represented by a graph (vertices and edges), which facilitates finding neighboring triangles and calculation of geodesic distances by our curvature estimation algorithm. Our algorithm combines two previously published algorithms that are based on tensor voting [1, 2]. Beside curvatures, it also provides a robust estimation of normals and principal directions.

We tested our method on several benchmark surfaces with known curvature. The benchmark results demonstrate the validity of our method and its robustness to quantization noise of triangle meshes originating from binned tomogram segmentations. We also applied the method on biological data: on endoplasmic reticulum (ER) and vesicle membranes in human cells for quantifying the curvature increase near huntingtin fibrils [3] and on cortical ER in yeast for quantifying its morphology. We suggest that our method can be applied to any segmented membrane compartments or even other structures from which a surface can be extracted, not necessarily originating from cryo-ET.

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SUPER-RESOLUTION IMAGING OF UNLABELED LIVING CELLS USING SUPEROSCILLATORY POLARIZATION CONTRAST

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KEYWORDS: unlabeled microscopy, superoscillations, super-resolution, polarization contrast.

The next grand challenge in biological imaging is unlabeled super-resolution. While stimulated emission depletion and single-molecule microscopies are shedding new light on previously invisible biological processes, they are still limited by the need for reporters (labels) within the sample. Another route to far-field subwavelength imaging is using optical superoscillations: engineered interference of multiple coherent waves creating an, in principle, arbitrarily small hotspot[1]. We show that far-field images taken with superoscillatory illumination are themselves superoscillatory and hence can reveal fine structural details of the object that are lost in conventional far-field imaging. We will demonstrate high-frame-rate polarization-contrast imaging of unmodified living cells with resolution beyond that achievable with conventional microscopes.

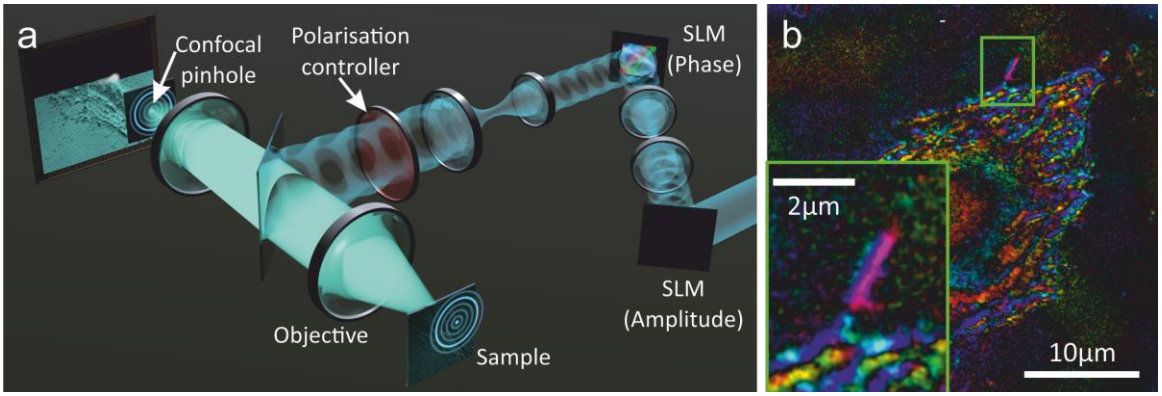

Figure 1: a) Schematic of our super-oscillatory microscope showing the key components. b) False colour polarization-contrast of an MG63 osteosarcoma cell line, with the inset showing a 200nm-wide image of a fillopodium.

We have constructed a practical superoscillatory microscope by upgrading a conventional commercial biological microscope (Figure 1a), with which we demonstrate that superoscillatory imaging: 1) provides greater spatial resolution than microscopy with conventional lenses; 2) gives radically more information on the fine details of the object than confocal microscopy; 3) can be combined with polarization-contrast imaging for unlabeled cell imaging; 4) can be performed simultaneously with epi-fluorescent imaging; 5) is possible at video frame rates and at low optical intensities. We will illustrate these features with in-vitro, high-frame-rate polarization-contrast images and videos of living unlabeled biological samples (mouse bone cells – as in Figure 1b – and neurons).

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Super-resolved live imaging for a wide range of biological applications using Random Illumination Microscopy (RIM)

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KEYWORDS: Superresolution microscopy, live imaging, macromolecule dynamic

We demonstrate the imaging of many sensitive and complex biological situations, from mitosis and cell cycle to 3D cell migration and cell differentiation in thick tissue, with a resolution close to 100nm. Our approach is inspired by Structured illumination Microscopy (SIM) in which the use of inhomogeneous excitation patterns gives access to object information that are inaccessible in widefield microscopy [1].

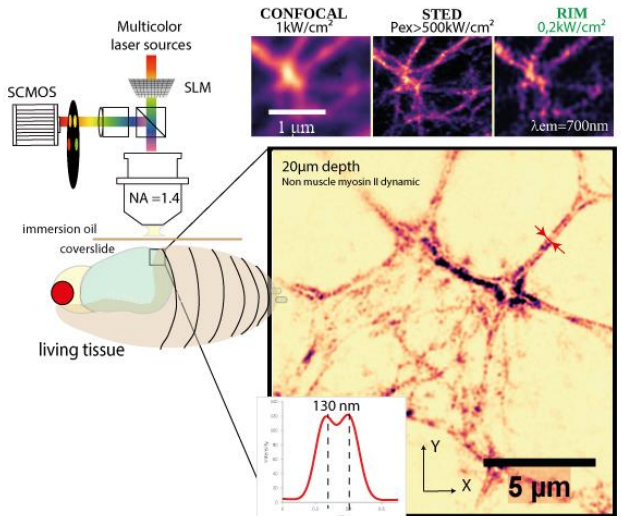

Figure 1. A versatile Random Illumination Microscopy (RIM).

To overcome the major issue, both from the experimental and algorithmic point of view, of the control and knowledge of the excitation patterns, we replace the periodic illumination of classical SIM by unknown speckles [2-3]. We develop a robust reconstruction scheme which necessitates only the knowledge of the statistical behavior of the random speckles. Our inversion approach avoids the widely used sparsity constraint [4] to better respect the dynamic range of the fluorescence density (especially in the case of high surface density of fluorochromes).

As a result, our images are less prone to reconstruction artefacts and appear in agreement with reference STED images. The low toxicity and high temporal resolution of Random Illumination Microscopy enables the imaging of critical biological function like mitosis, cell migration or subcellular force production during a morphogenetic drosophilia pupa and leg. A comparison with other super-resolution computational methods (SOFI, SRRF) and periodic or focused structured illumination microscopy shows that RIM provides a better resolved and more robust estimation of the sample whatever the biological situation.

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Photon count estimation in single-molecule imaging

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KEYWORDS: Imaging, single-molecule, 3D SMLM, aberrations.

A newly developed method was introduced by Franke, Sauer and van de Linde[1] to estimate the axial position of single-molecules. To this end, they compared the detected photon count from a temporal radial-aperture-based intensity (TRABI) estimation to the estimated count from Gaussian point-spread-function (PSF) fitting to the data. Empirically they found this photometric ratio to be around 0.7-0.8 close to focus and decreasing away from it. The ratio of these two estimates is proposed as a measure for defocus. In this work, we provide an explanation for this reported but unexplained discrepancy and quantify practical limitations.

We have investigated photons counts of 45 nm beads using three methods: Gaussian PSF fit, TRABI, and vectorial PSF fit[2]. The vectorial PSF performs the best but it becomes apparent that no method finds the true photon count for an fitting area used in practice, because the microscope PSF has a very long tail. This observation is supported by simulations using the vectorial PSF which for high NA imaging deviates substantially in the tail from the Airy PSF.

We further varied the axial position of the beads and evaluated the photometric ratio between the photon count estimate from Gaussian fitting and TRABI as a function of defocus (**Fig. 1**). We find a photometric ratio of 85% in contrast to the values around 75% in focus reported by Franke et al.[1], which we attribute to aberrations present in their experiment. To assess the influence of aberrations, we experimentally engineered PSFs with small amounts of aberrations[3].

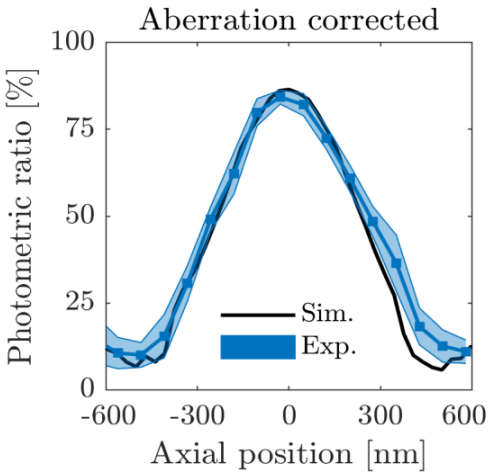

Fig. 1. The photometric ratio (Gaussain fit over TRABI value) over six bead measurements compared to a vectorial simulation as a functions of the axial position.

Here, we will show that the maximum value of the photometric ratio, overall value as a function of defocus strongly depends on the type and magnitude of aberrations. We used amounts of aberrations within diffraction-limited imaging, indicating that these aberration levels are seen in experiments. This is confirmed by inspecting seven microscopes for typical aberrations which results in axial errors on the order of ±50 to ±100 nm using TRABI without the aberration calibration. We therefore conclude that to convert the photometric ratio to a viable accurate, depth map the optical aberrations must be known to a very high degree.

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ADAPTIVE OPTICS FLOOD-ILLUMINATION OPHTHALMOSCOPE WITH STRUCTURED ILLUMINATION CAPABILITIES

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KEYWORDS: Adaptive Optics, Retinal imaging, Structured illumination

Adaptive Optics (AO) provides real-time correction of the wavefront aberrations introduced by the human eye to improve the contrast and resolution in retinal imaging. AO systems have been coupled to scanning laser ophthalmoscope (SLO) and flood-illumination ophthalmoscope (FIO) and have permitted to study small retinal structures such as photoreceptors and capillaries. These two types of instruments each have their advantages and their drawbacks. Whereas AO-SLO with the use of a confocal pinhole [1] obtains optical sectioning, higher image contrast and better 3D resolution than the AO-FIO, the latter provides a better temporal resolution, a larger field of view and non-distorted retinal images.

In this communication we present a custom-made AO-FIO [2] with structured illumination capabilities and its first experimental images. As its counterpart in microscopy [3,4], structured illumination should allow enhancing the lateral and axial resolution, through a tailored processing [5,6]. In our setup, incoherent sinusoidal fringe patterns are produced using a Digital Micro-Mirror Device (DMD). As the illumination pattern is pre-compensated of the ocular wavefront aberrations thanks to the AO, we manage to project high frequency fringe patterns onto the retina (Figure 1).

We present our first experimental retinal images with structured illumination and their preliminary reconstructions.

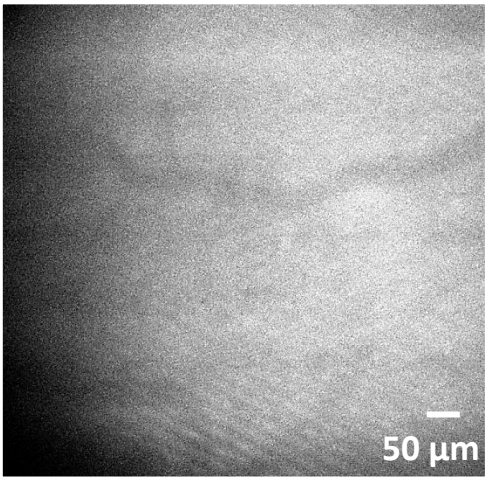

Figure 1. Acquired retinal image of the nerve fiber layer

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[4] M. G. L. Gustafsson, « Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. short communication », *J. Microsc.*, vol. 198, p. 82‑7, juin 2000.

[5] R. Baena-Gallé, L. M. Mugnier, et F. Orieux, « Optical sectioning with Structured Illumination Microscopy for retinal imaging: inverse problem approach », in *26ème Colloque sur le Traitement du Signal et des Images*, 2017.

[6] Y. Lai-Tim et. al., « Novel method for structured illumination in retinal imaging », in this conference.

NOVEL METHOD FOR STRUCTURED ILLUMINATION RETINAL IMAGING

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KEYWORDS: Structured illumination microscopy, retinal imaging

Structured Illumination Microscopy (SIM) is an imaging technique for achieving both super-resolution and optical sectioning in wide-field fluorescence and reflectance microscopy. It consists in illuminating the object with fringe patterns at different orientations and phase shifts. The reconstruction of the object is then performed using all of the acquired images.

In this communication we focus on the application of structured illumination (in particular sinusoidal incoherent illumination) to *in-vivo* retinal imaging. The lateral and axial resolution enhancement potentially brought about by structured illumination could benefit the study of the retinal 3D structure and function in the living eye. The classical SIM approaches [1], [2], which require accurate control of the phase shifts of the sinusoid are not suitable for *in vivo* retinal imaging due to uncontrolled eye movements. Nevertheless, these eye motion results in shifts of the observed retina and can be used as the required diversity of phase shifts for SIM.

A few authors tackled the problem of applying structured illumination to *in vivo* retinal imaging [3], [4]. We propose a novel approach using a Bayesian inverse problem formulation. This will allow us to process all the data jointly in a sound statistical framework so as to minimize noise amplification. It is based on a physical multi-layer model of the retinal image formation process initially proposed by [5], in which we distinguish the in-focus layer we want to reconstruct and the out-of-focus layers. The shifts of the object are estimated by a specific image registration method [6] and the modulation pattern parameters (spatial frequency, orientation, phase and contrast) accurately estimated for each acquired image. Then this permits to jointly process all the data to reconstruct the in-focus layer. We validate the performance of our method on realistic retinal image simulations for different noise levels.

[1] M. A. A. Neil, R. Juškaitis, et T. Wilson, « Method of obtaining optical sectioning by using structured light in a conventional microscope », *Opt. Lett.*, vol. 22, n° 24, p. 1905‑1907, déc. 1997.
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[5] R. Baena-Gallé, L. M. Mugnier, et F. Orieux, « Optical sectioning with Structured Illumination Microscopy for retinal imaging: inverse problem approach », in *26ème Colloque sur le Traitement du Signal et des Images*, 2017.
[6] L. Blanco, L. M. Mugnier, A. M. Bonnefois, et M. Paques, « Registration and restoration of Adaptive-Optics corrected retinal images », in *2014 International Workshop on Computational Intelligence for Multimedia Understanding (IWCIM)*, 2014, p. 1‑5.

Novel Tetrazine Probes for Fluorogenic Protein Labeling

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KEYWORDS: live-cell labeling

Protein labeling with organic fluorophores is a key component for many quantitative approaches in fluorescence microscopy. To ensure target labeling and minimize non-specific background signal bioorthogonal chemical reactions are employed to couple fluorophores to their targets. Essential prerequisites for the labeling reaction thereby are specificity, efficiency, reaction speed and live-cell compatibility. The inverse electron demand Diels-Alder reaction between ring-strained alkenes or alkynes and 1,2,4,5-tetrazines has proven to meet these demands and allow fast and specific protein labeling. Additionally, due to fluorescence quenching by the tetrazine moiety, according tetrazine-fluorophores can show fluorogenic properties – that is a significant increase of fluorescence emission upon reaction with the targeting group thereby showing reduced emission from unbound dye.[1]

With a new synthetic route we created a set of novel tetrazine-substituted dyes composed of fluoresceins, rhodamines and siliconrhodamines covering the visible spectrum from green to deep red emission and we employ the newly synthesized fluorogenic dyes to no-wash labeling in living cells.[2] Recently, we started exploiting the modular nature of the synthetic approach to create dyes for more sophisticated applications like multiplexing and live-cell single-molecule localisation microscopy. Furthermore, we study the kinetic parameters of the click reaction and the concomitant fluorescence enhancement in greater detail. For this purpose we use a bifunctional fluorogenic rhodamine for proximity-induced reaction with otherwise unreactive dienophiles.[3] Additionally, we started performing single-molecule studies to shed light on the molecular processes involved in the labeling reaction and to stimulate the development of fast-reacting fluorogenic labels with a pronounced fluorescence enhancement and minimal non-specific background.

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QUANTITATIVE 3D GEOMETRY FOR LARGE SCALE CONNECTOMICS DATA

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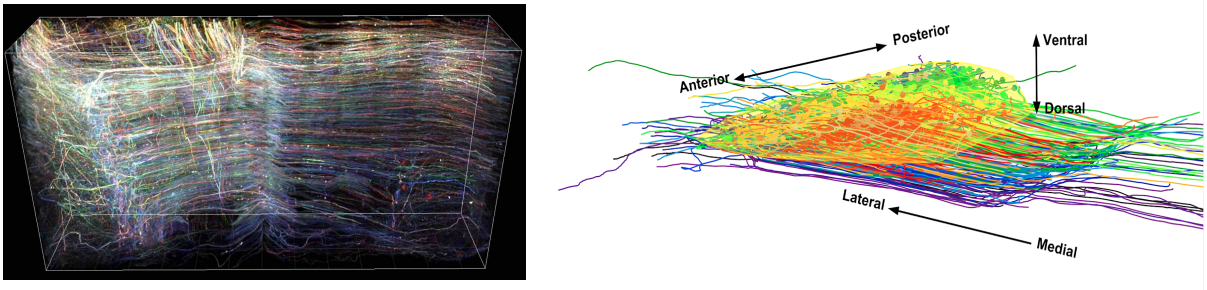
KEYWORDS: 3D data analysis, Medial Nucleus of Trapezoid Body, Connectome.

Recent developments in large-scale microscopy and fluorescent markers, combined with image analysis techniques allow us to extract quantitative data from large high resolution volumes. Such measurements, from the positions and shapes of neurons or glial cells to axon traces in different experimental or biological conditions, will be invaluable in neuroscience studies. However, we need to imagine and implement strategies in order to mine information and knowledge from such ensembles of measures. Here we present the development of an ensemble of quantitative 3D geometry methods and their application to the analysis of connectivity in the mouse Medial Nucleus of the Trapezoid Body (MNTB, part of the auditory pathway), based on a large scale, high resolution 3D image encompassing one thousand individual axon traces. Those methods from several fields including computational geometry, scale space methods and spatial statistics, acting on geometric data of all dimensionality -points, lines and surfaces- could be considered references to perform the quantitative analysis of 3D geometric data. The MNTB 3D shape, the large scale topography of incoming axons, or the axon pathway toward its target once it enters the MNTB were studied and provide insight into the development of this neural circuit.



Left: large scale, high resolution, multicolor microscopy of a thick Brainbow mouse brainstem sample. Right: the annotated axon traces and the MNTB outline in yellow shape.

ROBUST ADAPTIVE OPTICS FOR STRUCTURED ILLUMINATION MICROSCOPY FLUORESCENCE IMAGING

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KEYWORDS: Structured illumination imaging, sensorless adaptive optics, IsoSense.

Here we present 3D structured illumination imaging [1] fluorescence microscopy for robust live cell imaging enabled by adaptive optics (AO) aberration correction.
The set-up relies on both sensor-based interferometric wavefront sensing for deformable mirror control and on image quality based sensorless adaptive optics [2] for non-common path and sample aberration correction.

Artefact reduction and robust imaging within live cells is enabled through sensorless adaptive optics with the "IsoSense" illumination strategy. IsoSense solves a common limitation of sensorless AO where image based quality metrics lead to sub-optimal correction due to inadequate sampling of the optical transfer function caused by either preferential orientation of object structures, lack of sharp features, or poor signal-to noise ratio in the image. Also, we enable precise mirror control and fast deformable mirror flattening, by employing an interferometric wavefront sensor prior to imaging.

We will demonstrate the performance of our method in the SIM set-up for imaging live *Drosophila* macrophage cells, where our method effectively improved the accuracy of AO correction.

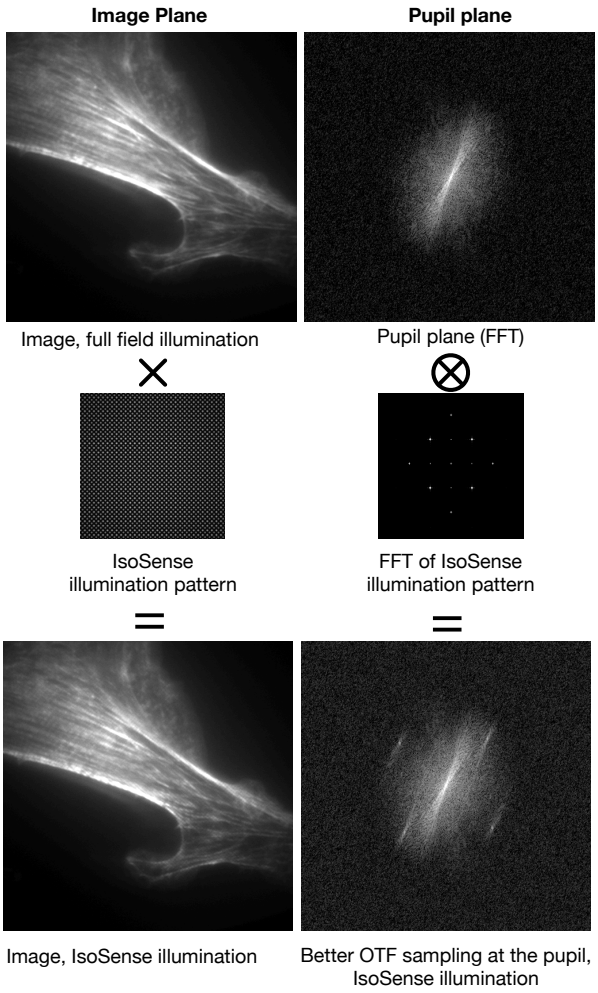

Figure 1. Sample-independent sensorless adaptive optics is enabled through isotropic sampling of the optical transfer function enabled by structured illumination

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DESIGN OF AN "ORTHOGONALITY-BREAKING" POLARIMETRIC CONFOCAL MICROSCOPE TO STUDY INTRACELLULAR ARCHITECTURE DYNAMICS

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KEYWORDS: Methods development - Optical methods development, multimodal imaging, intracellular dynamics.

Polarimetric imaging has been used in the past to characterize biological tissues and discriminate between healthy and cancerous samples [1]. However, due to the complexity of the extracellular matrix architecture, it is often difficult to finely interpret the polarimetric contrasts obtained as many structures within the tissues show anisotropic behavior that can affect the polarization of the incident light [2]. An impact on polarimetric signature has already been reported for some intracellular structures, such as the cytoskeleton (actin filaments and microtubules network) [3], however, the chromatin [4] or even the mitotic spindle [5] are much less documented.

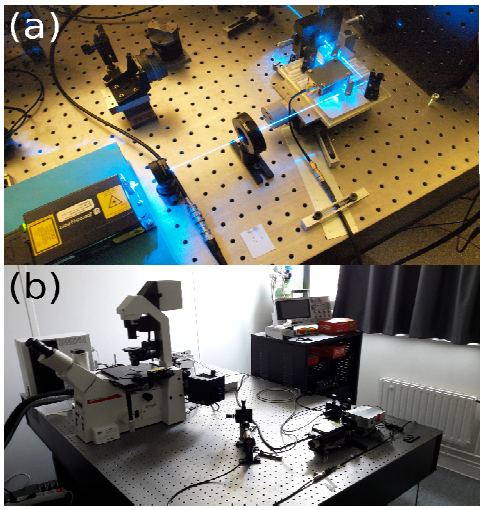

Figure 1. (a) Dual frequency dual polarization 488 nm source used in orthogonality breaking polarimetric sensing. (b) View of the polarimetric microscope setup settled in the BIOSIT-MRic platform in Rennes.

In order to progress in this way, we report the development of a polarimetric confocal microscope based on the "orthogonality breaking" approach, which relies on the original illumination of samples by a dual-frequency dual-polarization laser light beam (Fig. 1(a)), and the detection of polarimetric signature through a single measurement involving demodulation of light at radiofrequencies (80 MHz here) [6,7]. A confocal Leica SP2 setup (Fig. 1(b)) has been modified to couple this polarimetric imaging modality, in transmission, at 488 nm, with standard epifluorescence imaging, requiring careful synchronization between the Leica microscope laser scanning head with the homemade demodulation/detection circuit. First "orthogonality-breaking" polarimetric images of living cells are currently being acquired with this system settled in the BIOSIT-MRic facility in Rennes.

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QUANTIFYING PROTEIN OLIGOMERIZATION IN LIVING CELLS: A SYSTEMATIC COMPARISON OF FLUORESCENT PROTEINS AND APPLICATION TO DEVELOPMENTAL CELL-CELL FUSION

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KEYWORDS: Fluorescence correlation spectroscopy, Number&Brightness, Fluorescent proteins, Protein oligomerization, Protein counting, Cell-cell fusion

Fluorescence fluctuation spectroscopy has become a popular toolbox for non-disruptive studies of molecular interactions and dynamics in living cells. The quantification of e.g. protein oligomerization and absolute concentrations in the native cellular environment is highly relevant for a detailed understanding of complex signaling pathways and biochemical reaction networks. A parameter of particular relevance in this context is the molecular brightness, which serves as a direct measure of oligomerization and can be easily extracted from temporal or spatial fluorescence fluctuations. However, fluorescent proteins (FPs) typically used in such studies suffer from complex photophysical transitions and limited maturation, potentially inducing non-fluorescent states, which strongly affect molecular brightness measurements. Although these processes have been occasionally reported, a comprehensive study addressing this issue is missing. Here, we investigate the suitability of commonly used FPs (i.e. mEGFP, mEYFP and mCherry), as well as novel red FPs (i.e. mCherry2, mRuby3, mCardinal, mScarlet and mScarlet-I) for the quantification of oligomerization based on the molecular brightness, as obtained by Fluorescence Correlation Spectroscopy (FCS) and Number&Brightness (N&B) measurements in living cells [1]. For all FPs, we measured a lower than expected brightness of FP homo-dimers, allowing us to estimate, for each fluorescent label, the probability of fluorescence emission in a simple two-state model. By analyzing higher FP homo-oligomers and the Influenza A virus Hemagglutinin (HA) protein, we show that the oligomeric state of protein complexes can only be accurately quantified if this probability is taken into account. Further, we provide strong evidence that mCherry2, an mCherry variant, possesses a superior apparent fluorescence probability, presumably due to its fast maturation. We conclude that this property leads to an improved quantification in fluorescence cross-correlation spectroscopy measurements and propose to use mEGFP and mCherry2 as the novel standard pair for studying biomolecular hetero-interactions. Finally, we apply brightness and cross-correlation analysis to quantify protein-protein interactions involved in eukaryotic cell-cell fusion, a key process in developmental biology [2].

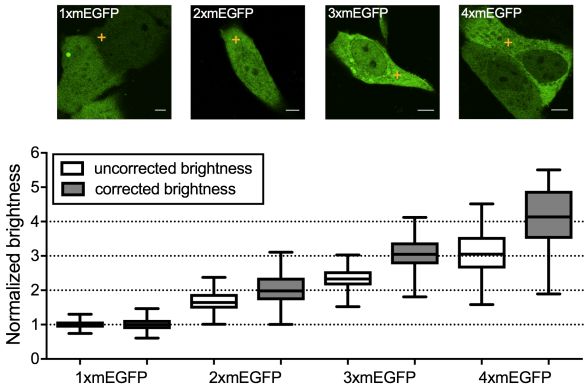

Figure 1. FCS brightness analysis of mEGFP homo-oligomers in living A549 cells.

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Structured Illumination – Localization Microscopy

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KEYWORDS: Structured Illumination Microscopy, Localization Microscopy

Visualization of cellular dynamics with sub-diffraction molecular resolution demands for advanced fluorescence imaging modalities that combine high resolution, sensitivity and speed with minimal photobleaching and phototoxicity for long-term cell observation. Super-resolution microscopy methods have pushed the diffraction limit of conventional imaging approaches (~250nm) by an order of magnitude down to the nanometer range. However, SR methods often compromise cell viability and long-term imaging, thereby limiting the application of SR imaging to fixed samples.
Structured Illumination Microscopy (SIM) has been established as an alternative minimal-invasive superresolution live-cell imaging approach. We developed an integrated SIM and single molecule localization microscopy (SMLM) platform for fast live-cell imaging of dense cellular structures such as actin cytoskeletal filaments in parallel with molecular dynamics at millisecond temporal resolution (<10ms). We further present SIMPLE – a SIM-based Point Localization Estimator – with superior localization performance and minimal photon budget compared to standard SMLM approaches based on ultra-fast beam shaping via digital micromirrors (DMDs). I will discuss the localization precision of SIMPLE based on computer simulations and experimental data. Finally, I will present potential applications of SIMPLE for studying the intimate crosstalk between the actin cytoskeleton and plasma membrane signaling dynamics in non-motile and migratory cells using dual color SIM combined with SIMPLE for long-term superresolution imaging of cellular structures and molecular dynamics.

A DYNAMIC RECONSTRUCTION OF THE ENDOCYTIC MACHINERY IN YEAST FROM STATIC SUPERRESOLUTION IMAGES

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KEYWORDS: Dynamic reconstruction, endocytosis, super-resolution, SMLM

The nanoscopic resolution of single molecule localization microscopy (SMLM) imaging enables understandings of macromolecular structures, but fixed samples are favourable to achieve optimal spatial resolution. As a result, temporal information is lost. In this study, we present an approach to extract dynamic information from static super-resolution data to reconstruct the architecture dynamics of a macromolecular machine. We demonstrate the approach by applying it to reconstructing clathrin-mediated endocytosis (CME) in budding yeast, extending a recent study on the radial structure of the CME machinery [1]. By selecting suitable protein pairs as reference structures, our framework automatically segments cells and endocytic sites, performs quality controls, analyses spatial descriptors of individual sites, and aligns the sites in space and sorts in time. By employing dual-colour imaging, we will be able to spatially and temporally map endocytic proteins to the reference structure. Our goal is to reconstruct a quantitative nanoscopic model involving more than 20 endocytic proteins from thousands of superresolution snapshot. Our study will expand the applicability of SMLM to dynamic and rapid but yet regular biological processes.

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HIGH-RESOLUTION 2D+t IMAGING OF CALCIUM SIGNALING MICRODOMAINS IN CARDIAC MYOCYTES

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KEYWORDS: cardiac myocyte, calcium, microdomain signalling, phosphorylation.

The heart pump function relies on the contraction of over 1 million cardiac myocytes that receive a timely electrical depolarization. The electrical stimulation opens voltage-gated calcium channels, which trigger larger calcium release from the sarcoplasmic reticulum (SR). High cytosolic calcium concentrations activate the contractile machinery and yield cell contraction. Ion exchangers and SR-ATPase clear cytosolic calcium to bring back to relaxation and allow the next contraction to occur at a rate higher than 1 Hz. Deep membrane invaginations (T-tubules) in cardiomyocytes ensure that the calcium store associates with the sarcomere contractile units.

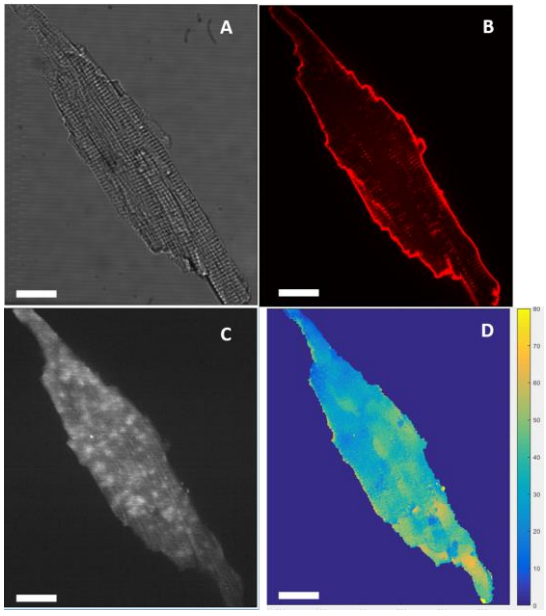

Figure 1: Dual imaging of calcium handeling and utrastructure in pig cardiac myocyte. (A) Brightfield image. (B) Live cell membrane stainging with an Alexa-647 conjugated WGA lectin. (C) Calcium imaging with Cal-520 AM, exposure time = 8 ms, electrical field stimulation, time of image corresponds to the initiation of calcium transient. (D) Example parametric map of time to peak. Scale bar = 20 µm.

Here we optimized a multi-beam array confocal microscope in order to image signaling microdomains at high spatial and temporal resolution (125 frames per second) in contracting cardiac myocytes. We fitted pixel-by-pixel the time evolution of calcium transients in intact myocytes and extracted key functional parameters. We registered calcium images to membrane staining thanks to a descriptor-based algorithm. When correlating structural and functional parameters, we showed that dense membrane invaginations facilitate local calcium handling. Moreover, we reveal the setting of local phosphorylation gradients and follow their evolution over time. Spatio-temporal patterns of phosphorylation seem to sustain synchronized signaling over the large cell volume.

ROBUST DISCRIMINATION OF ANTIGEN STAIN IN HISTOPATHOLOGY

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KEYWORDS: Immunohistochemical staining, Pathology scoring, Colorectal cancer

The prevalence of cancer and its impact calls for the creation of new therapies targeted to each individual patient that can reduce their convalescence time and suffering. Nevertheless, this personalized medicine approach is complicated and needs for enormous amounts of data to be analyzed and processed in order to reach significant conclusions. The recent progress of computational biology tools and hardware has the potential to facilitate the analysis of pathological images, thus promoting the creation of new, more effective cancer treatments. However, most of these developments have been thought for hematoxylin and eosin samples, while we still lack the tools for accurate analysis of immunohistochemical staining.

In this report we describe the development of a working pipeline based on a robust computational method for the segmentation and scoring of immunohistochemical samples capable of analyzing tumor biopsies fast and effectively. Two different automatic segmentation methods based on the color space of histopathology images were tested and compared with the conventional color deconvolution algorithm. These methods arise as modifications of Jacob S.Sarneki's non-linear tissue-component discrimination method [1] and are based on the bisection of the red-blue color joint histogram of the images through either their diagonal or through k-means segmentation. The methods were tested on twenty-two immunohistochemical biopsies of locally advanced colorectal cancer of three different patients stained with eight different antigens. The accuracy of the segmentations was assessed by comparison with a ground truth created from the same samples, both methods rendering over 0.9 precision score. The best method (i.e., diagonal-based) was then used to compute a biological scoring of each biopsy, which was compared to a previous visual evaluation by Hospital General Universitario Gregorio Marañón pathologists, thus assessing its validity.

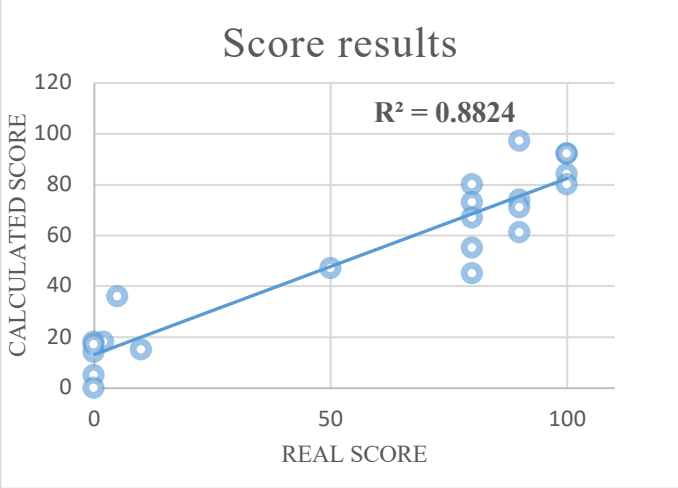

Figure 1: Comparison of the scoring obtained with the proposed algorithm and the original score by pathologists.

Our results *(Figure 1*) show that our proposed pipeline could effectively be used for the automatic scoring of immunohistochemical biopsies, improving the accuracy of conventional segmentation algorithms.

Acknowledgments: this work was partially funded by projects TEC2013-48552-C2-1-R, TEC2015-73064-EXP and TEC2016-78052-R from the Spanish Ministry of Economy and Competitiveness and a 2017 Leonardo Grant for Researchers and Cultural Creators, BBVA Foundation.

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Simulator of Benchmarking Image Datasets for Time-Lapse Lightsheet Microscopy

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KEYWORDS: image processing, segmentation, tracking, benchmarking, simulation

In fluorescence microscopy image analysis, cell tracking and segmentation algorithms are indispensable tools to, for instance, reconstruct lineages or for time-resolved analysis of cell characteristics or events. Although there are such algorithms in everyday use, most of them are not properly validated and their accuracy limits are not well understood. Provided testing data together with expected results (so called ground-truth annotations, GT) and suitable metrics would exist, much of the questions above could be addressed.

Lightsheet microscopy images come obviously without GT. Acquired datasets are displaying often time-lapse embryonic development in high-resolution, and easily reach 1 TB per one experiment. The number of displayed cells can be in thousands per single frame. In this setting, it is extremely difficult to manually annotate real datasets to obtain GT.

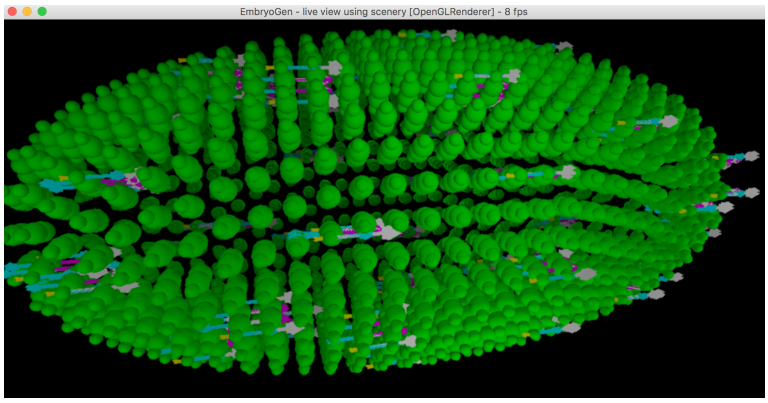

Figure 1. Example screen shot during a simulation of embryo: nuclei (as unions of 4 spheres in green) and various forces.

Here, I present the current status of my work on a generic open-source simulator of many-cells biological systems. In particular, the simulator produces GT annotated, time-lapse image sequences with artificial yet realistically looking and developing populations of nuclei-stained (simulation) cells. The cell development includes division and mutual interaction of cells. The simulator is based on my previous works: small nuclei population of dividing cells [1] that was also used during the Cell Tracking Challenge [2] and force based, see Fig. 1, cell interaction [3]. The created GT will be useful for developing, tuning and benchmarking segmentation, tracking and multi-view registration algorithms. Free benchmarking datasets are planned to be published.

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SPATIAL ANALYSIS OF PROTEINS OR LIPIDS DISTRIBUTIONS ON THE SURFACE OF SPHERICAL BEADS

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KEYWORDS: spatial organisation of membranes, protein-membrane interactions, in vitro reconstitution, image analysis

Lipids and proteins in membranes are known to compartmentalize and organize in spatial domains or patterns that often are prerequisite to the molecular function [1]. Elucidating the mechanisms how these spatial heterogeneities are generated and maintained is central to understanding the organization of membranes, both of cells and of intracellular compartments. *In vitro* reconstitution of membranes and membrane-associated proteins enables detailed quantification of the underlying molecular mechanisms of organization, which would not be feasible *in vivo*. Many *in vitro* reconstitution experiments make use of flat lipid bilayers or Giant Unilamellar Vesicles (GUV), however lipid membrane coated silica beads instead allow for higher experimental throughput (large numbers of beads can be imaged at the same time and, as a consequence, many different conditions can be tested in parallel) and reconstitute the correct surface topology (cells and organelles are usually homeomorphic to a sphere, whereas flat reconstituted flat bilayers require boundary conditions). Besides that, lipid coated beads allow for a complex and easily variable lipid composition as the difficulty of GUV formation increases dramatically with complexity of the lipid composition.

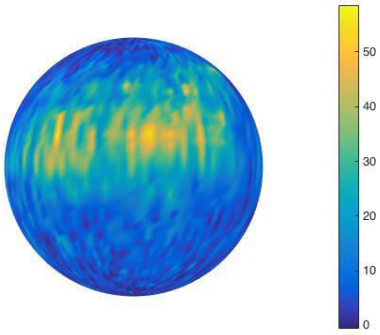

Figure 1. Example of reconstructed intensities of Rab5 protein on the surface of a unit sphere

To support high-throughput analysis, we have developed a quantitative image-analysis pipeline to characterize and compare the spatial organization of the surface of lipid-covered beads imaged by fluorescence microscopy. Our pipeline corrects for imaging artifacts and enables spectral analysis of patterns on the sphere. It consists of the following steps: (a) sphere fitting into the geometry of the bead to define the center of the bead and its radius, correcting for imaging blur; (b) reconstruction of the bead surface as a narrow band of particles, moment-conserving interpolation of the intensity values from the pixels to the particles, and radial maximum-intensity projection of the interpolated intensity values on the particles onto the exact surface of the sphere; (c) decomposition of the intensity signal on the surface into spherical harmonics series for filtering and statistical analysis; (d) construction of a 2D map, and 3D spherical reconstruction (Figure 1) of the filtered signal for visualization.

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1 NANOMETER PRECISION BY BAYESIAN GROUPING OF LOCALIZATIONS

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KEYWORDS: Super-resolution, Bayesian Inference

Single-molecule-localization-based super-resolution methods such as DNA-PAINT and (d)STORM result in multiple observed localizations from each dye or binding site that are not *a priori* assigned to the specific dyes or binding sites. We describe a Bayesian method of grouping and combining localizations from multiple blinking/binding events that can improve localization precision to better than 1 nm. The known statistical distribution of the number of binding/blinking events per dye/docking strand along with the localization precision of each localization event are used to estimate the true number and location of emitters in closely spaced clusters. The method uses Reversible Jump Markov Chain Monte Carlo to find the uncertainty of both the number of dyes/docking strands and their locations. The results can be returned from the most likely model or a posterior distribution that is a weighted average over all models. We show results from DNA origami structures and membrane proteins.

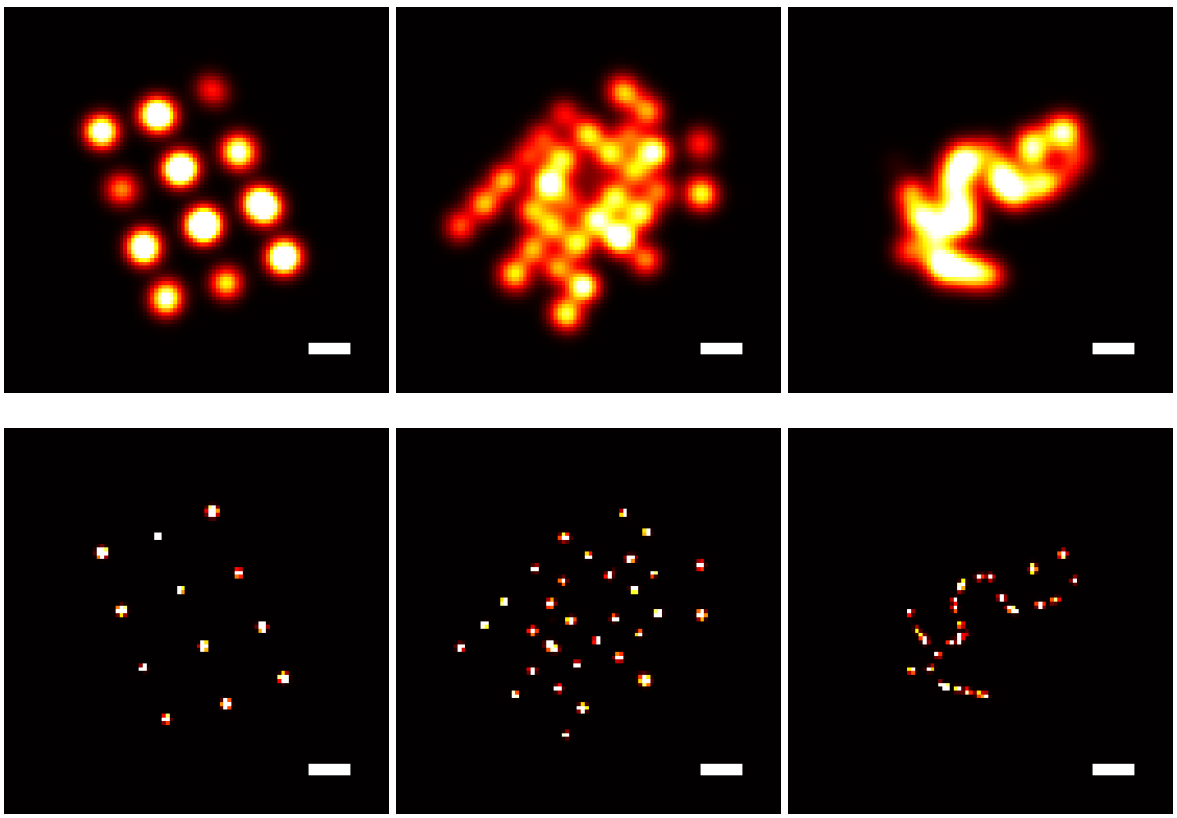

Figure 1. Example of Bayesian Grouping on DNA-origami Data. Top: Results of standard single emitter reconstruction. Bottom: Posterior probability of emitter locations. The 3x4 grids (left) are used to estimate the number of binding event per docking strand. Scale bar is 10 nm.

QuPath – a tool for quality assurance, enhanced user training, and improved reproducibility of image analysis in histopathology research

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KEYWORDS: QuPath, histopathology, histology, whole slide scanning, digital pathology, machine learning, 3D printing

QuPath is a multi-platform open-source whole slide image analysis software [1] offering excellent performance, reproducibility [2], and great flexibility. While clinical labs and many research histology facilities have been improving rigor and reproducibility in histopathology over the years, there are many smaller units that face challenges in implementing the best standards due to cost and complexity.

We will show how whole slide image analysis methods developed in QuPath can be used to aid in quality assurance by highlighting tissue imperfections, slide scanner errors, and assessment of staining quality and variability. User training can be enhanced by the use of machine learning to classify tissue elements. We will share how powerful but easy scripting allows for a more robust, rigorous and reproducible analysis that can be offered to histology lab customers for almost no additional cost. To this end, we have developed a set of open-source Groovy scripts that help less experienced users to better understand the images and get preliminary data that can be further refined by machine learning. More advanced users can quickly analyze substantial number of slides and extract the data for more sophisticated analysis from brightfield and fluorescence scans.

Additionally, we will discuss inexpensive additions to histology workflow that can help smaller labs with data management, and digitization such as the use of office label makers for slide barcoding. We will also explore the potential for 3D printed aids in specimen preparation.

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Computational pipeline for registration of 3D images of fixed specimens to 4D time-lapse recordings of developing biological systems

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KEYWORDS: Lineage Tracing, Registration, Fiji, Platynereis, Cell-fate

The development of an animal from an egg involves increase in cell number and cell fate specification accompanied by dynamic morphogenetic events. The generation of developmental cell lineages by cell tracing can help us understand when and where cell fates are specified during development and which cellular mechanisms govern morphogenesis on the tissue and organismal levels. In order to obtain gene expression atlases of development, gene expression data for key developmental genes need to be visualized in the context of the cell lineages. We are implementing a computational tool to perform alignment and registration between time-lapse recordings of developing biological systems and the static images of fixed specimen stained for gene expression marker genes. The pipeline will be demonstrated for the spiralian ragworm *Platynereis dumerilii*. This system offers the advantage of having a fixed cell lineage (similar to the widely used *C. elegans* model system), a transparent embryo developing into a larva with relatively few cells (approx. 700 cells). Validated in toto live imaging datasets of the early development in this system, together with the large body of 3D images of fixed specimen stained for gene expression of key developmental transcription factors, make this an ideal use case for the proposed registration pipeline. We will present the current state of the computational tool that is being developed in the Fiji platform and aims for general applicability in the developmental gene expression studies using a combination of live imaging and fixed 3D datasets.

Protein counting in T-cell receptor microclusters

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KEYWORDS: absolute protein counting, photon antibunching, T-cell receptor cluster

T-cell receptor clusters play a detrimental role in transmitting the innate immune response, e.g. on viral infections. However, the human immunodeficiency virus type 1 (HIV-1) manipulates T-cell receptor (TCR) clusters in infected cells in order to suppress immune response and support viral proliferation[1]. There is indication that the viral protein Nef reorganizes the TCR-clusters and changes their constitution. Thus, quantitative data on constitution and stoichiometry of the TCR-clusters can help understanding HIV1 pathogenesis.
A robust method for counting the number of fluorescently labelled proteins in cells is Counting by Photon Statistics (CoPS)[2, 3]. This single-molecule fluorescence spectroscopy technique exploits the photon antibunching effect, i.e. a dye as a quantum system may only emit one photon per excitation cycle. Multiple detection events, which are recorded by four single photon detectors of a confocal microscope setup, enable estimation of the protein copy number in the studied complex.
In our studies we currently analyze the time-resolved progress of CD3-antibody-induced T-cell activation. We investigate the number of LAT and SLP-76 proteins at different time points of the dynamic association of TCR clusters. Additionally we explore the impact of the virulence factor *Nef* on the cluster stochiometry to gain new insights in the working mechanism of this protein.

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A MECHANO-IMAGING METHOD TO QUANTIFY INTRACELLULAR BIOPHYSICS

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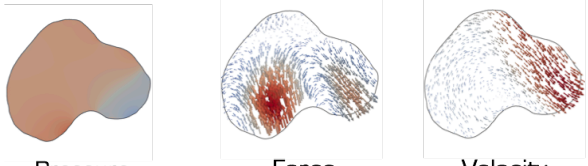
KEYWORDS: flow dynamics, forces, pressure, quantitative bioimaging, migrating cell.

Cellular movement in a tissue environment is generally triggered and/or guided by environmental stimuli (chemokine, nutrient or growth factor concentration gradient) and results in changes in cell morphology, which follow forces on the cell and within the cell.

Our project aims at developing an image based framework to model cell migration in complex 3D environments and to correlate cell morpho-dynamics features with intracellular events. To this end we have developed BioFlow, a method to extract intracellular biophysical quantities (flow dynamics, forces and pressure) using microscopy-imaging data (figure 1) [1]. We apply our method to analyse *Entamoeba histolytica* motility, a model for bleb-based amoeboid migration. Fluorescent amoebas were imaged with a spinning disk confocal microscope.

Figure 1. Biophysics quantities measured with Bioflow at one time point of amoeboid migration (cell migration is rightwards)



The spatiotemporal profile of the intracellular pressure during protrusion indicates that the pressure gradient precedes an increase in the cytoplasmic velocity. This supports the reported role of myosin II: an increase in pressure due to the contraction of the actomyosin cortex and, after the pressure stabilizes, a retraction of the cell rear accompanied by an increase in force. The pressure stabilization as the bleb fills is relatively fast and therefore suggests a process that is not solely based on actin re-polymerization. Furthermore, by studying the time between protrusions, we extend a period of 7.9s known only for confined *E. histolytica* to freely moving amoeba and discover a novel one of 4.6s that reflects the cytoplasmic streaming towards the bleb and could describe the actin cortex polymerization time at the edge of the protrusion. We showed that the pressure values differ across blebs, without disturbing the periodicity of bleb formation, indicating that pressure alone does not suffice to regulate bleb formation and stabilization. This is in agreement with recent evidence that bleb formation and regulation involves additional mechanisms such as Rho-GTPase activity. The importance of actin dynamics on intracellular flow, pressure and forces was showed by addition of Latranculin B leading to a stable decrease in the intracellular pressure gradient, velocity and force fields.

We expect to expand the reach of BioFlow by studying cell migration in 3D environments to describe the relationship between, shape and cytoskeleton dynamics and intracellular mechanics.

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A pyramidal PDE-constrained approach to quasi-static optical elastography

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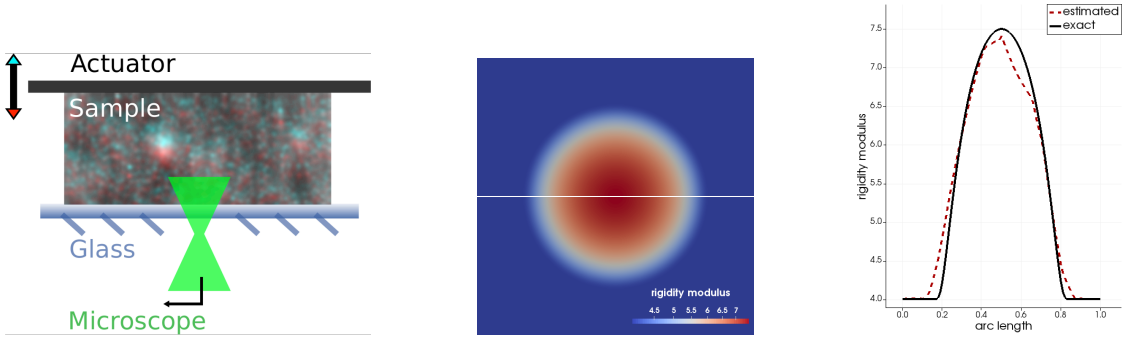
KEYWORDS: elastography, inverse problem, elasticity, optical flow, PDE-constrained optimisation.

Changes in the mechanical properties of organic materials are relevant to biological processes at many scales. For example, many diseases induce variations in the stiffness of the surrounding tissue that can be monitored and exploited towards more accurate diagnoses. At a smaller scale, the elasticity of the matrix where cells are embedded has been shown to have a direct influence on their differentiation and behavior. However, current elastographic methods, which allow measuring the spatial map of stiffness within a given material, do not yet cover all spatial scales and require complex and expensive experimental set-ups.

We present an image processing method that is general in scale and that can use any image pairs of a quasi-static compression where the pixel intensity is the relevant quantity. This methodology could be implemented in biology laboratories by using any available confocal fluorescent microscope, regardless of its resolution, and a piezoelectric actuator (Figure 1). In this case, the resolution of the map depends on the resolution of the image and could thus yield smaller scale measurements with the advent of super-resolution.



Figures 1, 2, 3. 1) Compression of a sample with an actuator. 2) Exact rigidity map of a sample. 3) Comparison between exact and recovered rigidity on the cross-section in Figure 2.

The technique consists in the minimisation of a customized functional (based on optical flow) that describes the movement of the pixel intensity within the images taking in account first-order out-of-plane motion, constrained to a set of elastic partial differential equations that describe the relation between the movement of the material and its elasticity. This variational framework allows the addition of several pairs of images taken from different compressions of the same material. This helps complement missing information, which is a common issue far from the compression boundaries. The algorithm is solved automatically via the finite element and the adjoint methods within a pyramidal multi-resolution scheme that helps detect movement at different scales. We present preliminary results with a linearly elastic model. Testing the algorithm on *in silico* images shows that this method is able to extract reliable maps of the rigidity modulus under experimental noise and deformations (Figures 2 & 3).

Accordion-like collagen fibrils suggested by P-SHG image modeling : implication in liver fibrosis

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ABSTRACT

Second-order non-linear optical anisotropy parameter $\rho = \chi_{33} / \chi_{31}$ is calculated for collagen-rich tissues considering both a single dominant molecular hyperpolarizability tensor element $\beta_{333} = \beta$ at single helix level and a priori known submicrometric triple helical organization of collagen molecules. Modeling is further improved by taking account of Poisson photonic shot noise of the detection system and simple supra-molecular fibrillar arrangements in order to accurately simulate the dispersion of ρ values in collagen-rich tissues such as tendon, skin and liver vessels. From combined P-SHG experiments and modeling, we next correlate experimental and theoretical statistical distributions of ρ. Our results highlight that the dispersion of experimental ρ values is mainly due to (i) Poisson photonic shot noise in tendon and skin, which proves to have a preponderant effect in P-SHG experiments (ii) variance of supercoil angles of accordion-like fibrils in vessels that is further reduced during the development of liver fibrosis therefore contributing to the rigidity of the tissue. These results open new avenue for future modeling correlating the dispersion of ρ values in P-SHG experiments and the fibrillar architecture as well as the mechanical stiffness of patho-physiological extracellular matrices in collagen tissues.

Abstract 143

Single Molecule at the age of Big Data: Probabilistic Pipeline and Unsupervised Learning

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Twenty years after its inception, the field of Single Molecule (SM) biology undergoes a transition towards a data-generating science [1-3]. At the nanometer scale, the dynamics of individual biomolecules is inherently controlled by random processes, due to thermal noise and stochastic molecular interactions. By accessing the distribution of molecular properties, rather than simply their average value, the great advantage of SM measurements is thus to identify static and dynamic heterogeneities, and rare behaviours.

In recent years, these experimental limits have been progressively alleviated with the advent of new, game-changing methods. Thanks to photoactivatable probes (protein-based or synthetic dyes), millions of individual trajectories can now be recorded in live cells in a few minutes. PALM/STORM images can be reliably acquired over many hours (or even days), yielding up to hundreds of millions of individual localizations.

As SM experiments enter the age of « big data », the development of a proper and unifying statistical framework becomes more necessary than ever. « big data » approaches certainly open up new research venues for our understanding of biological processes, as they enable the inference of molecular dynamics. Yet they also come with a price. Often, adding more data brings both more information and more variability and noise. Specific tools are required to handle the complex structure of results associated to large datasets and to account for the sources of experimental and systemic variability.

Here, we show a global probabilistic pipeline: TRamWAy [4-7] that automatically analyse single molecule experiments from images to random walk analysis. TRamWAy relies on deep neural network to deconvolve single molecule images, Belief propagation coupled to ghost graph summing to perform probabilistic assignments between images, and both supervised and unsupervised Bayesian analysis to extract information from random walks.

We demonstrate the approach on two datasets: Glycine receptors in synapses and GAG dynamics during the formation of the Virion in HIV-1 [4]. We demonstrate two ways of applying the probabilistic pipeline TRamWAy. In the first we use model-based learning with automated results extraction and statistics. In the second we show that unsupervised learning with structured inference allows full analysis without assigning a model to the biomolecules dynamics.

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A Human-in-the-Loop Approach To Image Analysis

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Today we are able to volumetrically image living systems from the scale of individual molecules to that of entire human beings. A major challenge for researchers and doctors alike is giving sense to complex, non-standardized and unstructured data that scientific images typically embody. A solution to this problem comes from placing users in a context that combines virtual reality, physical interaction and artificial intelligence.

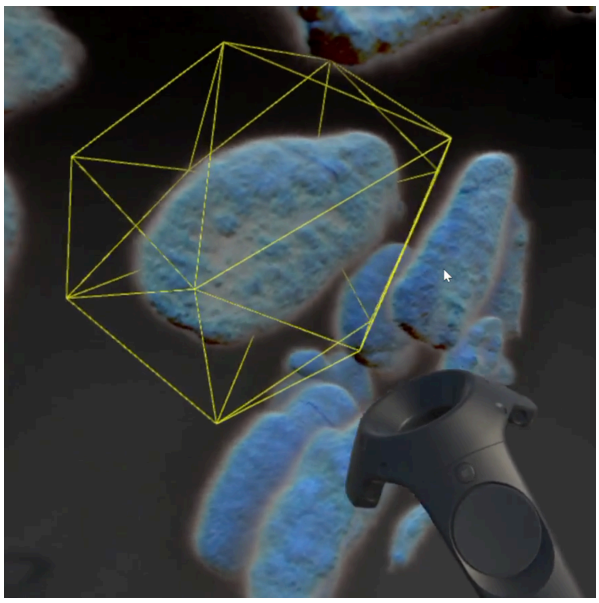

Figure 1. An example of 3D region selection using virtual reality

We present a *human-in-the-loop* means of performing image analysis based on a software platform entitled DIVA, developed in collaboration between the Institut Pasteur and Institut Curie. With this approach, the user and the machine (i.e. learning algorithm) work simultaneously to achieve a specific image treatment task, be it 3D segmentation, conformal projections or probabilistic inference.

We present the use of our platform with neurobiologists at the Institut Pasteur as well as with surgeons at the Institut Curie hospital. Moreover, we demonstrate that the use of rich and freely-interactive representations of imaging data gives way to new forms of analyses and enhances existing image processing protocols.

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NUCLEAR PORES AS UNIVERSAL REFERENCE STANDARDS FOR QUANTITATIVE MICROSCOPY

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KEYWORDS: Super-resolution microscopy, quality control, resolution standard, labelling efficiency, protein counting

Recent advances in superresolution microscopy now allow us to address structural questions in cell biology with optical methods. A quantitative interpretation however is often limited by sub-optimal performance and calibration of the microscope, undetermined performance of the fluorescence label and imaging conditions, unknown labeling efficiencies and systematic errors in counting protein numbers.
Here we show that the use of reference standards can overcome these limitations and greatly improve quantitative microscopy. To this end we exploit the precise 3D arrangement and stoichiometry of proteins in the nuclear pore complex.
We present a set of genome edited cell lines in which we endogenously labeled the nucleoporin Nup96 with eGFP, SNAP- or HALO-Tag or the photoconvertible fluorescent protein mMaple. We demonstrate their use as a) simple and robust resolution standards for calibration and quality control, b) accurate assays to quantify absolute labeling efficiencies in superresolution microscopy and c) precise counting reference standards for absolute stoichiometry measurements.
As a resource shared with the community, these cell lines will enable many groups to assess the quality of their microscopes and labels and to perform quantitative, absolute measurements.

A tutorial in spatial statistics for microscopy data analysis

Ed Cohen, Imperial College London

Spatial statistics plays a crucial role in the analysis of microscopy data and our understanding of the biological structures and mechanisms being imaged. This tutorial is an introduction to some of the fundamental theory and methodology for the modelling and analysis of spatial data of the type typically encountered in fluorescence microscopy.

FUNCTIONAL INSIGHTS INTO THE INTRINSICALLY DISORDERED PROTEIN α-SYNUCLEIN

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KEYWORDS: Disordered protein function, FRET, Photobleaching, Membrane, Synuclein.

Alpha-synuclein (αS), an intrinsically disordered protein, is thought to be the major player in synucleinopathies such as Parkinson's disease. The exact function of αS is not known. In vitro experiments show that αS is disordered in buffer and that in the presence of model lipid membranes it can adopt alpha-helical structure. In cells αS has been shown to colocalize with membranes. Taking into account the membrane bound structure found in vitro, one would expect that αS adopts alpha-helical structure on cellular lipid bilayers. In contrast, NMR and EPR studies indicate that αS remains disordered inside the cell [1,2].

We address this controversy. We showed that cellular αS is associated with membranes inside neuron like cells. We used ultrasensitive microscopy and photobleaching to quantify the number of αS-GFP on vesicles. We found a number of 70 αS-GFP/vesicle which is an extraordinary high number [3]. To identify if this membrane bound αS adopts α-helical structures, we microinjected cells with small amounts of αS labelled with a FRET pair sensitive to the membrane bound conformation (Fig. 1 top). We use the FRET signal as a readout for protein conformation and observed two significant different conformations of αS in cells, one in the cytoplasm and the other on cellular vesicles (Fig. 1 middle and bottom panels) [4]. This clearly shows that there are at least two structurally distinct subensembles of αS inside cells: 1) a disordered form in the cytosol, 2) a membrane associated form. Our data shows that the disordered nature of monomeric αS is not fully preserved in cells.

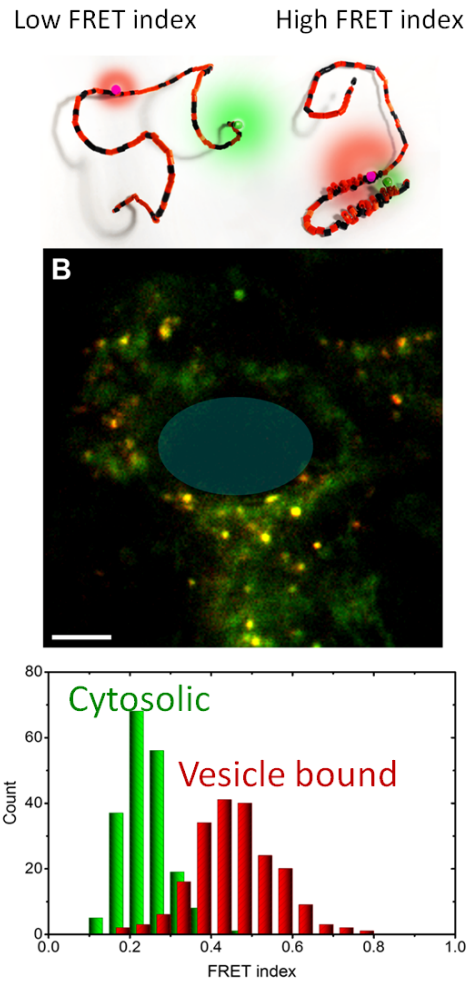

Figure 1. *Top:* Schematic representation of the disordered vs ordered conformations of the αS FRET probe. *Middle:* Microinjected cell imaged for donor (green) and acceptor emission (red). *Histogram*: FRET imaging of the microinjected SH-SY5Y cells shows two distinct conformations in the cytosol and on vesicles [4].

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High-speed, real-time reconstructed structured illumination microscopy of living cells

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Super-resolved structured illumination microscopy (SR-SIM) is among the most flexible, fastest and least perturbing fluorescence microscopy techniques capable of surpassing the optical diffraction limit. Current custom-built instruments are easily able to deliver two-fold resolution enhancement at video-rate frame rates. SR-SIM is, however, often perceived as a two-step process, with raw data acquisition and subsequent, time-consuming post-processing for image reconstruction. In contrast, wide-field and (multi-spot) confocal techniques produce immediate, high-resolution results. Such immediacy is also possible with SR-SIM by tight integration of the structured illumination microscope capable of video-rate image acquisition with fast image reconstruction software. Here, I will present our latest efforts in realizing multi-color SR-SIM at video frame-rates (and beyond), with minimal delay time between measurement and displaying the resulting SR-SIM image. This is achieved by modifying and extending the fastSIM approach based on utilizing spatial light modulators, and combining it with a new, graphics processing unit (GPU) enhanced, network-enabled version of our SIM reconstruction software. Recent results imaging live cell dynamics in multiple fluorescence colors, in particular with regard to the dynamics of liver cell fenestrations or mitochondria motility will be shown and discussed.

SIMULTANEOUS DETECTION OF 3D ORIENTATION AND 3D SPATIAL LOCALIZATION OF SINGLE EMITTERS FOR SUPER RESOLUTION STRUCTURAL IMAGING

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Measuring a single molecule's 3D orientation behaviour is a challenge that, if solved in addition to 3D localization, would provide key elements for super-resolution structural imaging. Orientation contains information on the local conformational properties of proteins, while orientational fluctuations are signatures of local steric, charges or viscosity constraints. Both these properties are not perceptible in pure super resolution imaging, which relies on position localization measurements. Imaging 3D orientation together with 3D localization is however not easily achieved due to the intrinsic coupling between spatial deformation of the single molecules' point spread function (PSF) and their off-plane orientations, as well as the requirement to measure six parameters which are not directly distinguishable (two angles of orientation, aperture of angular fluctuations, and three spatial position coordinates). In this work, we report a method that is capable of resolving these six parameters in a modality that is compatible with super resolution imaging. The method is based on the use of a stress-engineered spatially-variant birefringent phase plate placed in the Fourier plane of the microscope detection path. This phase plate modifies the PSF of single emitters in a way that can be non-ambiguously decomposed onto the nine 3D-analogs of the Stokes parameters. Moreover, the use of two complementary co/counter circular polarizations projections provides a non-ambiguous determination of the 3D spatial position of single emitters with tens of nanometres precision. This method, which opens the door to nanoscale structural imaging of proteins organization, is presented on model nano-beads emitters and applied to single fluorophores used for actin filaments labelling.

Fixation and Sample Processing Issues for Super-Resolution Microscopy

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Paraformaldehyde (PFA) is the most commonly used fixative for immunostaining of cells, but has been associated with various problems, ranging from loss of antigenicity to changes in morphology during fixation. Numerous other fixatives have been employed over the last decades, ranging from dialdehydes (such as glutaraldehyde) to protein-unfolding reagents such as methanol. All fixatives need to address two problems: to maintain the sample morphology in an in vivo-like condition, and to enable the penetration of imaging probes, such as antibodies. Here I discuss potential optimization procedures, including the use of a small dialdehyde, glyoxal, that can successfully replace PFA. Glyoxal acts faster than PFA, cross-links proteins more effectively, and improves the preservation of cellular morphology. Interestingly, it also enables better antibody penetration into samples. At the same time, the use of smaller probes, such as affibodies or nanobodies, also provides further improvements of sample imaging, for multiple super-resolution techniques. I also present recent progress in this respect, comparing classical antibody labeling to small probe-labeling.

Why to Automate Your Microscope and How to Control It

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KEYWORDS: tutorial, hardware control, automation, super resolution, confocal imaging, software development

The development of new bioimaging methods often depends on custom-built microscopes, with capabilities that go beyond commercially available solutions. Computerized control of such systems presents a challenge, however, since custom hardware necessitates custom acquisition and control software, and few off-the-shelf solutions exist.

Large, reproducible and standardized data-sets are essential foundations for quantitative cell biology research. Automated light-microscopy is the method of choice for collecting such data-sets of images of cells and whole organisms: it is both faster than manual approaches and more flexible than dedicated high-content systems.

In this tutorial, we will discuss design approaches to microscope automation and control software. For experiments requiring a high degree of customization, software control of individual hardware elements, their integration into a more complex instrument, and the development of efficient user interfaces will be presented.

Furthermore, we will present examples of how control and automation approaches can be tailored to different experimental needs, discussing the benefits and the limitations of customized control software vs. commercial solutions. Several groups combine both methods by extending commercial systems for specialized applications with dedicated workflow code. The large datasets produced by automated microscopes also require data management solutions, which are best considered during the design phase of the whole system and will also be covered in the tutorial.

After the tutorial we will offer break-out sessions to discuss specific topics in detail.

HIGH AND DEEP IMAGING FLOW CYTOMETRY: A POTENTIAL DIAGNOSTIC TOOL FOR HEMATOLOGICAL DISORDERS

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Flow cytometry has long been used routinely in clinical diagnoses, especially in hematological disorders. The introduction of imaging flow cytometry (IFC) brings even greater diagnostic potential thanks to its high content and high-throughput capability, where hundred thousand of images of individual cells are captured in minutes.

Based on previous open-source protocol for high-content IFC data analysis [1-3], we extended its implementation to detect and classify abnormal cells in collected blood samples of leukemic and allergic patients in 2 independent clinical trials. We utilized both classical machine learning and deep learning to examine high dimensional feature spaces extracted from IFC images, and found out that bright field (forward scatter) and dark field (side scatter) signals in fact contained valuable morphological information that is often overlooked by conventional image analysis methods. We were able to use this label-free information to differentiate leukemic blasts from normal lymphocytes and granulocytes; as well as differentiate activated eosinophils from non-activated population. We developed a robust workflow to perform feature extractions, feature selections, and classifications using supervised (random forest, support vector machine) and unsupervised learning (t-SNE, PCA, diffusion map) as well as deep learning (ResNet convolutional neural networks) on IFC imagery data. The results encourage the adoption of IFC as a potential diagnostic tool in clinical practice [4].

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The problem of orientation-induced mislocalizations of single emitters in cryo-fluorescence microscopy

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KEYWORDS: single molecule localisation microscopy, cryogenic temperature, super-resolution microscopy, fluorescence anisotropy.

Single molecule localization microscopy techniques have become one of the most successful and widely applied methods of super-resolution fluorescence microscopy. The obtained resolution is closely related to the number of photons emitted from a single fluorescent molecule and roughly scales with the inverse square root of the detected photon number. The photobleaching behaviour of the fluorophores is the fundamental bottleneck that limits the achievable resolution. We have recently designed and built flexible cost-efficient wide-field cryo-fluorescence microscope with exceptionally high thermal and mechanical stability, excellent single molecule imaging quality, and the capability to perform a sample change at cryogenic temperatures [1]. Using this system, we investigate a variety of fluorescent dyes in the red spectral region and find an improvement of the photostability of these molecules by more than two orders of magnitude, which corresponds to a theoretical localization precision around 0.1 nm at liquid nitrogen temperatures [2]. In the super-resolution microscopy imaging at the cryogenic temperature we are dealing with a rotationally fixed dipole emitters, which can cause significant mislocalization while using common fitting approaches [3]. Here we present a simple experimental solution to this problem based on implementing the polarization beam splitter in the detection part of the microscope.

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MEASURING THE PHASE AND INTENSITY OF THE LIGHT: FROM QUANTITATIVE LABEL-FREE IMAGING TO FLUORESCENCE 3D SUPER-RESOLUTION DEEP IN TISSUES.

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KEYWORDS: Quantitative electromagnetic-field imaging, label-free, cytoskeleton imaging, 3D single-molecule localization, fluorescence super-resolution.

Having access not only to the intensity of the light but also to its phase grants many possibilities in optical imaging microscopy. I will show in this presentation that quantitative electromagnetic-field imaging (i.e. phase+intensity) has powerful applications both in the scope of label-free microscopy and for 3D fluorescence super-resolution. In particular, I will show that quantitative electromagnetic-field imaging allows to identify in living cells and without labeling organelles and cytoskeleton fibers at high frame-rate and for any duration[1]. I will then demonstrate that the quantitative electromagnetic-field radiated by single particles is intrinsically carrying the 3D localization of each particle. First, I will discuss the case of 3D localization of absorbing nanoparticle and its application to stabilize a super-resolution microscope[2] in a single-shot manner and with sub-nanometer precision in 3D. Then I will move to fluorescence single emitter and demonstrate that the concept of measuring the electromagnetic field is also particularly efficient for fluorescent single molecule 3D localization. Indeed, it grants the capability to reach a quasi-isotropic 3D super-resolution even deep in biological samples without any adaptive optics[3].

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LECTIN-DRIVEN AND GLYCOSPHINGOLIPID-DEPENDENT CONSTRUCTION OF ENDOCYTIC PITS

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KEYWORDS: Endocytosis, lectin, glycosphingolipid, lattice light sheet microscopy, galectin, Shiga toxin, integrin, CD44

Several endocytic processes do not require the activity of clathrin, and it has been a major question in membrane biology to know how the plasma membrane is bent and cargo proteins are sorted in these cases. Our previous studies have allowed us to propose the GL-Lect hypothesis: nanodomain construction by GlycoLipid-binding cellular or pathological Lectins induces membrane curvature changes and drives the formation of endocytic pits for the cellular uptake of glycosylated membrane proteins with critical roles in cell migration (CD44, α5β1 integrin…), of pathogens (polyoma viruses, norovirus) or pathogenic factors (Shiga and cholera toxins). We are now analyzing how cortical actin dynamics contributes to the clustering of glycosphingolipid-lectin complexes on active membranes, thereby facilitating the nucleation of endocytic tubules exploiting fluctuation forces that had not been linked before to endocytosis. Furthermore, we are identifying mechanisms by which the GL-Lect mechanism is acutely controlled at the plasma membrane. Finally, we study how GL-Lect domain construction at the plasma membrane programs the intracellular distribution of cargo molecules, notably via the retrograde transport route.

Recent publications:

Pezeshkian et al. (2017) ACS Nano 11: 314-324
Shafaq-Zadah et al. (2016) Nat Cell Biol 18: 54-64
Bhatia et al. (2016) Nat Nanotechnol 11: 1112-1119
Renard et al. (2015) Nature 517: 493-496
Lakshminarayan et al. (2014) Nat Cell Biol 16: 595-606

Uncovering transients in single molecule motion: Application to cell surface proteins

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KEYWORDS: motion estimation, protein diffusion, single-particle tracking.

The analysis of molecule movement, as revealed by live-cell imaging and particle tracking, has helped uncover important information about how molecules interact with their environment. As these interactions are often transient and might change within the window of observation, leading to changes in molecule movement, accurate motion analysis often requires transient (i.e. sub-track) motion classification. A prime example are cell surface proteins and lipids, which can exhibit multiple motion types depending on their plasma membrane and juxta-membrane environment.. Therefore, for a full understanding of the dynamic nature of plasma membrane organization, it is essential to identify not only the different motion types of cell surface molecules, but also the lifetimes of these motion types and transition rates between them.

Here we present our efforts to develop accurate and computationally efficient algorithms for transient diffusion analysis, primarily focusing on a recently developed algorithm, termed "divide-and-conquer moment scaling spectrum" (DC-MSS) [1]. DC-MSS works in a multistep fashion: 1) it utilizes a local movement descriptor throughout a track to divide it into initial segments of putatively different motion classes; 2) it classifies these segments via moment scaling spectrum (MSS) analysis of molecule displacements [2]; and 3) it uses the MSS analysis results to refine the track segmentation. This strategy uncouples the initial identification of motion switches from motion classification, allowing DC-MSS to circumvent the sensitivity-accuracy tradeoff of classic rolling window approaches for transient motion analysis, while at the same time harnessing the classification power of MSS analysis.

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VOLUMETRIC IMAGING OF WHOLE-TUMORS REVEAL CANCER MALIGNANCY

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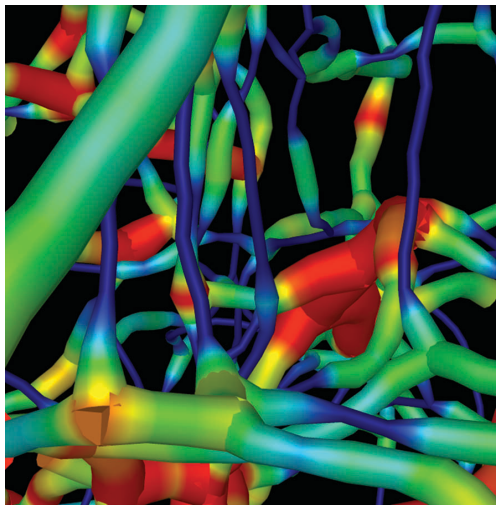
KEYWORDS: Cancer diagnosis, Histopathology, Light-sheet microscopy, 3D imaging

Intratumoral heterogeneity is a critical factor when diagnosing and treating patients with cancer [1]. Marked differences in the genetic and epigenetic backgrounds of cancer cells have been revealed by advances in genome sequencing, yet little is known about the phenotypic landscape and the spatial distribution of intratumoral heterogeneity within solid tumors. Our research group has developed a new diagnostic pipeline termed DIPCO, using three-dimensional light-sheet microscopy of cleared solid tumors, to identify structural abnormalities of phenotypic heterogeneity, in the epithelial-to-mesenchymal transition and in angiogenesis, at single-cell resolution in whole formalin-fixed paraffin-embedded biopsy samples [2]. We also show that the DIPCO pipeline can identify structural abnormalities to determine tumor stage and stratify patient prognosis from clinical samples with higher accuracy than current diagnostic methods and map the three-dimensional lymphatic microvasculature of tumors [3]. Now we are improving and further developing our 3D imaging method to identify novel tumor features. Just as 3D visualization with CT and MRI have revolutionized medical diagnostics, we expect that 3D microscopy will revolutionize histopathology to determine tumor stage and stratify patient prognosis with greater accuracy than current diagnostic methods, which will improve the design of more effective cancer therapies, resulting in positive effects on society.



Patterns of vascular heterogeneity in a cleared solid tumor identified by light-sheet microscopy

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Br J Cancer Apr;118(7):995-999 (2018)

Unraveling the Rules of Life a few Photons at a Time

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Although single molecule experiments provide unique spatiotemporal information, both theoretical modeling of single molecule behavior and verifying predictions from theoretical models remain open challenges. Fundamentally, this is because at the length and time scales of relevance to single molecules, thermal fluctuations in addition to the measurement process itself, are intrinsically entangled with the properties of the biological system that we care about.

The approach we present herein exploits a novel branch of Statistics – called Bayesian nonparametrics (BNPs) – first proposed in 1973 and now widely used in data science as the important conceptual advances of BNPs have become computational feasible in the last decade. BNPs are new to the physical sciences. They use flexible (nonparametric) model structures to efficiently learn models from complex data sets. Here we will show how BNPs can be adapted to learn models of single molecule dynamics often just a few photons at a time.

ANALYZING THREE-DIMENSIONAL BIOLOGICAL SPECIMENS OBSERVED WITH LIGHT SHEET MICROSCOPY UNDER NEAR-NATURAL CONDITIONS

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KEYWORDS: SPIM, DSLM, spheroids, organoids, fluorescence, quantitative biology

A major objective of the modern life sciences is to perform experiments under near natural conditions (NNC), i.e. to rely on three-dimensional biological specimens such as cysts, organoids, spheroids, embryonic bodies, tissue sections and small model organisms. Scientific projects relate to developmental biology, including embryogenesis and tissue formation, as well as to cell biology, e.g. investigating specific pathways in 3D rather than in 2D.
This talk concentrates on the development of new microscopes and image processing pipelines that are capable of handling millions of large-scale images for various different applications, which stem from our own research and our collaborations.
In general, fluorescence microscopy provides a high contrast, since only specifically labelled cellular components are observed while all other structures remain "dark". However, the optical sectioning capability is fundamental for dynamic three-dimensional imaging. One of the very few instruments, with this property is light sheet-based fluorescence microscopy (LSFM).
Fundamental issues of fluorescence microscopy that have to be addressed are: 1) Excitation light degrades endogenous organic compounds and bleaches fluorophores. 2) Specimens provide only a finite number of fluorophores, which limits the number of collectable emitted photons. 3) Organisms are adapted to a solar flux of 1.4 kW/m^2. Thus, irradiance should not exceed a few mW/mm^2 or $nW/\mu m^2$ in live imaging assays.
LSFM makes the most sincere effort to address these challenges by decoupling the excitation and emission light pathways. The significance of the illumination-based optical sectioning property is that the viability and the fluorescence signal of a living specimen are retained while millions of images are recorded for days or even weeks.
Particular benefits of LSFM are: (i) good axial resolution, (ii) imaging along multiple directions, (iii) deeper tissue penetration due to the low numerical aperture of the illumination objective lens, (iv) high signal-to-noise ratio, (v) unrestricted compatibility with fluorescent dyes and proteins, (vi) reduced fluorophore bleaching and (vii) photo-toxicity at almost any scale, (viii) millions of pixels recorded in parallel and (ix) excellent specimen viability.

www.researcherid.com/rid/A-7648-2011 scholar.google.com/citations?user=EV5RvqkAAAAJ

Three-dimensional single molecule imaging, with cellular context, of an engineered FcRn antagonist using remote focusing multifocal plane microscopy

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Three-dimensional (3D) single molecule fluorescence microscopy enables the observation and analysis of subcellular trafficking of individual molecules. Molecule trajectories imaged using this technique, however, often reveal only limited information about the underlying biological process. Information about the cellular environment, in particular the cellular structures and organelles with which the molecules of interest interact, is often lacking when imaging is carried out using conventional modalities. We recently described a 3D fluorescence microscopy imaging modality that enables the simultaneous imaging of the trajectories of fast-moving molecules and the associated cellular context. This imaging modality, called remote focusing multifocal plane microscopy (rMUM), extends multifocal plane microscopy (MUM) with a remote focusing module (r-module). Its MUM component allows the simultaneous imaging of distinct focal planes within a biological specimen, while its r-module allows the cellular context to be imaged in the form of z-stacks. Using rMUM, we visualize the dynamics of an engineered FcRn antagonist at the single molecule level, together with the sorting endosome with which it interacts. Tracking the single molecule in conjunction with the sorting endosome provides insight into the behavior of the engineered antibody in various intracellular sorting processes associated with sorting endosomes. We show that the precise dynamics of the engineered antibody can be estimated using rMUM by properly compensating for the movement of the sorting endosome with which it interacts. We also describe the dynamics of the engineered antibody as it interacts with tubular and fusing endosomes, and perform a detailed diffusion analysis on the trajectories.

Combining optical imaging of cleared tissue with mathematical modelling and *in vivo* imaging to predict drug delivery and therapeutic response

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KEYWORDS: *Optical imaging, tumour, blood flow, interstitial flow, solute delivery*

Understanding how drugs are delivered to diseased tissue, and their subsequent spatial and temporal distribution, is a key factor in the development of effective, targeted cancer therapies. Preclinical tools to better understand drug delivery are urgently required, which incorporate the inherent variability and heterogeneity between tumour types and deposits, and even within individual tumours[1]. However, few (if any) experimental techniques exist that can quantify drug delivery across whole tumour samples purely through experimental imaging.

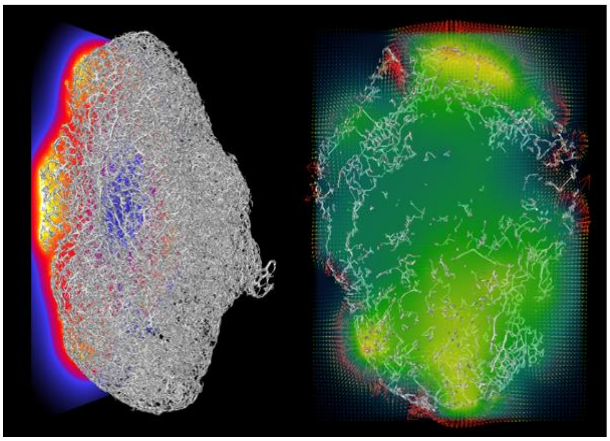

Figure 1. REANIMATE simulation of an LS174T tumour, showing blood flow (left) and interstitial transport (right), overlaid on blood vessels.

To meet this need, we have developed the REANIMATE (REAlistic Numerical Image-based Modelling of biologicAl Tissue substratEs) framework[3], which integrates optical imaging of intact biological tissue with computational modelling. Specifically, REANIMATE enables the microstructure of these large samples to be virtually reconstructed in 3D, on the scale of microns. These resultant data act as substrates for our mathematical model which is parametrised and validated against *in vivo* ASL-MRI perfusion data, thereby enabling physiological simulations of fluid delivery through the vasculature and into the surrounding tissue[3].

REANIMATE was applied to imaging data from two murine models of colorectal cancer (LS147T and SW1222) to: 1) simulate steady-state fluid dynamics (such as intravascular and interstitial fluid pressure (IFP)), 2) uptake of the MRI contrast agent Gd-DTPA, and 3) uptake and response to vascular-targeting treatment (Oxi4503). REANIMATE predictions were found to be consistent with the magnitude and spatial heterogeneity of *in vivo* measurements, both in steady-state (blood flow, IFP) and transient (drug delivery) models[3] (see **Figure 1**). Simulations predicted that, whilst the traditionally elevated IFP in the tumour core[4] can occur, vascular spatial heterogeneity can also induce spatially heterogeneous IFP[3]. Lastly, loss of vessels as a result of administration of Oxi4503 resulted in a subtle spatial pattern of perfusion loss in significant tissue volumes that is tumour-type dependent.

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Analyzing development, function and pathofunction of the vascular systems using light sheet microscopy

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KEYWORDS: VIPAR, VOREEN, vascular biology, lymphatic endothelial cells, ultramicroscopy

Blood and lymph vessels form two organs of considerable size, central to the function of all other organ systems. Being systemically disseminated organs the spatial structure of blood and lymph vessels is particularly relevant to understand their function in tissue space. Consequently, a comprehensive 3-dimensional interrogation of vascular development and structure is paramount to understanding vascular biology.

Using ultramicroscopy, we visualized developing blood and lymphatic vessels in intact wholemount-stained midgestation mouse fetuses. Our analysis revealed that lymphatic endothelial progenitor cells emerge from the largest fetal venous vessel, the cardinal vein, as streams of connected but non-lumenized cells. This resolved a long standing dispute on the mechanism of lymph vessel formation and identified novel structures in the developing lymphatic system that had so far eluded the analysis by tissue sections [1]. In the meanwhile, we proceeded to evaluate this information more quantitatively to derive new insights in the mechanism of this central developmental process [2, 3].

Mainly due to their relative invisibility the lymph vessels have so far eluted extensive structural investigation. A lack of investigatory and diagnostic tools has been a major contributing factor to the failure to mechanistically understand lymphoedema and other lymphatic disorders in order to develop effective drug and surgical therapies. A central difficulty in understanding the true changes in lymph vessel pathology has arisen from the use of standard 2D tissue sections.

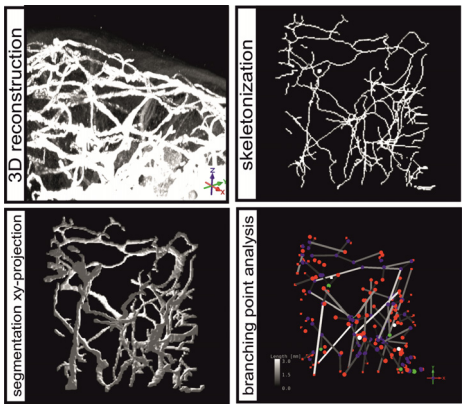

Fig. 1. Example of analysis of light sheet visualization of dermal lymphatic vessels.

VIPAR (volume information-based histo-pathological analysis by 3D reconstruction and data extraction), a light sheet microscopy-based approach for the analysis of tissue biopsies is based on digital reconstruction and visualization of microscopic image stacks [4]. VIPAR allows semi-automated segmentation of the vasculature and subsequent non-biased extraction of characteristic vessel shape and connectivity parameters. We applied VIPAR to analyse biopsies from healthy, lymphoedematous and lymphangiomatous skin. Digital three-dimensional reconstruction provided a directly visually interpretable, comprehensive representation of the lymphatic and blood vessels in the analysed tissue volumes. The most conspicuous features were disrupted lymphatic vessels in a lymphoedematous and hyperplasia in a lymphangiomatous skin sample. Both abnormalities were quantitatively evaluated by connectivity analysis based on extracted vessel shape and structure data (see Fig. 1). VIPAR is a volume-based tissue reconstruction, data extraction and analysis approach that is not limited to the vascular systems or dermal samples.

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SEGMENTING 3D VASCULAR NETWORKS WITH DEEP LEARNING

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KEYWORDS: Deep learning, 3D image analysis, fluorescence imaging

High Resolution Epsicopic Microscopy (HREM) is a block-facing modality capable of imaging at high resolution (~1μm) over a large field of view (up to 2cm^3) [1]. Images of resin-embedded samples are captured serially, while thin sections are removed from the block surface [2]. The resultant image stacks are inherently aligned, avoiding much of the post-processing need in classical histology. Therefore, HREM offers an alternative to histology for applications where understanding tissue morphology in 3D is crucial, for example, modelling of drug delivery and perfusion in tumours.

Quantitative analysis of HREM data has proven challenging due to the large volume of data, as well as uneven background illumination common in HREM images. A multilayer Convolution Neural Networks (CNNs) was developed to segment full vessel networks in 3D. It was first trained on manually segmented microvascular cast CT data in a proof-of-principle study, and then re-trained on HREM data (figure 1).

Despite the differences in contrast, resolution and pathology of the vessel networks between these two modalities, it was found that pre-training the CNN on micro-CT data decreased the number of epochs required to train the network to accurately segment HREM data. This suggests that it is possible to train CNNs to detect vascular structures in a way that is not specific to a single modality or pathology. This has the potential to reduce the need to manually segment large amounts of training data for different applications which has been a bottleneck in many machine learning applications to date [3].

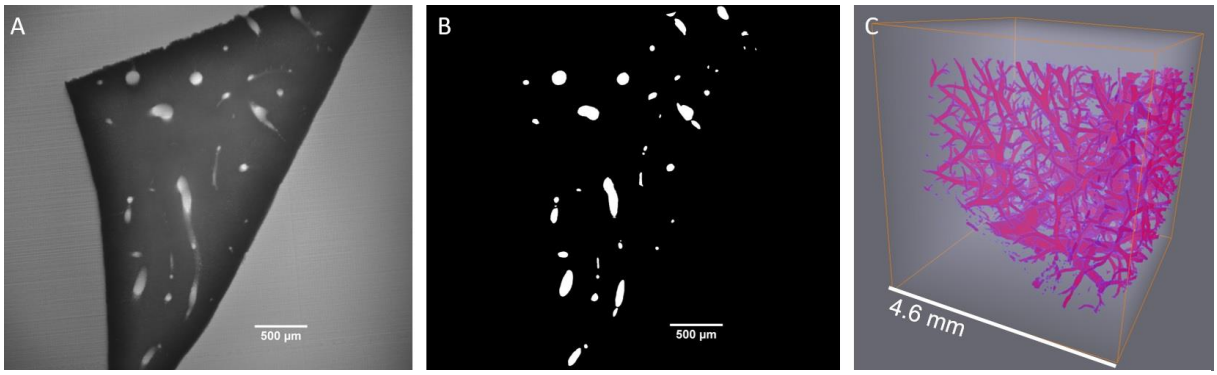

Figure 1. (A) a sinlge HREM image of a murine liver sample stained with eosin B. (B) Manual segmentation fo the same image. (C) 3D vessel network segmented by our CNN

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Structure and dynamics of endosomes analyzed by imaging-based methods

Marino Zerial and Yannis Kalaidzidis
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Endosomes are important organelles for the transport and sorting of endocytosed cargo but also for other functions, such as signal transduction, regulation of metabolism and stress response. We have been developing a pipeline of microscopy and image analysis methods to study the organization, dynamics and mode of function of early endosomes. By correlative super-resolution light and electron microscopy (SuperCLEM), we could map cargo molecules to the ultra-structure of early endosomes. Importantly, we found that the Rab5 GTPase membrane tethering and fusion machinery is compartmentalized into discrete domains on the endosomal membrane. We are now applying quantitative imaging and functional genomics approaches to explore the mechanisms underlying the biogenesis, structure, function and dynamics of endosomal sub-compartments, both in cultured cells and tissues. However, super-resolution methods have two major drawbacks when applied to live cell imaging: 1) high illumination intensity that triggers phototoxicity, and 2) slow acquisition rate (0.01÷1 Hz) that leads to image blurring due to fast intracellular motility. To overcome these obstacles, we developed a new method, *DeconSVD*, that achieves super-resolution without sacrificing the time resolution and is applicable to low intensity conventional fluorescence microscopy modalities. We will report how *DeconSVD* was applied to study intracellular organelles dynamics in cultured cells and by intra-vital microscopy (IVM) in mouse liver tissue.

Quantitative visualization of SNARE complex formation in living cells.

Geert van den Bogaart (Radboud University Medical Center, Nijmegen, Netherlands)

Members of the SNARE protein family are essential for secretion and transport between organelles in all eukaryotic cells. However, for most of the intracellular trafficking routes, the SNARE proteins remain unidentified, because SNARE proteins are highly expressed and have multiple overlapping functions. This makes it complicated to identify the sets of SNARE proteins for specific intracellular membrane trafficking routes. Here, I will describe a novel technique based on Förster resonance energy transfer (FRET) fluorescence lifetime imaging microscopy (FLIM) allowing for quantitative visualization of SNARE complexes within living cells with organellar resolution. We used this FRET-FLIM technique to investigate which SNARE proteins are responsible for the secretion of the inflammatory cytokine interleukin-6 by dendritic cells. We found that upon bacterial encounter, dendritic cells have increased complex formation of the SNARE proteins syntaxin 4 and VAMP3 specifically at the plasma membrane. Silencing of the gene for VAMP3 reduced the amount of secreted interleukin-6.

Structure and dynamics of endosomes analyzed by imaging-based methods

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Methods and applications of quantitative dynamic full-field OCT

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KEYWORDS: OCT, fluorescence, multimodal imaging, retina, stem cells, machine learning, cell segmentation, classification.

We present a recent technic adapted from full field optical coherent tomography to capture the dynamic in samples. We applied quantitative dynamic full-field OCT (qDFFOCT) to imaging of human induced pluripotent stem cell retinal organoids which are a platform for investigating retinal development, pathophysiology, and cellular therapies. In contrast to histological analysis and immunofluorescence staining in which multiple specimens fixed at different times are used to reconstruct developmental processes, qDFFOCT imaging can provide repeated images and analysis of the same living organoids with a contrast created by intracellular organelle motion and linked to metabolism. In order to quantify the dynamic signal, we computed each image in Hue-Saturation-Value color-space and benefitted from the latest advances in GPU computing to accelerate the process. We performed time-lapse acquisitions in a locked plane, highlighting cell differentiation, division and mitosis with a sub-micrometer resolution. By moving deeper into the samples, we were also able to acquire series of planes in depth to reconstruct the organoid 3D organization. We also applied qDFFOCT on a damaged macaque cornea and developed algorithms to track cells motion and successfully reconstruct a migration map of epithelial wound healing. This could help understand the healing mechanism and have great interest in cell therapy.

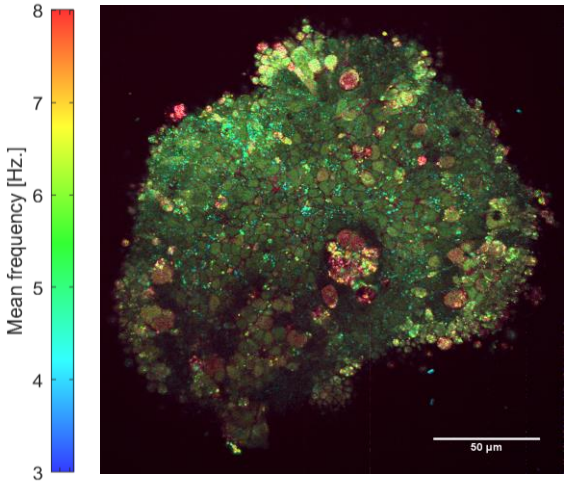

Figure 1. En-face dynamic image of 28 days old retina organoid.

We will explain the signal processing chain we developed to compute quantitative dynamic images where the colors code continuously for dynamic frequencies. Our overall aim is to use the dynamic signal as a non-invasive marker to predict cell type and cell cycle phases, making qDFFOCT a new label-free imaging method. In addition we recently proposed a multimodal setup to combine fluorescence with full-field OCT. We acquire both *en-face* quantitative dynamic full-field OCT (qDFFOCT) and fluorescence image with labelled cells. We then construct a cell data-set by using cutting edge cell segmentation algorithms. At different points of their development the retinal organoid's stem cells differentiate into neural cells, photoreceptors and retinal pigment epithelium. We performed non-supervised clustering techniques that successfully grouped cells by type. Besides being able to record time-lapse movies highlighting cell differentiation, division and mitosis we plan to train supervised algorithms to predict cell type only using the dynamic information with fluorescence images as ground truth, disregarding any information on shape, reflectivity, optical index, etc. Interestingly, the intrinsic high dimensionality of dynamic signals contains much more information compared to traditional images and allow us to use a

Fisher information matrix for molecules with stochastic trajectories

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KEY WORDS: object tracking, single molecule microscopy, stochastic differential equation, maximum likelihood estimation, Fisher information matrix, Cram´er-Rao lower bound.

The advent of single molecule microscopy enables the tracking of single molecules in cellular environments. Estimation of the parameters of the model that describes the molecule trajectory in such environments is a critical task in the analysis of single molecule tracking data. In most available methods, the motion of the molecule is limited to a Brownian motion model. Here, the motion of the molecule is modeled more generally by stochastic differential equations, and measurements are the detected photons emitted by the moving fluorescent molecules, which occur at discrete time points, corresponding to the arrival times of a Poisson process, in contrast to uniform time points which have been commonly used in similar dynamical systems. Whereas most available methods only focus on Gaussian measurements, our method enables the use of different measurement models, e.g., Born and Wolf models for out-of-focus molecules. We propose a framework to calculate the maximum likelihood estimates of the parameters of the motion model of the molecule, e.g., drift and diffusion coefficients. More importantly, we calculate the Cramér-Rao lower bound (CRLB)-based limit of accuracy, given by the inverse of the Fisher information matrix, for the estimation of the unknown parameters. Even for Gaussian measurements, there currently exists no systematic methodology to evaluate the standard deviations of the estimates using the CRLB for the general motion model considered here. We apply the developed methodology to single molecule trajectory data and show that the standard deviation of the estimates matches well with the square root of the CRLB.

Practical Aspects of Adaptive Optics for Microscopes

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KEYWORDS: Adaptive optics, microscopes

Aberrations frequently affect the performance of high-resolution microscopes. They can arise from imperfections in the optics, but are often introduced by the specimen, particularly when imaging thick specimens [1]. One common source is a planar mismatch in refractive index, such as that between the microscope coverslip and the specimen mounting medium, which introduces spherical aberration. Biological specimens also exhibit variations in refractive index that arise from the three-dimensional nature of cells and tissue structures. In general, these aberrations become greater in magnitude and more complex in form as the focusing depth is increased [2]. The induced wavefront aberrations distort the focus causing a reduction in resolution and, often more importantly, reduced signal level and contrast.

Adaptive optics (AO) has been implemented in microscopes to compensate the effects of system imperfections and specimen induced aberrations. These AO systems employ a dynamic element to correct aberrations, restoring image quality. Adaptive optics has been demonstrated in a range of microscope modalities including conventional widefield microscopes as well as laser scanning systems, such as confocal and two-photon microscopy with various applications in biomedical imaging and other areas [3,4]. It has been further developed for super-resolution microscopes – or nanoscopes – which enable resolutions smaller than the diffraction limit of light [5].

AO requires a dynamic optical element for wavefront control and a method of aberration measurement. Deformable mirrors (DM) or liquid crystal spatial light modulators (SLM) have variously been used in AO microscopes. SLMs provide versatile wavefront control and are best suited to modulation of laser illumination, whereas DMs are best compatible with correction of broadband unpolarised fluorescence emission. Aberrations have been measured using wavefront sensors and via indirect optimization methods. These elements have been combined to great effect in for a range of microscope applications.

This tutorial will provide practical guidance on the implementation of AO in microscopes. We will cover the selection of correction and sensing methods that are appropriate for particular microscopes and aspects of their implementation. This will include important points for consideration in the design of the optical system, in addition to software aspects for calibration and control of the AO devices. This will provide an appreciation of the challenges encountered in the implementation of AO and a suite of solutions that enable the effective use of AO in a range of bio-imaging applications.

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Transmembrane pickets corral bystanders in the plasma membrane by tethering to the submembrane cytoskeleton

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Phagocytic receptors must diffuse laterally to become activated upon clustering by multivalent targets. Receptor diffusion, however, can be obstructed by transmembrane proteins ("pickets") that are immobilized by interacting with the cortical cytoskeleton. The molecular identity of these pickets and their role in phagocytosis have not been defined. We used single-molecule tracking to study the interaction between Fcγ receptors and CD44, an abundant transmembrane protein capable of indirect association with F-actin, hence likely to serve as a picket. CD44 tethers reversibly to formin-induced actin filaments, curtailing receptor diffusion. Such linear filaments predominate in the trailing end of polarized macrophages, where receptor mobility was minimal. Conversely, receptors were most mobile at the leading edge, where Arp2/3-driven actin branching predominates. The corralling of bystanders by CD44 in other cell types –namely that of selectins in the apical/lumenal domain of endothelial cells– will also be discussed. Here, we find that limiting the diffusion of adhesion receptors and their ligands supports rolling adhesion and may explain the requirement for CD44 in the process.

Label-Free Refractometry and Pathogen Detection by Super-Critical Angle Fluorescence

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KEYWORDS: refractometry, supercritical angle fluorescence, refractive-index, bio-detection, microfluidics, bacterial growth

The refractive index (RI) is a material property that describes its interaction with light. This can be used to extract information about concentrations, material structure, and much more. Recently, sensitive methods for RI measurements of small volumes have been applied to detection of pathogens and quantification of protein-ligand interactions. Here we describe a method for label-free sensing of the RI: a device composed of a glass coverslip coated with a thin, dense layer of fluorophores inside a microfluidics channel.

The channel is imaged using a fluorescence microscope, where the detector is placed in a conjugate back focal plane of a high-NA objective lens. The RI is then calculated using a robust, custom algorithm for determining the transition between the under-critical and super-critical fluorescence. This is done by an iterative circle fitting method that estimates the radius of maximum intensity in the observed pattern, which scales linearly with the RI of the medium (Fig. 1).

We validate the methodology over a broad range of indices with ultra-sensitivity. Our microfluidic-based device and analysis technique for refractometry, enable label-free measurements of tiny amounts of liquid sample (picolitres of imaged volume) with high throughput and an order of magnitude higher precision than was previously reported. The sensitivity of our device enables detection of RI changes smaller than $4 \cdot 10^{-5}$ RI units, corresponding to a change of less than a 0.1% glycerol concentration in a water-glycerol solution. We furthermore demonstrate our system's applicability for biosensing, by measuring E. coli bacteria growth in the chamber and scanning over a sparse sample for single bacteria detection (Fig. 2).

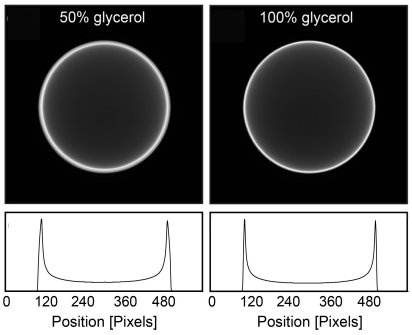

Figure 1. (top) Experimentally measured intensity images for two refractive indices and (bottom) associated image cross-sections.

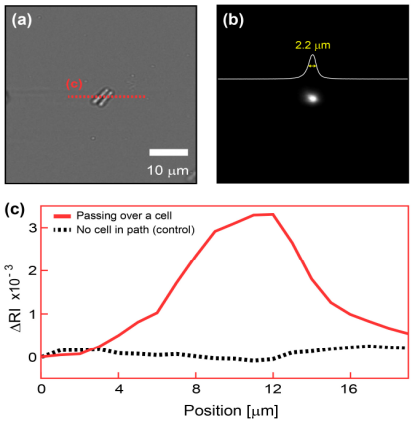

Figure 2. (a) White light image of *E. coli*. The sample was translated horizontally across the highlighted line. (b) Illumination laser-spot, overlaid with the beam profile cross section. (c) The index change corresponding to the probed region (dashed line in (a)).

Determining biophysical parameters in living cells with Icy

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KEYWORDS: Cell population analysis

Over the past 20 years plenty of developments in quantitative fluorescence microscopy techniques have emerged as well as fluorescent probes and genetically engineered fluorescent proteins and conjugates. These tools allow the scientific community to study cell behaviour and biological processes such as cell motility and morphology deformation.

The purpose of our work is to provide a robust method that permits to highlight the heterogeneity within a cell population via different descriptors. Some of the addressed questions are the following. Do cells follow Brownian or directed motion? Do they interact? Can we observe a quorum sensing, a synchronicity in their behaviour?

To this end we have developed an approach based on the combination of several biological techniques (e.g. transfection, cell cultures or fluorescent labelling) and informatics tools (such as tracking, speed, internal pressure). To compare the properties of our cell population of *Entamoeba histolytica* amoebas, I have used many plugins of the Icy software (Spot detector, HK Means, Spot Tracking and Bioflow) followed by statistical tests to assess the significativity of data. Icy is a community platform for bioimage informatics. It provides the software resources to visualize, annotate and quantify bioimaging data. (http://icy.bioimageanalysis.org)

The correlation between these properties is presented in the following figure. Perimeter, area, intensity of fluorescence, speed, roundness and homogeneity have been correlated and we can conclude that the smaller the cell is, the faster it goes.

We propose this framework as an useful protocol for the community so that biologists can perform their own image analysis and help the development of new plugins.

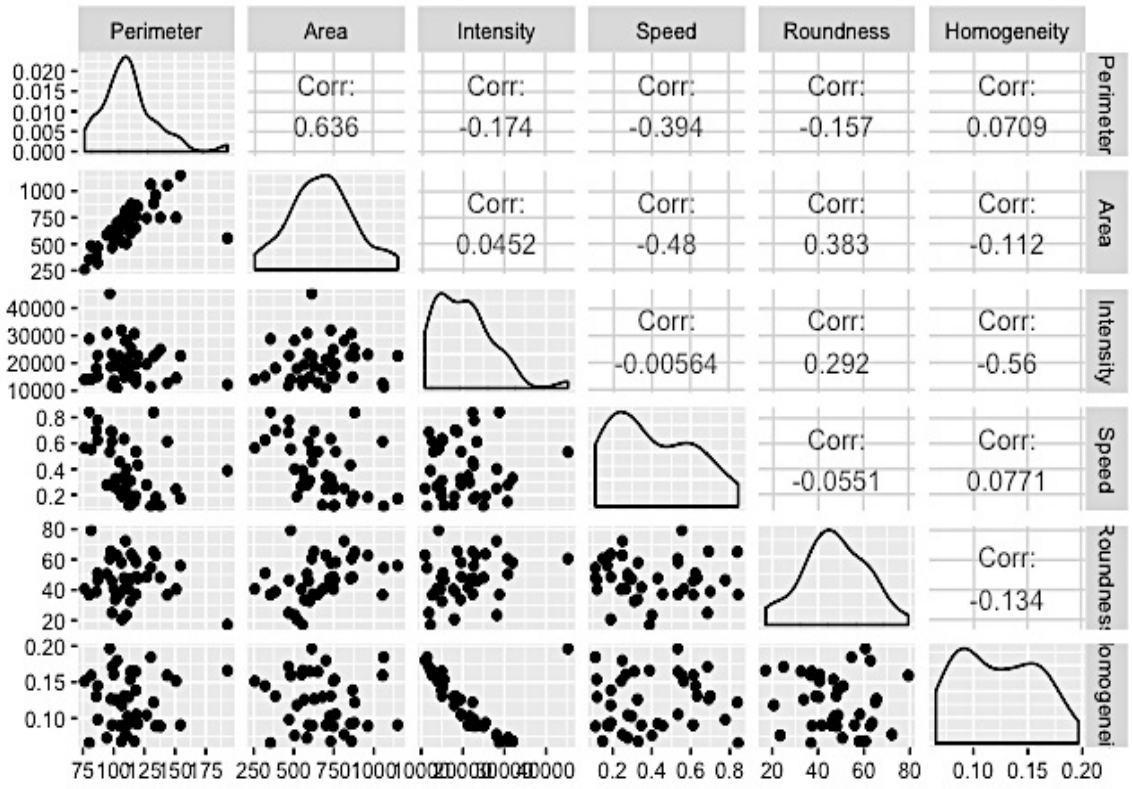

Figure: Correlation between different properties of cells behaviour.

Deep learning for dense 3D single molecule localization microscopy

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KEYWORDS: superresolution microscopy, 3D single molecule localization microscopy, PSF engineering, deep learning.

In conventional microscopy, the spatial resolution of an image is bounded by Abbe's diffraction limit, corresponding to approximately half the optical wavelength. Localization microscopy methods, namely (f)PALM and STORM have revolutionized biological imaging in the last decade, enabling the observation of cellular structures at the nanoscale.

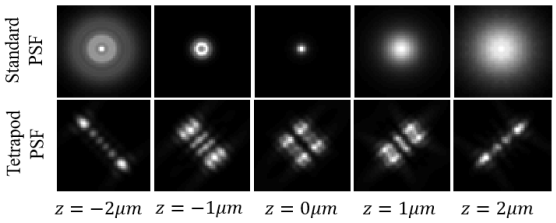

Figure 1. Tetrapod PSF axial profile. By altering the standard system PSF the axial position of an emitter can be encoded in the shape of the PSF, allowing depth recovery from 2D data.

Localization microscopy methods have been extended to 3D via the application of PSF engineering (Fig. 1). In localization microscopy, regions with a high density of overlapping emitters pose an algorithmic challenge. Specifically, the overlap problem is more severe in 3D, since encoding the axial position of an emitter introduces additional complexity into the PSF shape and inceases its size.

Recently, we demonstrated 2D superresolution image reconstruction by harnessing Deep-Learning [1]. Here, we extend our method to 3D by training a net to recieve an image of densely-spaced Tetrapod PSFs and output a list of 3D localizations (Fig. 2). The net localizes emitters with resolution comparable or better than existing methods; furthermore, the method is extremely fast, and our software can leverage GPU computation for additional speed enhancement. Moreover, Deep-STORM 3D is parameter free, requiring no expertise from the user, and importantly, Deep-STORM 3D is general and does not rely on any prior knowledge of the structure in the sample, making the method applicable to any single-molecule blinking dataset.

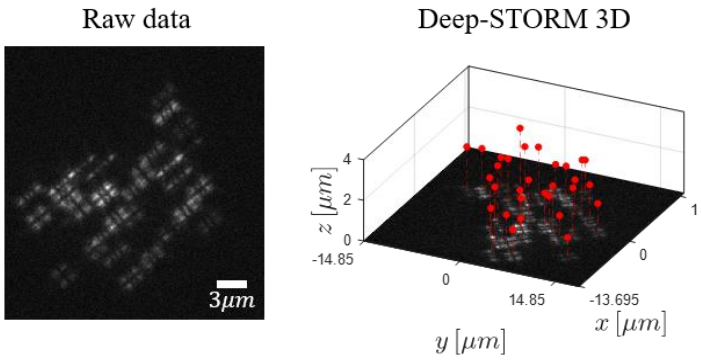

Figure 2. Deep-STORM 3D recovery (simulation). The net recieves a raw 2D image of overlapping Tetrapod psfs, and outputs a list of localizations in 3D including the depth of each

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Improved single molecule localization from dual-objective microscopes with ZOLA-3D

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KEYWORDS: Dual-objective microscope, Single molecule, Localization microscopy, Phase retrieval.

In single molecule super-resolution microscopy, the resolution is limited by the number of photons that is collected from individual fluorophores. Using dual opposing objectives to collect photons from both sides of the sample enables collection of twice more photons, hence allowing an improvement of the localization precision [1,2]. Introduction of astigmatism by means of cylindrical lenses permits to reconstruct 3D images, but sample thickness leads to spherical aberrations. We recently developed ZOLA-3D, a software that enables 3D SMLM image reconstruction using a realistic modelling of engineered PSFs, and which accounts for spherical aberrations [3]. Here, we describe an extension of ZOLA-3D for the 3D localization of molecules imaged by a dual-objective microscope equipped with two cameras. The localization method is optimal as it combines the images from both cameras while using realistic PSF models. Cramér Rao bounds confirm that both the theoretical localization precision and the axial range are improved using dual objectives. Simulated data show that our software achieves optimal precision on single emitters, and that the use of two cameras allows to improve localization precision for high emitter density by reducing detection ambiguities. The software also performs registration of the two images and drift correction. Reconstructions of tubulin filaments in 3D show that the dual objective system improves resolution not only because more photons are collected, but also because detection ambiguities are reduced.

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Computational boosts to single molecule localization microscopy

Christophe Zimmer, Institut Pasteur

Single molecule localization microscopy (SMLM) has matured into one of the most powerful and widely used super-resolution imaging methods. In this talk, we'll highlight recent developments of our lab to push the limits of SMLM using computational approaches.

One long-standing challenge is to visualize cells at high resolution and with high throughput. SMLM delivers exquisite spatial resolution, but at the price of very low throughput. Previous approaches to accelerate SMLM typically trade off spatial resolution. We present ANNA-PALM, a computational technique based on deep learning that can reconstruct high resolution views from strongly under-sampled SMLM data and widefield images, enabling considerable speed-ups without any compromise on spatial resolution[1]. We illustrate ANNA-PALM's robustness and potential for high throughput super-resolution imaging and highlight a dedicated web platform (annapalm.pasteur.fr). We will also discuss limitations and perspectives of ANNA-PALM.

Another challenge is to extend SMLM to 3D imaging of entire cells. While many approaches for 3D SMLM have been proposed, the need remains for a more accessible and flexible technique. We present ZOLA-3D a combined optical and computational method that enables versatile 3D super-resolution imaging over up to ~5 um depth[2]. Software and sample data are freely available from github.com/imodpasteur/ZOLA-3D.

Finally, the microscopy field could greatly benefit from easier access to SMLM data generated by the community, especially to train machine learning models. We will briefly highlight shareloc.xyz, an online platform to facilitate the sharing and reanalysis of SMLM data.

Complex diffusion analysis challenge

Maxime Woringer,[1,2,&] Hugues Berry,[3,4,&] Dominique Bourgeois,[5,&] Cyril Favard,[6,&] and Ignacio Izeddin[7,&]

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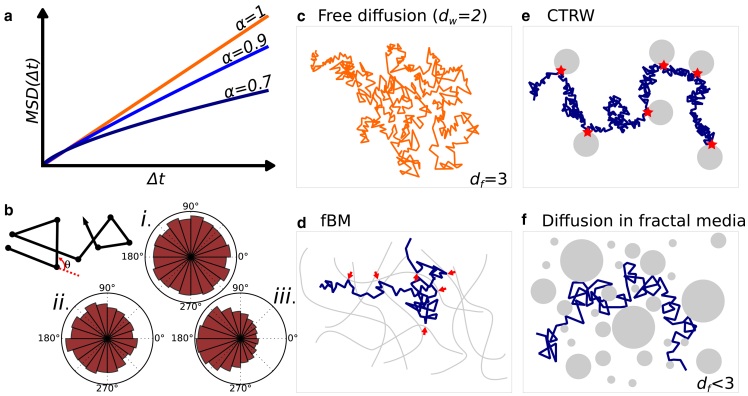
The life of a cell is governed by highly dynamical nanoscopic processes. Two notable examples are the diffusion of membrane receptors and the kinetics of transcription factors governing the rates of gene expression. Different fluorescence imaging techniques have emerged to study (macro)molecular dynamics in the living cell. Among them, fluorescence correlation spectroscopy (FCS) and single-particle tracking (SPT) have proven to be instrumental to our understanding of cell dynamics and function down to the molecular level.

Beyond insights on the biochemistry of the cell, these techniques have also unraveled an unforeseen complexity and diversity of mechanisms of protein diffusion. Many efforts have been devoted to analyze datasets generated by FCS or SPT, ranging from diffusion coefficient estimations to inference approaches. Among this array of algorithms, choosing the appropriate software for a given biological question can be challenging. Indeed, the richness of experimental data often makes it difficult to determine which are the models to be considered and the relevant biophysical parameters to be estimated.



Models of anomalous diffusion and plausible underlying physical structures. From Woringer and Darzacq, Biochemical Society Transactions 46 (2018)

Within this context, the CNRS GdR ImaBio proposes the ***Complex diffusion analysis challenge 2019***. Our goal is to foster the development of new state-of-the-art analysis algorithms. We aim at providing a unified data benchmark based on a set of biologically and physically relevant metrics in order to compare the diffusion analysis software available for the community.

Following an international call, we will provide researchers from the fields of image analysis, statistics and machine learning with a series of computer-generated datasets emulating realistic acquisitions produced by the single-molecule imaging and correlation spectroscopy communities. Part of this reference dataset will disclose the generating model and parameters used to generate the trajectory, to be used as training set. The performance of trajectory analysis algorithms will be quantified based on their ability to correctly infer the model used to generate a set of unlabeled trajectories (test set).

With this challenge, we hope to provide the molecular imaging community with a comprehensive set of data and metrics allowing to objectively evaluate existing and new analysis tools, as well as instigating an open discussion about the limitations and challenges of analyzing and modeling diffusion of molecules in the complex environment of the cell.

RSMLM: AN R-PACKAGE FOR POINTILLIST BASED ANALYSIS OF SINGLE MOLECULE LOCALIZATION MICROSCOPY DATA

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KEYWORDS: Single Molecule Microscopy, Clustering, Topological Data Analysis

After performing localization, the data from a single molecule localization microscopy (SMLM) experiment is represented by a set of spatial coordinates, each corresponding to a single detection, that form a point cloud. This can be analyzed either by rendering an image from these coordinates and using image-based analysis methods, or by analyzing the point cloud directly.

Here we present an RSMLM: an R-package for pointillist based analysis of single molecule localization microscopy data. This framework allows users to easily and efficiently design customised analysis workflows for high throughput and efficient analysis of complex 2D and 3D single molecule datasets. Our package includes methods for segmentation, specifically persistence based clustering [1], DBSCAN, Ripley K based clustering [2] and Voronoi diagram based segmentation [3]. For each method a Baysian engine can be employed to set key parameters [2].

Moreover, the package allows users to probe the topological or "shape" of biological nano-structure through the use of persistent homology [1]. Simple simulation of dSTORM datasets can also be performed. Binder ready Jupyter notebook tutorials are provided to facilitate easy use of the package. The methods from the library can be included within KNIME workflows using simple R-snippets. This enables users without any scripting knowledge to access the core functionality.

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Mesoscopic organisation of polarity proteins in epithelial cells

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KEYWORDS: single-molecule imaging, DNA-paint, protein clusters, self-organisation

Cell polarity is reflected by asymmetric distribution of cell membrane-associated proteins along an axis. In epithelial cells we distinguish apical and lateral domains, both occupied by different polarity proteins. The apical domain contains a transmembrane protein and its numerous cytoplasmic interacting proteins.

Confocal imaging revealed that these polarity proteins are not homogeneously distributed but form diffraction-limited clusters. Here we take advantage of in vivo super-resolution imaging (using the DNA-PAINT approach) of fixed cells to determine the mesoscopic characteristics of protein clusters. We use mean shift algorithm to identify clusters and analyse their size (i.e. number of proteins). Based on the cluster size distribution we also try to predict the mechanism of cluster formation using computer simulations.

Icy: A powerful and user friendly platform for Bio image analysis.

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KEYWORDS: BioImaging, Image Analysis, Quantitative, 3D, Visualization, Open Source.

Icy[1] is a free open-source bio-image analysis software. Its development started in 2010 and it has been continuously improved along its life time and had continuously brought new features to users and developers. Today Icy is used by about 2 thousand of regular users which appreciate its intuitive GUI, its powerful ray-traced 3D visualization and its cutting-edge analysis methods. Users can also adapt and create new algorithms with script and the graphical programming protocol designer and store them on the Icy website which centralize all resources: http://icy.bioimageanalysis.org

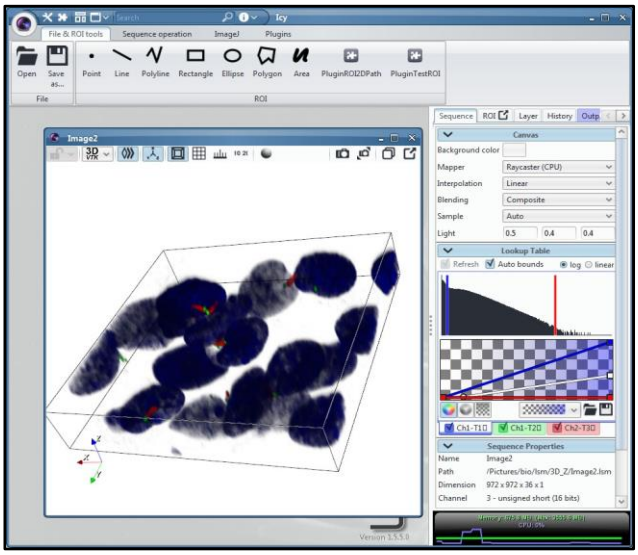

Figure 1. Icy graphical interface

Thus it makes available plug-ins[2], protocols[3] and scripts to everybody. Centralization also allows searching directly from within the application for specific features and enables it in a one-click install.

[1] **Icy: an open bioimage informatics platform for extended reproducible research.** (2012)
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Nature communications 9(1):698. doi:10.1038/s41467-018-03053-x

Nanostructured substrates for super-resolution imaging

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KEYWORDS: Strcutured Illumination, Super-resolution microscopy.

Fluorescence microscopy is an essential tool for studying sub-cellular dynamics, but image resolution is diffraction-limited. Super-resolution microscopies overcome this deficiency, either by point-spread function engineering (as for STED [1]) or by localizing individual fluorophores and accumulating their positions over time (in the case of PALM [2] and STORM [3]). These techniques attain lateral resolutions of a few tens of nm, and when combined with Total internal Reflection microscopy (TIRF), also high axial resolution. However, they either impose important constraints on the fluorophores that can be used, cannot easily provide multi-color co-localization information, or are too slow for imaging large fields. Many biological applications, however, require simultaneously a large field-of-view and a high frame rate because signalling events, organelle dynamics and/or membrane fusion events can happen at any place, anytime. For such live-cell time-lapse imaging, structured illumination microscopy (SIM) [4] appears ideal because it offers a compromise between spatial and temporal resolution. Using wide-field excitation with the projection of a diffraction-limited fringe pattern, SIM doubles lateral spatial resolution over the entire field-of-view, and combined with TIRF an isotropic 100-nm resolution can be obtained [5]. In this case, the final super-resolved image is typically computationally reconstructed from nine wide-field images acquired at three grating orientations and three phase shifts each.

In SIM the spatial resolution is directly related to the illumination pattern period, the shorter the period the higher the resolution. Typically, this pattern is created by the interference of two incident beams. Therefore the pattern period is given by $\Delta=\lambda/2n\sin(\theta)$, where λ is the excitation wavelength, n is the refractive index of the substrate (typically glass, n=1.52) and θ is the half-angle between both beams. Hence the minimum period that can be attained is $\Delta=\lambda/2n$ (i.e., 160 nm for λ=488 nm). A straightforward way to decreasing the pattern period (and increasing then the resolution) is the use of a higher-index medium, such as TiO_2 (n~2.5). But only replacing the glass by TiO_2 it is not enough for decreasing the pattern period, because of Snell's law. So we took a thin layer of TiO_2 that we use as a waveguide. In order to couple the excitation light inside this waveguide we designed and fabricated a periodic nanostructure acting as a diffraction grating [6]. The structure features a triangular lattice of holes on a glass coverslip in consequence it presents the 3-fold symmetry necessary for rotating the illumination pattern. The pattern phase shift is achieved by tuning the phase of the two counterpropagating evanescent waves.

To validate the expected resolution gain, we deposited sub-resolution fluorescent beads of 47-nm diameter on the surface of this substrate. Images with different azimuthal angle of incidence were taken, Fig. 1. The low frequency stripes, more visible in the Fourier Transform images (inset), show the coupling of the incidents beams into the waveguide, putting into evidence the possibility for this structure to reduce the illumination pattern period, increasing then the lateral resolution of the images. Ongoing work aiming at reconstructing the super-resolved image involves the testing and optimization of different reconstruction algorithms.

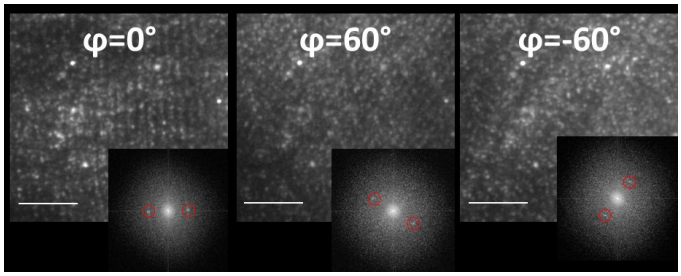

Fig.1: Images of fluorescent beads deposited on the surface of the grating and illuminated at different azimuthal angles. Insets: FFT of the images show the presence of a pattern period of ~800nm in the respective excitation directions evidencing the coupling of the excitation light into the waveguide. Scale bar: 5 µm.

In conclusion, we have shown the validity of our approach to further increase resolution in TIRF-SIM to 75 nm. Far from being "poor-man's super-resolution", SIM, as wide-field technique, compatible with standard fluorophores labelling protocols and multi-color imaging holds an enormous potential for biological nanoscopy.

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Quantifying molecular confinement using sSTED-FCS: The case of lipid trapping during HIV-1 assembly in living T cells

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KEYWORDS: STED, scanning FCS, lipid trapping, HIV-1 self-assembly.

Most of the functional biological processes induce changes in the dynamics of the involved molecules. Two main methods in optical microscopy (single particle tracking (SPT) and Fluorescence Correlation Spectroscopy (FCS)) have been developed at the level of single or few molecules to measure and monitor change in this dynamic. These methods have recently taken benefits of super resolution approaches (spt-PALM and STED FCS). Contrarily to SPT where molecular confinement can (hardly sometimes), FCS lack of spatial information that can be overcome by scanning on the sample [1] or varying waist of observation [2,3].
Scanning STED FCS allow to monitor molecular motion below the diffraction limit [4,5]. One of the benefits of scanning FCS is the fast, spatially distinct, multiplexing of acquired correlograms, allowing a statistically relevant analysis for each of the positon. We used this approach to monitor the T cell membrane differential lipid trapping during HIV-1 assembly (see figure 1). For this, we determine HIV-1 assembly locations and simultaneously measure the dynamics of different lipids (PI(4,5)P_2, sphingomyelin, cholesterol, phosphatidylethanolamine) in and out of these HIV-assembly sites. We observed that in Gag only transfected as well as in HIV-1 infected cells, PI(4,5)P_2 and cholesterol are nano-clustered during virus assembly while the sphingomyelin is not, demonstrating that the viral Gag protein is the driving force for T cell plasma membrane lipid clustering at the assembly site as it was observed on biomimetic membranes [6]. These results strongly confirm that lipids are spatially sorted in order to facilitate viral assembly.

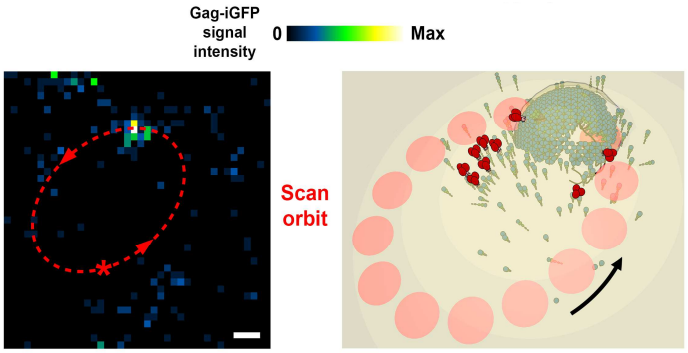

Figure 1 : Scanning STED FCS over HiV-1 Gag assembly sites

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Using microscopy to dissect endolysosomal trafficking and function

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We have used quantitative fluorescence microscopy to develop a general model of endocytic trafficking in non-polarized mammalian cells. Key initial findings included the initial demonstration that endosomes are acidic and a mapping of the pH of various endosomes. We have also used multiple fluorescent lipids and proteins to kinetically map the pathways for endocytosis and post-endocytic sorting. These studies led to key concepts such as the delivery from early to late endosomes by organelle maturation and a "default" pathway of membrane lipid recycling. We also found under-appreciated pathways such as the return of about half the internalized lipids to the plasma membrane with a $t_{1/2}$ of 1-2 min. Many of these studies were designed to understand how ligands such as LDL are delivered to lysosomes for degradation. Recently, we have used novel quantitative assays to understand how macrophages and related cell types use lysosomal hydrolases to degrade objects that are too large to be phagocytosed. These objects include lipoprotein deposits, dead adipocytes, and Alzheimer's amyloid deposits. We have used quantitative microscopy to document a novel process, exophagy, in which cells create a tight seal on the target, acidify the sealed region, and secrete lysosomal enzymes into the sealed region to digest the target. These processes are intrinsically asynchronous, so they need to be examined by quantitative single cell microscopy.

STRUCTURED ILLUMINATION IN LASER SCANNING MICROSCOPY FOR SUPER-RESOLVED MULTIPHOTON IMAGING: THEORY AND SIMULATIONS OF SCANNING SIM

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KEYWORDS: Structured illumination microscopy, super-resolution imaging, multiphoton microscopy.

Two-photon microscopy modalities such as second harmonic generation (SHG) scattered signal imaging and two-photon excitation fluorescence imaging are important tools for deep live tissue imaging. The requirement of a very high photon flux is in general met by spatially focusing the pulsed laser source while scanning an object. Conventional structured illumination microscopy (SIM) is a widefield based super-resolution method and its principle can therefore not straightforwardly be translated to a scanning setup.

Structured illumination in a laser scanning microscope can be achieved by temporally modulating the laser power while scanning the sample, resulting in a spatial modulation pattern [1, 2, 3]. The fluorescence emission or SHG is collected with a camera. Although this idea may appear to be equivalent to widefield SIM, there is a critical difference between the two implementations. An image *I* recorded with scanning SIM (sSIM) can be described as

$$I = \left[\left((P \otimes h_1) \cdot t\right) \otimes h_2\right].$$

Here, *P* is the sinusoidal illumination pattern, h_1 and h_2 are the excitation and emission intensity point-spread-functions (PSF), respectively, *t* describes the sample structure, and $\otimes$ is the convolution product.

The excitation PSF h_1 in sSIM, unlike in widefield SIM, plays an important role. Its convolution product with the illumination pattern *P* reduces the effective pattern modulation depth at the sample plane, complicating the SIM analysis.

We simulated the process of sSIM for several phantom samples, pattern periodicities and signal-to-noise ratios. We showed that, because of the 'modulation smoothing' effect, the pattern spatial frequency must be set to a practical value that is significantly lower than the objective cut-off frequency. As a result, the twofold resolution improvement that can be attained in widefield SIM is not feasible in sSIM. In contrast to what is claimed in the literature, a more modest resolution improvement can be obtained which heavily depends on the signal-to-noise ratio.

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Adaptive Holographic Region of Interest Illumination with Oblique Angles for use in Single Molecule Localization Microscopy

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KEYWORDS: SMLM, Image Processing, Computer Generated Holograms, ROI

Candida albicans and *Aspergillus fumigatus* cause often infections in humans, especially in immunocompromised patients. Both fungi have developed a pathogen mechanism to alter the membrane of the phagosome/ phagolyosome after being phagocytosed [1, 2].

However, even with Single-Molecule-Localization-Microscopy (SMLM) [3] which provides the most precise position of fluorophores, it is not possible to measure the membrane before and after the infection process, preferably in vivo.

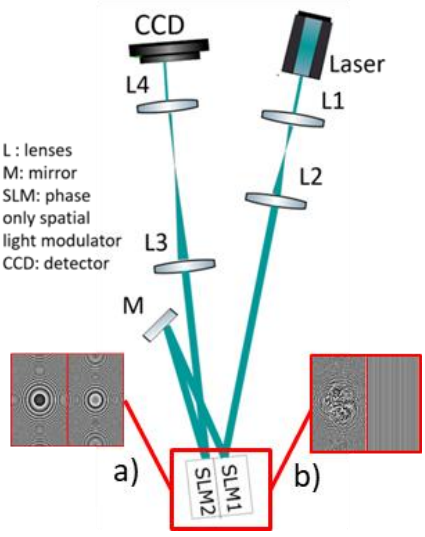

Figure 1. Illumination setup with two different modes to choose.

To improve the localization further and to reduce the overall photo-damage in SMLM, we introduce a holography based region-of interest (ROI) illumination [4] with two different application modes. Both modes are realized by illuminating a phase-only spatial light modulator (SLM) twice (see Fig. 1). They allow to adaptively modify the size and the (excitation) angle of the ROI illumination, resulting in reduced out-of focus signal and less overall phototoxicity.

The first mode (see Fig. 1a) generates an (almost) speckle-free circular ROI with selectable size and illumination angle. It is realized by focussing the excitation beam in the back focal plane (BFP) of the objective using SLM1/2. This spot can be changed in diameter to control the ROI size. In addition the spot can be laterally moved in the BFP controlling the (oblique) illumination angle. The second mode (see Fig. 1b) allows more flexibility with respect to the shape of the ROIs at the cost of more speckles in the illumination. An Iterative Light Propagating Algorithm (ILPA) retrieves the phase distribution which the SLM displays to generate the desired amplitude field (user defined pattern). To obtain the oblique illumination angles, an additional phase term is added. To optimize the ILPA in terms of speed and results, an integration in TensorFlow™ - a library for high performance numerical computation- will be realized.

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SINGLE-MOLECULE LOCALIZATION MICROSCOPY: PERFORMING 3D SUPER-RESOLUTION RECONSTRUCTION USING OPEN-SOURCE SOFTWARE

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The 3D SMLM reconstruction is a challenging computational task in term of performances, parametrization and runtime. The goal of this workshop is to present and to experiment some of the existing software solutions. We selected some software based on their usability and their accessibility (open-source) on ImageJ or Matlab. We will experiment software in four different modalities: 2D, astigmatism, double-helix, and biplane, both on simulated datasets and real datasets.

- Introduction of techniques for 3D SMLM and software
- Analysis methods, performances, limitations, density
- Calibration (3D)
- Post-processing: wobble correction, drift correction, temporal grouping, rendering
- Presentation of QuickPLAM or ThunderSTORM
- Presentation of SMAP by Jonas Ries, EMBL
- Presentation of ZOLA-3D by Benoît Lelandais, Pasteur Institute
- Demonstration on selected software (if time allows): QuickPALM, ThunderSTORM, EasyDHPSF, SMAP, ZOLA-3D

Software framework for Advanced Single Molecule Data Analysis

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Keywords: software, single molecule microscopy, data analysis, localization-based super-resolution, single molecule tracking

Modern microscopy, particularly single molecule microscopy, often involves diverse types of experiments and large volumes of complex data. Analyzing the acquired data typically requires several processing steps, many of which use advanced algorithms. Therefore, microscopists rely on software tools in order to effectively analyze microscopy data.

Designing such tools is challenging considering the diversity of experiments, the analysis complexity, and the varying levels of software expertise among microscopists. Currently available analysis tools often have limitations in these areas, requiring microscopists to use several different tools to complete a single analysis. However, this complicates the documentation and reproducibility of the results. A further challenge in the design of analysis tools is the need for easily updating and expanding the software based on changes in the computing environment or in requirements that users have for the software.

Here, we present a software framework that addresses these challenges in developing effective software solutions for microscopy, and particularly single molecule microscopy, data analysis. Using this software framework, microscopists can perform a complete analysis on data acquired from diverse types of experiments within a single software platform, beginning with the raw data to the generation of the final results.

SIMTOOLBOX 2.0: AN OPEN-SOURCE TOOLBOX FOR STRUCTURED ILLUMINATION MICROSCOPY

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KEY WORDS: super-resolution microscopy, structured illumination, SIM, CUDA, MATLAB, toolbox

There are numerous easy-to-operate commercial structural illumination microscopy systems, e.g. Nikon super-resolution microscope (N-SIM)[1], Zeiss ELYRA super-resolution microscopy[2], or DeltaVision OMX system[3]. MATLAB toolbox SIMToolbox [1] and ImageJ/Fiji plugin fairSIM [2] are two commonly used open-source tools designed for SIM reconstruction. In this work, the second generation of SIMToolbox is presented.

Several different reconstruction methods can be used for SIM imaging [3]. SIMToolbox is capable of optical sectioning SIM (OS-SIM) [4], super-resolution SIM (SR-SIM) [5], and maximum a posteriori probability image estimation (MAP-SIM) [6] methods. MAP-SIM as an alternative reconstruction method can potentially reduce unwanted reconstruction artifacts in conventional SIM approach. Moreover, SIMToolbox 2.0 now exploits high precision spot detection methods [1] for localization of pattern spectral peaks. Correct phase estimation is ensured by Single-step Correlative Phase Determination method [7]. In order to achieve faster processing of long image sequences of live cells, the second generation of SIMToolbox includes a new implementation of MAP-SIM. The new version takes advantage of computer graphics cards (GPUs) and the CUDA programming environment. To illustrate processing time gain of the new implementation, Table 1 shows the processing times for various size input images when SIMToolbox 2.0 is running with or without CUDA acceleration.

Despite precise estimation of illumination pattern, reconstructed images may contain artifacts. SIMToolbox achieves the best performance using calibration file. In this work, the results with and without calibration are discussed as well as the most common reconstruction artifacts in SIM will be presented. We expect that the second generation of SIMToolbox might be of great interest to the microscopy community.

SIMToolbox 2.0 and complete documentation are publicly available for download at Multimedia Technology Group website[4].

Table 1: Processing time for CPU and CUDA implementation of MAP-SIM, (CPU Intel Core i5-750, 2.67GHz, GPU nVidia GeForce GTX960)

Image Size, px	GPU Time, s	CPU Time, s
100×100	0.54	0.11
300×300	0.56	0.43
500×500	0.61	1.64
1000×1000	0.82	6.85
1500×1500	1.32	14.95
2000×2000	1.75	24.97

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Fluorescence studies of sterol transport between cellular membranes

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KEYWORDS: FRAP, deconvolution, multiphoton microscopy, probe development

Cholesterol is a small, water insoluble lipid molecule, which regulates the permeability, fluidity and bending flexibility of cellular membranes. Its intracellular distribution is very heterogeneous with the majority of cholesterol residing in the plasma membrane (PM). The endoplasmic reticulum (ER) is the site of cholesterol synthesis, but the ER harbors only very little cholesterol compared to the PM and endosomes (1). Recent studies have shown that cholesterol moves by vesicular and non-vesicular mechanisms, and a major question is how the strong cholesterol concentration gradients can be maintained between cellular membranes (1, 2). Quantitative fluorescence microscopy of suitable cholesterol analogs is an important tool to address this question. However, linking a fluorescent dye to cholesterol often perturbs its membrane properties and can lead to miss-targeting and deviant behavior in living cells (3, 4). In this talk, intrinsically fluorescent cholesterol analogs will be introduced, which differ from cholesterol only in having few additional double bonds in the steroid ring system. Such polyene sterols (P-sterols) resemble the biophysical properties of cholesterol closely, show similar binding affinities to sterol transporters and can replace natural sterols in sterol auxotroph organisms. However, this comes to the price of their weak fluorescence in the ultraviolet (UV), high bleaching propensity and low quantum yield (4-6). In this talk, it will be shown how such P-sterols can be visualized in living cells using UV sensitive wide field and multiphoton microscopy (5). It will be shown, how inter-compartment transport kinetics can be determined for P-sterols using dynamic multi-color imaging and fluorescence recovery after photobleaching. This will be discussed for healthy cells and for cells of patients suffering from a lysosomal storage disease (7, 8). Finally, a rational design strategy for development of new P-sterols with extended conjugated system and thereby improved optical properties will be presented (9-10).

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MACHINE LEARNING WORKSHOP
8th January 2019

11:00 – 12:30 Seth Flaxman (Imperial College London)
What is machine learning?

- Supervised vs unsupervised learning: k-nearest neighbors, k-means clustering
- From linear to nonlinear methods: transformations, feature engineering, and the kernel trick
- Bias vs variance, and how to deal with the problem of overfitting.
- Regularization, lasso, ridge regression, and the elasticnet

12:30 – 13:30 Lunch

13:30 – 14:30 Seth Flaxman
Advanced machine learning

- Ensemble methods
- Gaussian processes

14:30 – 15:45 Julien Mairal (INRIA)
Optimization
In this lecture, we will discuss a few techniques recently introduced in machine learning and optimization to deal with large amounts of data. We will focus on regularized empirical risk minimization problems, which consists of minimizing a large sum of functions, and cover also stochastic optimization techniques for minimizing expectations. Concepts we are planning to cover include

- stochastic gradient descent techniques with variance reduction,
- Nesterov's acceleration
- Quasi-Newton techniques.

We will also consider variants that allow dealing with nonsmooth regularization such as the l1-norm, which is useful for sparse estimation in high dimension.

15:45 – 16:45 Christophe Zimmer (Institute Pasteur)
Deep Learning

- what deep learning can do
    - image classification
    - other applications

how deep learning works

- forward propagation
- convnets
- training
- babysitting neural nets

DIY deep learning with Keras

Biological Deep Thermal Imaging with Fluorescence Lifetime of Rare-Earth-Based Ceramics Particles that Emit Near-Infrared Light in the Second Biological Window

Takumi Chihara[1], Masakazu Umezawa[1], Keiji Miyata[1], Shota Sekiyama[1], Naoki Hosokawa[1], Kyohei Okubo[1], Masao Kamimura[1,2] and Kohei Soga[1,2]*

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KEYWORDS: Time-gated imaging, Temperature, Biological deep imaging, Near-infrared fluorescence, Fluorescence lifetime, Rare-earth-doped $NaYF_4$.

Fluorescence thermometry realizes contactless thermal sensing by detecting temperature-dependent changes in emission properties of fluorophores. In development of fluorescence thermometry, the use of near-infrared (NIR) light is receiving much attention due to its high bio-permeability and less scattering loss as well as potential application for measuring temperature at deep biological tissues [1]. Here, we present an NIR fluorescence time-gated imaging (TGI) thermometry technology for deep biological tissues that is based on determining fluorescence lifetime, an intrinsic time-constant of fluorophore unrelated to observation depth. A TGI system was constructed to monitor pixel-level fluorescence decay realized by controlled delay of the timing between laser excitation and detection. An NIR fluorophore, $NaYF_4$ co-doped with Nd^{3+} and Yb^{3+} that emits 1000-nm fluorescence in the NIR biological window, was embedded in an agar gel-based phantom as a mimic of biological tissues. The phantom was covered by sheets of meats (0.7-mm-thick per sheet) to vary the observation depth. We confirmed that the temperature can be measured with independence of depth by sequences of NIR fluorescence decay images, while the temperature-dependent change in the fluorescence lifetime was almost constant (−1.0 to −1.3% per 1°C) at depth ranging from 0 to 1.4 mm. This indicates that there is a possibility to visualize temperature even in deep biological tissues and suggests that higher spatial resolution in temperature can be achieved in microscopic system.

[1] Smith, A. M., Mancini, M. C. and Nie, S., *Nat. Nanotechnol.,* **4**, 710-711 (2010)

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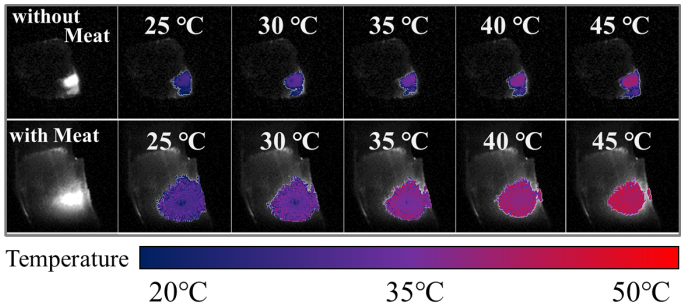

Figure 1. Thermal images rendered using the lifetime of fluorophores in biological deep tissue.

Temperature Sensing of Deep Abdominal Region in Mice by Using Over-1000 nm Near-Infrared Luminescence of Rare-Earth-Doped $NaYF_4$ Nanothermometer

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KEYWORDS: Temperature, Biological deep imaging, Near-infrared fluorescence, Ratiometry, Rare-earth-doped $NaYF_4$.

Luminescence nanothermometry is receiving much attention as a contactless thermal sensing technique. However, it is not widely explored for *in vivo* applications owing to the low transparency of tissues for the light to be used. In this study, we performed biological temperature sensing in deep tissues using β-$NaYF_4$ nanoparticles co-doped with Yb^{3+}, Ho^{3+}, and Er^{3+} ($NaYF_4$: Yb^{3+}, Ho^{3+}, Er^{3+} NPs) [1], which display two emission peaks at 1150 nm (Ho^{3+}) and 1550 nm (Er^{3+}) in the >1000 nm near-infrared wavelength region, where the scattering and absorption of light by biological tissues are at the minimum. The temperature-dependent change in the I_{Ho}/I_{Er} of $NaYF_4$: Yb^{3+}, Ho^{3+}, Er^{3+} was evident at the peritoneal cavity level (observation depth: approx. 1.5 mm), which is deeper than the subcutaneous tissue level, in mice [2]. The change in the luminescence intensity ratio of the emission peaks of Ho^{3+} and Er^{3+} (I_{Ho}/I_{Er}) in the $NaYF_4$: Yb^{3+}, Ho^{3+}, Er^{3+} nanothermometer differs corresponding to the thickness of the tissue, possibly due to the change in attenuation of fluorescence by water in the biological tissues by temperature change. Therefore, the relationship between I_{Ho}/I_{Er} ratio and temperature needs to be calibrated by the depth of the nanothermometer *in vivo*. The designed experimental system for temperature imaging will open the window to novel luminescent nanothermometers for *in vivo* deep tissue temperature sensing.

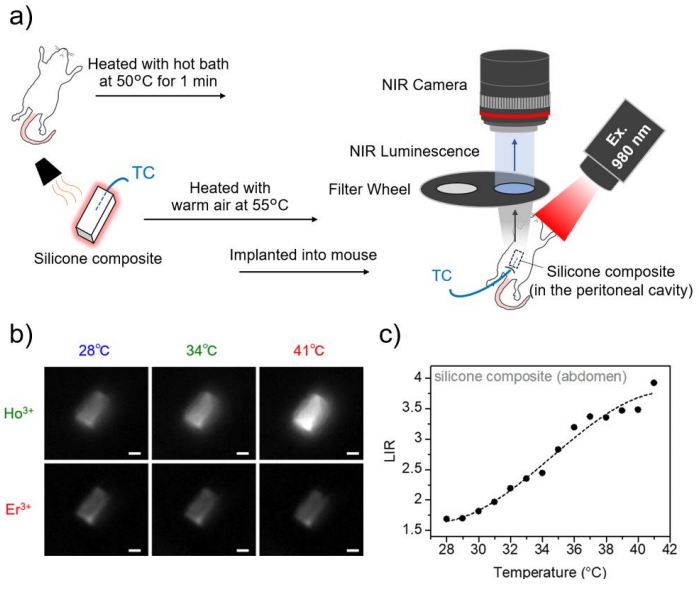

Figure − Temperature-dependent change in OTN-NIR fluorescence images of the silicone composite placed in the peritoneal cavity under 980-nm laser excitation.
a) Schematic representation of the methods. **b)** Ratiometric fluorescence images of the implanted area in the peritoneal cavity acquired using band-pass filters at 28, 34, and 41°C. Scale bars represent 5 mm. **c)** Luminescent intensity ratio (LIR: I_{Ho}/I_{Er}) of the fluorophore in the peritoneal cavity.

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[2] S. Sekiyama et al. *Sci. Rep.*, **8**: 16979 (2018)

INTRODUCTION TO DEEP LEARNING

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KEYWORDS: deep learning.

Deep learning is fueling the current renaissance of artificial intelligence thanks to breakthrough successes in a wide range of applicative domains. The purpose of this presentation is to introduce deep learning and demystify it for a broad audience. No prior knowledge of machine learning is assumed and an effort will be made to explain the main principles and terminology from scratch. We will also highlight tools that make deep learning accessible. The outline of the presentation is as follows:

1) What deep learning can do
   a) image classification
   b) other applications
2) How deep learning works
   a) forward propagation in neural nets
   b) convnets
   c) training
   d) babysitting neural nets
3) Do-it-yourself deep learning with Keras

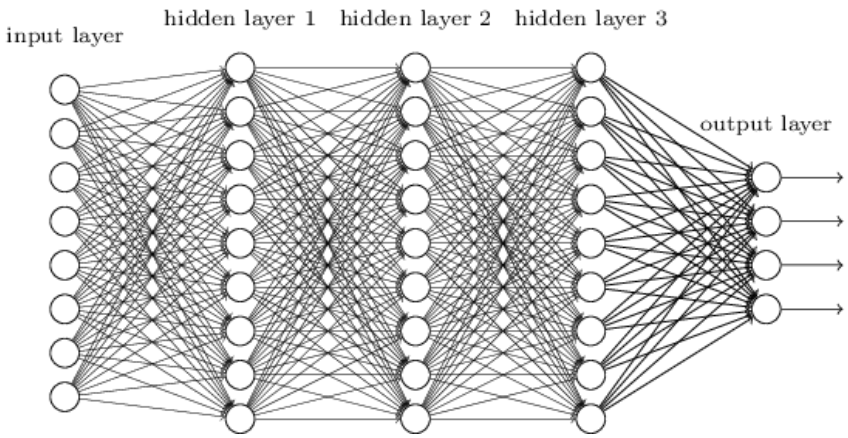

Figure 1: A fully connected deep neural network with 3 hidden layers, 8 neurons in the input layer and 4 neurons in the output layer.

Time resolve intensity photo bleaching (TRIP) as a tool for quantify bound /unbound fraction of lamin A protein *in vivo.*

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Abstract

Here we present a method for exploring proteins dynamics while revealing a significant number of its quantitative properties including its diffusion coefficient, bound and free fraction, protein concentration and binding-unbinding kinetics. The method is based on the combination of live-cell imaging modalities combined onto a single experiment that we term time-resolved continuous photobleaching (TRIP). By applying the method on structural proteins in the nucleus, we demonstrate the complex function of lamin A that is of crucial important for maintaining the genome organization in the nucleus.

IMAGE PROFILING EXPOSES A PHARMACOLOGICAL WINDOW FOR MODIFIERS OF NEURONAL NETWORK CONNECTIVITY

Marlies Verschuuren[1] & Peter Verstraelen[1], Ines Cilissen[4], Emma Coninx[2,3], Mieke Verslegers[3], Andreas Ebneth[4], Peter Larsen[4], Rony Nuydens[4], Winnok H. De Vos[1,5]

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KEYWORDS: Phenotypic fingerprints, High-throughput microscopy, Image analysis, Live cell calcium imaging, Neuronal Connectivity

Therapeutic developments for neurodegenerative disorders are redirecting their focus to mechanisms that contribute to synaptic plasticity and the loss thereof. Identification of novel regulators requires a method to quantify neuronal network connectivity over time with high accuracy and throughput. To meet this demand, we have established a microscopy-based pipeline that integrates morphological - based on nuclear, cytoskeletal, pre- and postsynaptic markers - and functional - spontaneous synchronous calcium activity - correlates of connectivity in primary neuronal cultures [1].

Using clustering and classification approaches, we unveiled a connectivity signature that was specific to the cultured cell type (hippocampal or cortical) and maturation stage (days *in vitro*). We established a metric, termed connectivity score, that accurately reports on the degree of neuronal connectivity and we validated this score using previously characterized perturbations of microtubule stability [2]. As expected, both pharmacological (nocodazole) and genetic (MAPT-P301L overexpression) perturbations caused a significant reduction in the connectivity score. We found a similar reduction in cultures that had been depleted from anti-oxidants.

Using a focused compound screen, we discovered that inhibition of dual leucine zipper kinase activity [3] could rescue MAPT-P301L- and antioxidant deprivation-induced defects, but also that – within a specific dose range - it increased the connectivity score in otherwise healthy cultures. Thus, our results illustrate that image profiling enables sensitive interrogation of neuronal connectivity and allows exposing a dose and time window for pharmacological interventions. Therefore, the current approach holds promise for identifying pathways and treatments that preserve or rescue neuronal connectivity in neurodegenerative disorders.

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FLUOSIM: SIMULATOR OF SINGLE MOLECULE DYNAMICS FOR FLUORESCENCE LIVE-CELL AND SUPER-RESOLUTION IMAGING

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KEYWORDS: Simulation, single molecule dynamics, SPT, FRAP, FCS, SRI

Critical cellular functions such as membrane adhesion, receptor-mediated signaling, or synaptic transmission, involve the diffusional trapping of specific molecules in sub-cellular compartments. To quantitatively describe such molecular dynamics in living cells, several fluorescence imaging techniques are available, including single particle tracking (SPT), ii) photo-activation and photo-bleaching methods (PAF/FRAP), and Fluorescence Correlation Spectroscopy (FCS). Recent approaches based on single molecule localization (PALM, STORM, PAINT), yield images of protein distribution at improved resolution, giving unprecedented information about the nanoscale organization of biological structures. Despite such progress in imaging power, many experimental parameters remain difficult to estimate or control, including protein expression levels, probe labeling density, potential fixation artifacts, spatial and temporal sampling of the recordings, and protein motion below the system resolution, such that results are sometimes difficult to reconcile.

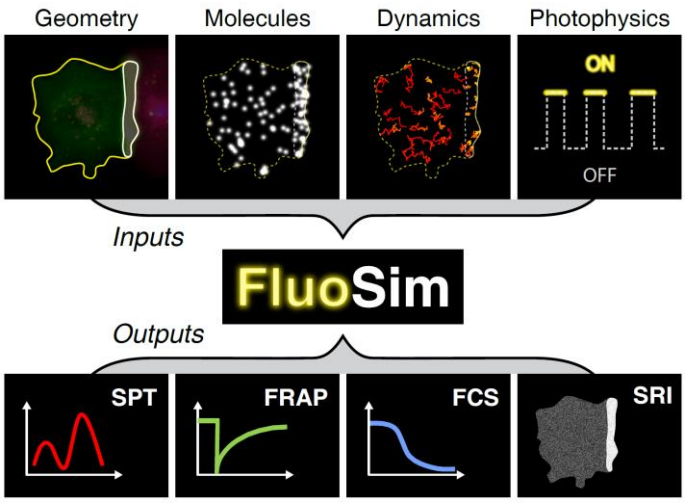

Figure 1. FluoSim general principle

In this context, we provide here a fast and robust software (**FluoSim**) allowing the simulation of a wide range of live-cell imaging modes **(Fig. 1)**. The program uses protein diffusion coefficients and binding rates, as well as fluorophore photo-switching rates to calculate in real time the motion and intensity of thousands of independent molecules in arbitrary 2D cellular geometries, providing simulated data of protein dynamics (SPT, FRAP, PAF, and FCS). Moreover, since the positions of molecules are known with near-infinite accuracy, the program can generate super-resolved localization maps of fixed samples directly comparable to PALM or STORM images.

We validated FluoSim against experiments performed on the canonical neurexin-neuroligin adhesion complex in COS-7 cells, using a unique set of parameters extracted from published in-vitro studies and/or taken from our own measurements, thereby giving strong credit to the correlative approach. The program should be applicable to model a wide range of dynamic molecular systems experiencing membrane diffusion and transient confinement. The software can also be used to test the robustness and predictions of single molecule tracking and image reconstruction algorithms that have been implemented those past years. Overall, FluoSim is intended to help biologists adjust and interpret their experiments on a variety of cellular systems, and serve as a teaching resource in bio-imaging programs.

Correlative STED and SMLM, without demons

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KEYWORDS: superresolution microscopy | single molecule localization microscopy | non-coherent illumination source | STED | UV activation

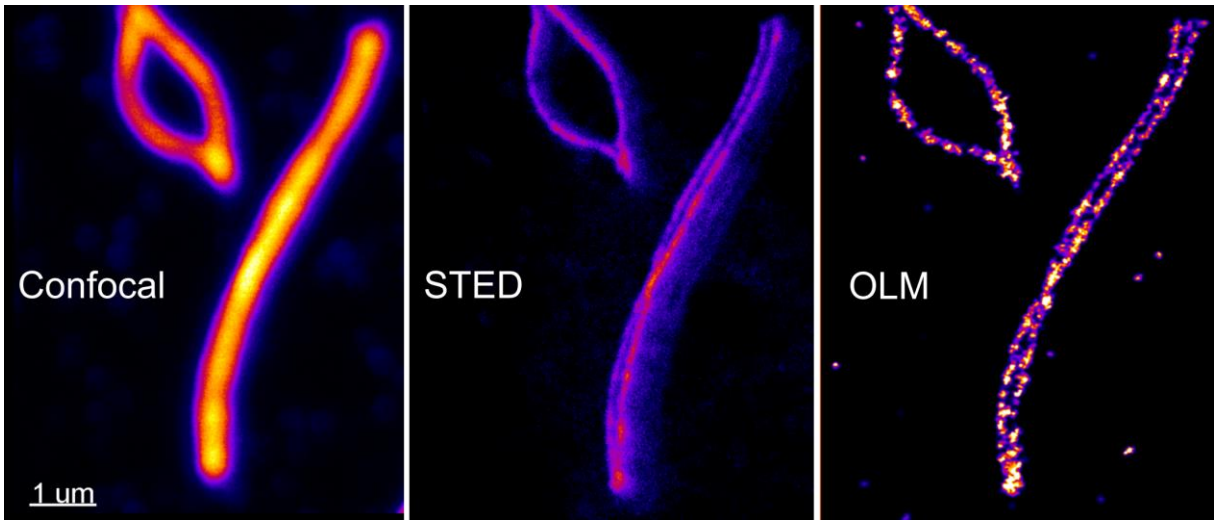

Figure 1: Synaptonemal complex (SC) proteins from mouse stained with Alexa 594. (A) The confocal image shows the entire complement of SC proteins. (B) The same chromosome imaged with STED and (C) OLM (Omnipresent Localisation Microscopy, see Prakash 2017). The two halves of the SC that unite the meiotic chromosome pair are not resolved with confocal but with STED and OLM.

The spatial resolution of light microscopy is continuously being pushed with the development of new technologies. The significant milestones have been Confocal, 4Pi, Two-photon microscopy, STED, Zero point STED, SIM, PALM, STORM. Recently, correlative light microscopy techniques have further pushed the spatial resolution down to a few nanometers. MINFLUX, a correlative STED and SMLM technique, requires very few photons and is based on adaptive updating of positions and localisation precision. The perfect localisation is described by the authors to be achievable upon the aide from "a supernatural being, a demon (1)." While the requirement of fewer photons for accurate localisation is much desired, the access to a demon might not be readily available. Moreover, the technique has only been demonstrated on DNA origami and bacterial samples (for tracking) which can be a limiting factor for biological research.

Here, I demonstrate that using deep UV reactivation (350 to 380 nm lamp excitation) instead of 405 nm laser, correlative STED and SMLM can be performed on real biological samples (mouse meiotic chromosomes) using a simple imaging medium (2). This technique, termed as inSTED, can be used by any scientist to generate high-resolution images and provides an open framework for correlative microscopy. Lastly, I hope the new photophysical observations reported here will pave the way for more in-depth investigations on the mechanisms underlying the excitation, photobleaching and photoactivation of a fluorophore.

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PyMOL Molecular Graphics System for Immunoglobulin Cross-Reactivity Analysis

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KEYWORDS: Cross-reactivity, 3D, PyMOL, Protein, antibody

Cross-reactivity can invalidate the results of an experiment and there by impact scientific reproducibility. Recent developments in techniques for modeling, digitizing and visualizing 3D shapes has led to an explosion in the number of available 3D models on the Internet and in domain-specific databases. We describe here a superposition method using PyMOL for comparing the surfaces of antibody binding sites that cross-react with two fractions of proteins.

PRACTICAL IMPLEMENTATION OF THE OFF-AXIS HOLOGRAPHIC MICROSCOPE

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KEYWORDS: digital holographic microscope, microscope setup

In this contribution we present in detail the inverted design of transmission off-axis digital holographic microscope [1] (DHM) implemented from scratch on optomechanical table. We provide description of different optomechanical parts and their importance in the DHM design. The results are presented by imaging of the USAF resolution chart.
The main features of our design are following:

1) Large number of degrees of freedom of the optomechanical setup – different kinematic mounts are used throughout the design, which enable easy and precise setting of the optical path.
2) Telecentric system – the configuration of microscope objective (MO) and tube lens creates a telecentric system with afocal configuration [2], which significantly suppress the spherical phase factor in resulting phase map. Furthermore, MO can be easily change because the distance between MO aperture stop and tube lens remains the same.
3) Easy possibility of extension – the "system on the table" enables easy integration of other microscopic techniques (e.g. fluorescence).

Figure 1 shows the layout of our system. The main components are: 534nm laser source, beam spatial filter, reference path with the beam expander, sample path with MO and tube lens, rotating beamsplitter and fast CMOS on-board sensor.

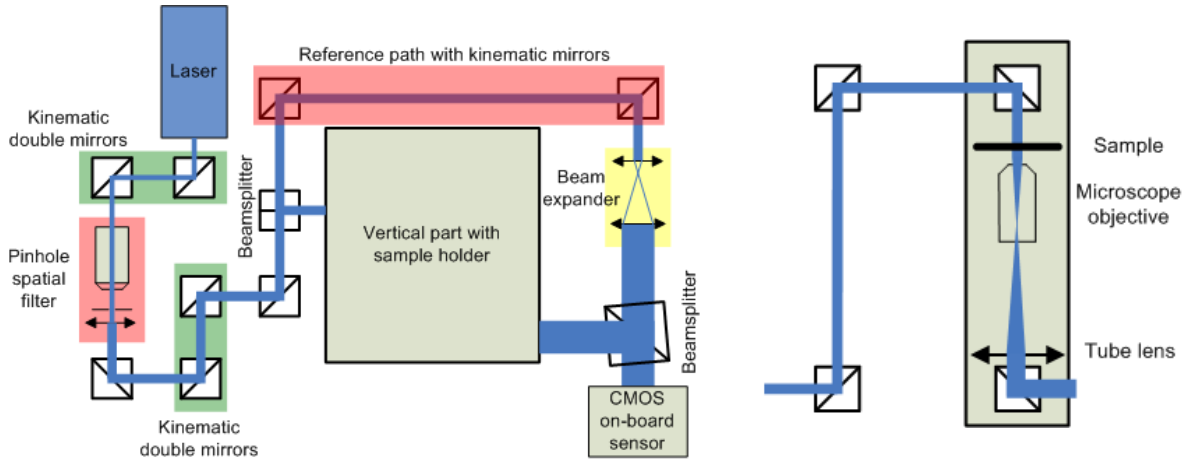

Figure 1. (Left) The microscope layout with the main parts, (Right) The vertical part of the microscope.

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[2] E. Sánchez-Ortiga, P. Ferraro, M. Martínez-Corral, G. Saavedra, and A. Doblas, *Digital holographic microscopy with pure-optical spherical phase compensation*, J. Opt. Soc. Am. A **28**, 1410 (2011).

LABEL-FREE PREDICTION OF SUBCELLULAR ORGANIZATION: CAPTURING VARIATION AND INTEGRATING OBSERVATIONS

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Determining the organization of cells under normal and pathologic conditions is critical for our understanding of cell biology. Due to the limitations of live-cell imaging, it is difficult to gather long time series with multiple labeled subcellular structures. In contrast, transmitted light microscopy is a low-cost alternative that drastically reduces sample preparation time and phototoxicity although it lacks the specificity of other imaging methods. Here, we present a deep neural network-based approach to automatically determine the localization of subcellular structures directly from transmitted light images. We show that our model can be trained on relatively few examples, approximately 30 spatially registered transmitted light and fluorescence image pairs, for most subcellular structures of interest. Our model can be applied to a variety of contexts, including extended live-cell imaging sessions, automated detection of rare events and quantification of uncertainty in the predicted fluorescence images, as well as identification of highly resolved complex subcellular structures in differentiated phenotypes. Building models across multiple experiments allow us to integrate data and generate multi-structure images. We characterize our model on a range of cell types, subcellular structures, and imaging conditions and apply our label-free imaging method to array tomography immunolabeling, image registration, and denoising contexts, greatly expanding its potential applications.

Automated brain region recognition in fluorescent tissue slices using deep learning

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KEYWORDS: Machine learning, deep learning, brain atlas, fluorescent tissue slices

Many neurodegenerative disorders, such as Alzheimer's Disease, pertain to or spread from specific sites of the brain. Hence, accurate disease staging or therapy assessment in mouse models demands automated analysis of specific brain regions. To address this need, we have recently developed an algorithm, termed SliceMap, that enables contextual quantification by mapping anatomical information onto microtome-cut brain slices [1]. This algorithm relies on a combination of affine and elastic registration procedures. However, SliceMap demands a library of pre-annotated reference slices and has no internal quality checks, which may compromise delineation of morphologically deviant samples. To improve the performance of our method, we have therefore turned to deep learning. A deep convolutional neural network based on Mask R-CNN (DeepSlice) was optimized to segment brain regions in high-resolution DAPI-stained fluorescent images. The network was trained on 200 sagittally-cut pre-annotated brain slice images originating from a comparable cut region. In comparison with SliceMap, DeepSlice proved significantly more robust at mapping regions to distorted slices, whilst yielding a minor improvement in the overall accuracy and keeping the computational load within limits (time to predict a single slice on the magnitude of a minute). Thus; DeepSlice allows for a faster and more accurate delineation of the regions, increasing the accuracy of any downstream analysis. Current work focuses on improving the performance by using data augmentation and combining the network with a high resolution tiled network.

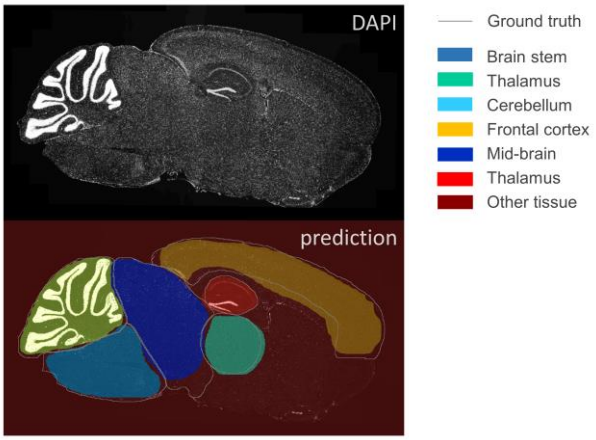

Figure 1. Example of a brain tissue slice sample (DAPI channel) together with an overlay of predicted regions and manually delineated region outlines

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IMAGING RELEASE OF siRNAs FROM VESICLES DAMAGED BY MEMBRANE-DISRUPTING DRUGS

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Small interfering RNAs (siRNAs) is a promising class of new therapeutics for targeting of disease-causing genes by RNA interference. For most siRNA-delivery approaches however, endosomal trapping of endocytosed siRNAs severely hampers the therapeutic effect. Only a small fraction ever exits the endosomes, which poses a significant barrier in the development of new siRNA therapeutic. Exploring strategies to improve the endosomal release of siRNAs, we investigated a collection of membrane destabilizing drugs reported to mediate lysosomal membrane permeabilization. These compounds were found to trigger the recruitment of cytosolic galectins to intracellular vesicles. The galectins are a family of β-galactoside-binding lectins, serving as sensors of membrane damage in live-cell microscopy experiments. The formation of galectin foci correlated with release of macromolecular dextran from later endosomal compartments. To study these processes in detail, we established a sensitive live-cell imaging approach for visualization and quantification of vesicle damage and release of conjugated siRNAs from individual endosomes. Using in-house developed data handling and analysis tools we are able to assess the disruption frequency of siRNA-containing vesicles, siRNA escape efficiency and release kinetics. In addition, we used supervised machine learning classification of time series measurements to characterize the compartment-identity of vesicles damaged by membrane disrupting drugs. Surprisingly, only a subset of damaged vesicles show clear presence of the lysosome associated membrane protein 1 (LAMP1). This indicates that membrane destabilizing drugs are diverse and not lysosome specific. The imaging and analysis platform used here could be extended for investigating vesicle damage in other contexts, as well as endosomal release of other macromolecules.

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Towards self-driving super-resolution microscopes for live-cell imaging

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KEYWORDS: super-resolution, structured illumination, fluorescence microscopy, automation, smart microscopy.

Super-resolution fluorescence microscopy has enabled un unprecedented insight into the workings of biological samples below the diffraction limit. However, it still faces big challenges when imaging living samples. The capabilities of live-cell super-resolution fluorescence microscopy are constrained and present a trade-off between sample health, amount of detected light, spatial and temporal resolutions[1]. This is in part due to limitations imposed by photobleaching and phototoxicity. How we balance the imaging parameters is a compromise that in turn sets the experimental conditions – which usually remain fixed and ignore changes in the sample.

Here, we propose a "sample-oriented" approach where experimental conditions are tailored to and adapt to changes in the sample. As proof of principle, we consider the specific case of mitochondrial division – a relatively fast but infrequent event. We combine the biological molecular marker Drp1 with on the fly image processing to identify mitochondrial division sites as events of interest. Simultaneously, we use the flexible acquisition speed of an instant structured illumination microscope (iSIM)[2] – spanning from timescales of 10 milliseconds to 100s of seconds – to dynamically adapt the temporal resolution of the acquisition, to better capture events of interest, while preventing unnecessary photobleaching, phototoxicity and acquisition of redundant data. Such adaptive temporal resolution allows for better optimization of the experimental conditions and more efficient acquisition of only the most essential data.

Overall, we develop a general framework outlining the three requirements for self-driving microscopes: (1) sensing; (2) computation or interpretation; and (3) actuation or adaptation to the sample. More generally, such self-driving microscopes will produce more useful, efficient and better quality data.

[1] N. Scherf and J. Huisken, 'The smart and gentle microscope', *Nat. Biotechnol.*, vol. 33, no. 8, pp. 815–818, 2015.

[2] A. G. York *et al.*, 'Instant super-resolution imaging in live cells and embryos via analog image processing', *Nat. Methods*, vol. 10, no. 11, pp. 1122–1126, 2013.

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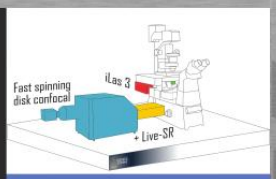

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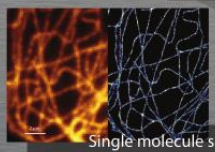
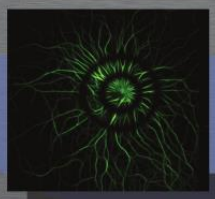
- Laser Launch (up to 8 lines)

Multi-angle TIRF acquisitions with penetration motorization



Single molecule super resolution



TIRF/FRAP

New Modular software

Fluorescence Detection

Visiview Software

Metamorph Software

Yokogawa CSUX1/W1

Photometrics Qimaging

X-Light Spinning Disk

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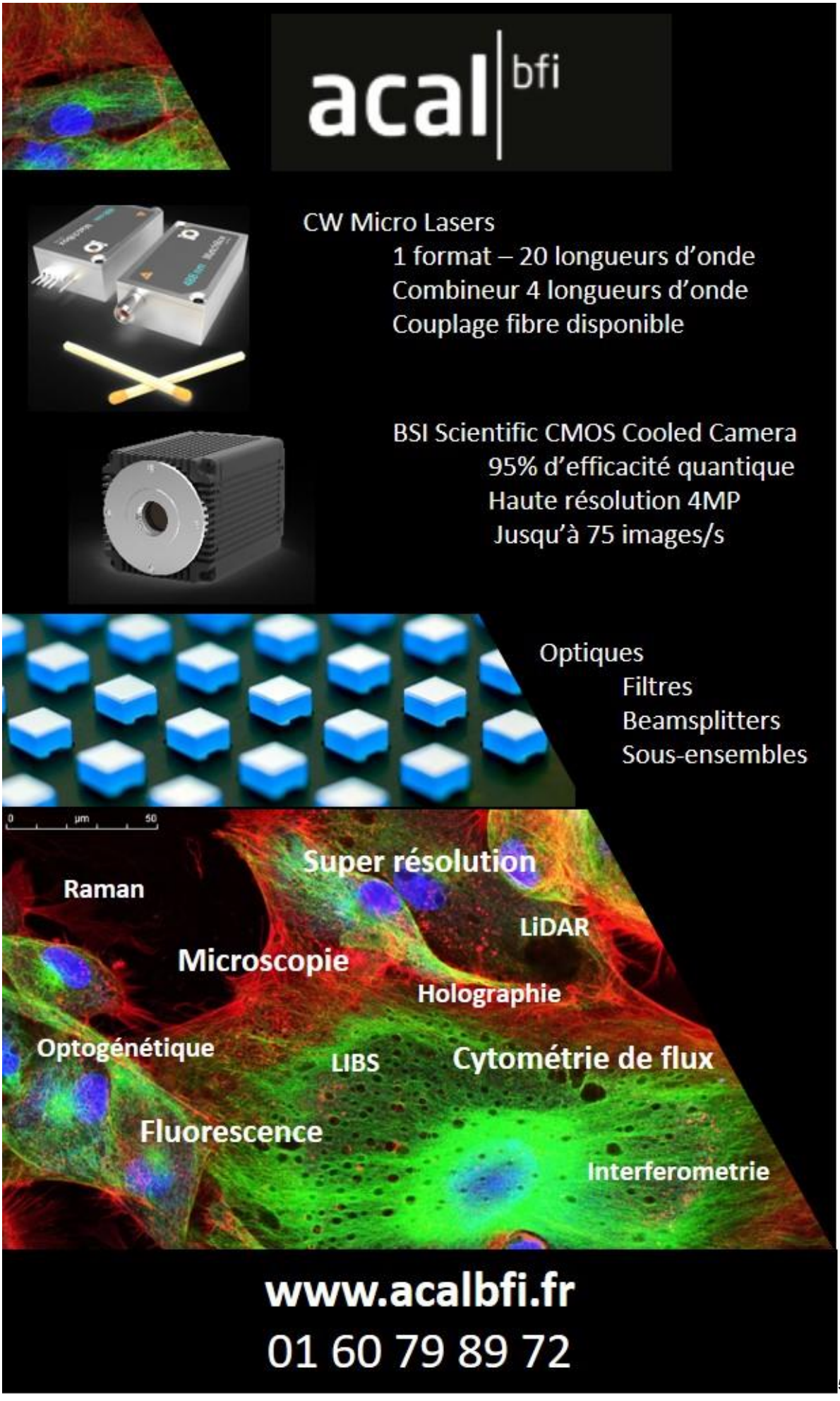
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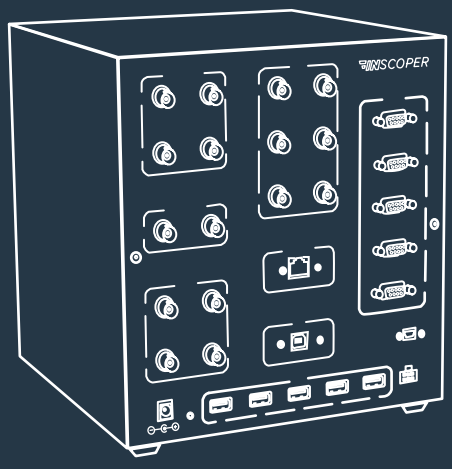
Fluorescence microscopy is an important tool in life science research, particularly because of its remarkable sensitivity and capability to image molecular events in living cells.

However, for many systems involving multiple hardware devices (such as cameras, shutters, filter wheels, stages, spinning disks), software-based solutions to control these devices always lead to latency effects which slow down the rate of image acquisition.



Inscoper, a spin-off of the Institute of Genetics and Development of Rennes (IGDR), develops solutions for life science microscopy systems, allowing user-friendly image acquisition and significantly increased acquisition speeds.

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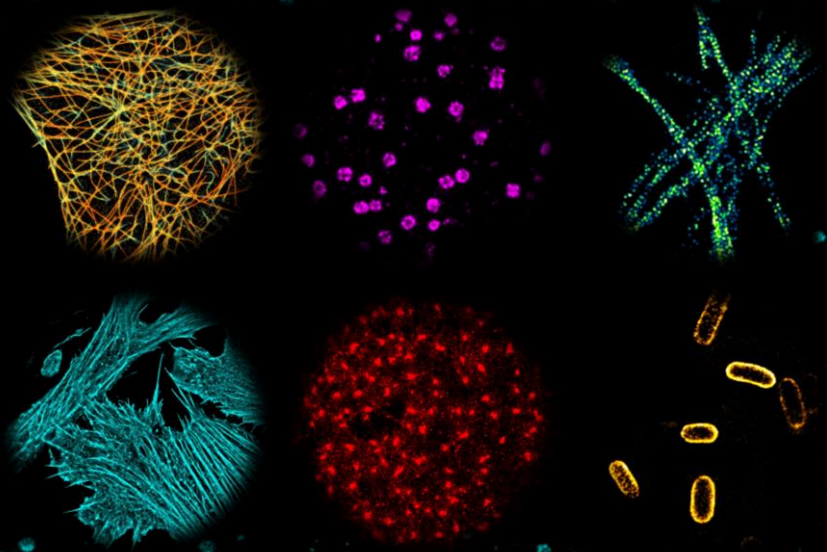

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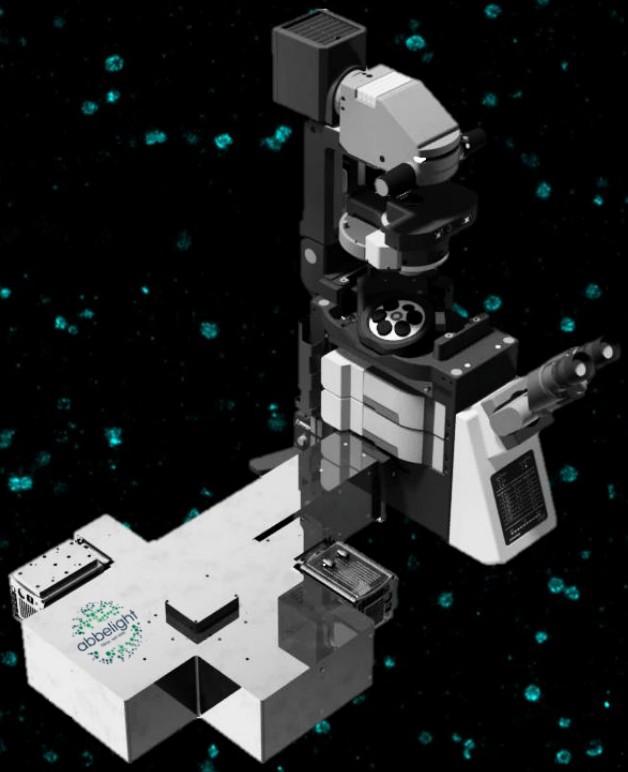

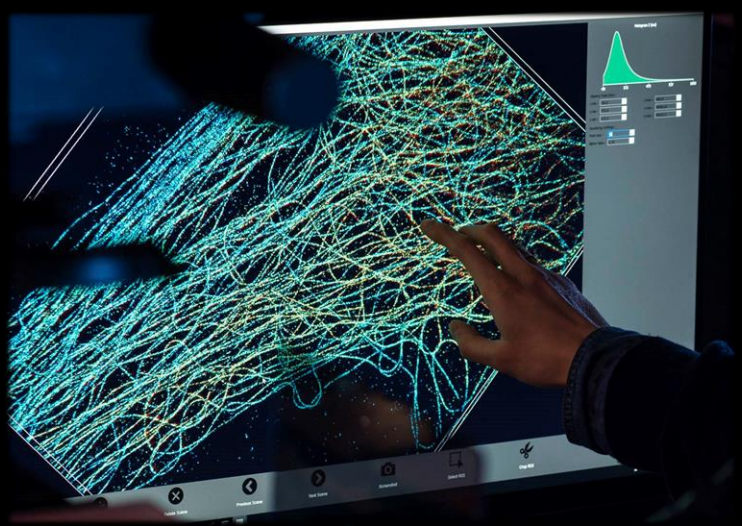

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