From 3D Light to 3D Electron Microscopy
EMBL Workshop

EMBL Advanced Training Centre, Heidelberg, Germany
13 – 16 March 2016
Expand Your Lab Capabilities with Latest Products from ZEISS

ZEISS Crossbeam Series
Look Deep Into the Secrets of Life

ZEISS Crossbeam technology provides you a complete 3D imaging system for your biological samples. It combines the GEMINI column’s well-established excellence in imaging with the milling of a superior FIB. Designed for high throughput and resolution, Crossbeam makes it quick and simple to bridge the gap between the micro and nano worlds. Each Crossbeam has a modular platform concept, an open and easily-extendable software architecture and unique solutions for challenging samples.

ZEISS ZEN Correlative Array Tomography
3D Correlative Light and Electron Microscopy For Serial Sections

With the software module ZEISS ZEN Correlative Array Tomography you automatically image hundreds of sections across length scales and combine them into one single correlative volume data set. Cut your resin embedded tissue samples with an ultramicrotome and image them. After automated image acquisition in the light microscope (LM), you transfer the sample to your electron microscope (EM) where you find the same software tools. With Array Tomography you use serial sections to reconstruct your sample volume.

From 3D Light to 3D Electron Microscopy

In Life Sciences, imaging in three dimensions is a prerequisite for the understanding of the fine organisation of cells and tissues. Whilst key biological functions are addressed by a large choice of fluorescence microscopy techniques, volume imaging by scanning electron microscopy offers now an unprecedented understanding of the ultrastructure of large biological objects. Correlating both imaging modalities, in 3D, is therefore a powerful way to link function to structure, even in complex biological systems.

EMBL EM Core Facility

The facility provides advanced expertise in electron microscopy, from sample preparation to image analysis, for a large variety of biological samples.

The EMCF activities cover a large spectrum of EM techniques with a major focus on sample preparation, immunolocalisation of proteins, ultrastructural analysis in 2D and 3D, correlative light and electron microscopy and data processing. Staff in the facility can help you to define optimal experimental conditions for your project – we have experience spanning virtually the full spectrum of biological specimens, with high-level resources for both research and training.

Advanced equipment: We offer access to a set of high-pressure freezing machines that are routinely used to vitrify biological samples. Specimens can then be dehydrated, stabilised and embedded in resins in specific freeze-substitution units. Strong expertise has been developed in yeast cells, adherent cultured cells, Drosophila embryos, nematodes, zebrafish embryos, and mouse tissues. A microwave-assisted sample processor, used for chemical fixation, dehydration and embedding, greatly reduces time spent preparing the samples (from days to hours). The electron tomography equipment includes a transmission electron microscope (microscope with a field emission gun and a direct electron detection camera) and computing set-up with programs for 3D reconstruction and cellular modelling. Specialised EM engineers have expertise in tomography data acquisition and processing.

This workshop is jointly organised by ZEISS and EMBL

EMBL Advanced Training Centre, Heidelberg, Germany, 13 – 16 March 2016

Scientific Organisers:

Yannick Schwab
EMBL Heidelberg, Germany

Nicole Schieber
EMBL Heidelberg, Germany

Robert Kirmse
Carl Zeiss Microscopy GmbH, Germany

Hans-Jürgen Oberdiek
Carl Zeiss Microscopy GmbH, Germany

Chris Guerin
VIB Ghent, Belgium

Saskia Lippens
VIB Ghent, Belgium

Conference Organiser:

Diah Yulianti
EMBL Heidelberg, Germany

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Session Overview

Sunday 13 March 2016
14:00 – 15:30 Arrival and Registration
ATC Auditorium

15:30 – 16:00 Welcome Remarks
Jan Ellenberg
EMBL Heidelberg, Germany

Markus Weber
Member of the Management Board, Carl Zeiss Microscopy GmbH, Germany
ATC Auditorium

16:00 – 17:00 Structural Neurobiology: Goals, Tools, Pitfalls
Winfried Denk
Max Planck Institute of Neurobiology, Germany
ATC Auditorium

Fred Hamprecht
University of Heidelberg, Germany
ATC Auditorium

18:30 – 19:30 Welcome Reception
ATC Foyer

Monday 14 March 2016
09:30 – 12:30 Session 1
3D Correlative Light and Electron Microscopy
Chair: Graham Knott, EPFL Lausanne, Switzerland
ATC Auditorium

12:30 – 13:30 Lunch and Poster Session 1
Odd Numbers
ATC Foyer

13:30 – 15:00 Workshops
Various Location* (s. page 13)

15:00 – 16:30 Workshops / Panel Discussion
Various Locations* (s. page 13) / ATC Auditorium

16:45 – 19:30 Session 2
The Challenges of Sample Diversity
Chair: Chris Guerin, VIB Ghent, Belgium
ATC Auditorium

Tuesday 15 March 2016
09:00 – 12:00 Session 3
From Large Volume Imaging to Data Analysis and Processing
Chair: Anna Krestuk, IWR and Heidelberg Collaboratory for Image Processing (HCI), University of Heidelberg, Germany
ATC Auditorium

12:00 – 13:00 Lunch and Poster Session
Even Numbers
ATC Foyer

13:00 – 14:30 Workshops
Various Locations* (s. page 13)

14:30 – 16:00 Workshops / Panel Discussion
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16:15 – 18:30 Session 4
How 3D CLEM Connects to other 3D Imaging Techniques
Chair: Peter O’Toole, University of York, United Kingdom
ATC Auditorium

18:30 – 20:30 Conference Special Dinner
EMBL Canteen

Wednesday 16 March 2016
09:00 – 16:00 Session 5
Diversity of Applications in Biology
Chair: Richard Webb, University of Queensland, Australia
ATC Auditorium

12:00 – 13:00 Lunch and Anonymous Feedback
ATC Auditorium

13:00 – 14:30 Workshops
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Various Locations* (s. page 13) / ATC Auditorium
**Sunday 13 March 2016**

14:00 – 15:30  
**Arrival and Registration**  
ATC Auditorium

15:30 – 16:00  
**Welcome Remarks**  
*Jan Ellenberg*  
EMBL Heidelberg, Germany  
*Markus Weber*  
Member of the Management Board, Carl Zeiss Microscopy GmbH, Germany  
ATC Auditorium

16:00 – 17:00  
**Structural Neurobiology: Goals, Tools, Pitfalls**  
*Winfried Denk*  
Max Planck Institute of Neurobiology, Germany  
ATC Auditorium

17:15 – 18:15  
**Machine Learning in Automated EM Volume Tracing**  
*Fred Hamprecht*  
University of Heidelberg, Germany  
ATC Auditorium

18:30 – 19:30  
**Welcome Reception**  
ATC Foyer

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**Keynote Lecture Abstracts**

**Structural Neurobiology: Goals, Tools, Pitfalls**  
*Winfried Denk*  
Max Planck Institute of Neurobiology, Germany

Mapping the wiring diagram of the brain requires nanometer resolution and millimeter, even centimeter, fields of view, both in 3 dimensions. I will discuss how serial block-face electron microscopy can achieve this and what might go wrong in the way to a circuit diagram of a whole mouse brain.

**Machine Learning in Automated EM Volume Tracing**  
*Fred Hamprecht*  
University of Heidelberg, Germany

Now that connectomics electron volume images are available in unprecedented quality and quantity (imaging of the first full fly brain was accomplished a few weeks ago) accurate and preferably dense tracing becomes a pressing issue. While humans manage to correctly trace the bulk of an EM volume, they are too slow. Algorithms are tireless, but do not reach human accuracy yet. I will discuss the role that machine learning (and artificial neural networks specifically) can play in automated tracing, and I will explain our latest strategy, which currently gives the best results in the two major connectomics tracing challenges.
Programme

Monday 14 March 2016

09:30 – 12:30  Session 1
3D Correlative Light and Electron Microscopy
Chair: Graham Knott, EPFL Lausanne, Switzerland
ATC Auditorium

1 09:30 – 10:15  Analysing Neuronal Plasticity using Focused Ion Beam Scanning Electron Microscopy
Graham Knott
EPFL, Switzerland

2 10:15 – 10:30  No two CLEM Experiments are the same: A Diversity of Challenges
Jemima Burden
MRC Laboratory for Molecular Cell Biology, United Kingdom

10:30 – 11:15  Coffee Break and Poster Session
Odd Numbers
ATC Foyer

3 11:15 – 11:45  A 3D CLEM Workflow for Cultured Cells
Lucy Collinson
The Francis Crick Institute, United Kingdom

4 11:45 – 12:15  Caught in the Act: High-Resolution 3D Imaging of Single Tumor Cells Invading Mouse Tissue
Matthia Karreman
EMBL Heidelberg, Germany

5 12:15 – 12:30  Advancing Array Tomography for Mapping of 3D Subcellular Localization in C. elegans
Sebastian Markert
University of Wuerzburg, Germany

12:30 – 13:30  Lunch and Poster Session
Odd Numbers
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# Programme

## Tuesday 15 March 2016

### 09:00 – 12:00
**Session 3**
**From Large Volume Imaging to Data Analysis and Processing**
Chair: Anna Kreshuk, IWR and Heidelberg Collaboratory for Image Processing (HCI), University of Heidelberg, Germany
ATC Auditorium

### 09:00 – 09:45
**EM Image Processing: Ilastik News and Algorithms with Priors**
Anna Kreshuk
University of Heidelberg, Germany

### 09:45 – 10:00
**3D Electron Microscopy of Biological Samples**
Minh Huynh
Australian Centre for Microscopy & Microanalysis, The University of Sydney, Australia

### 10:00 – 10:45
**Coffee Break and Poster Session**
Even Numbers

### 10:45 – 11:15
**Microscopy Image Browser: An Open Source Platform for Segmentation and Analysis of Multidimensional Datasets**
Ilya Belevich
University of Helsinki, Finland

### 11:15 – 11:45
**EcCLEM: an Open Source Plugin for CLEM Image and Volume Registration**
Perrine Paul-Gilloteaux
CNRS University of Nantes / France Bio Imaging, France

### 11:45 – 12:00
**From a Biological Sample to Highquality Digital Image Representation: Avoiding and Restoring Image Artifacts through proper Acquisition and Image Restoration**
Joris Roels
Ghent University iMinds VIB, Belgium

### 12:00 – 13:00
**Lunch and Poster Session**
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### 13:00 – 14:30
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### 14:30 – 16:00
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### 16:00 – 16:15
**Coffee Break**
ATC Foyer

### 16:15 – 18:30
**Session 4**
**How 3D CLEM connects to other 3D Imaging Techniques**
Chair: Peter O’Toole, University of York, United Kingdom
ATC Auditorium

### 16:15 – 17:00
**Making Light Work of 3D Bioimaging**
Peter O’Toole
University of York, United Kingdom

### 17:00 – 17:30
**Correlative Microscopy - Spectroscopy Studies of Bone Materials**
Silke Christiansen
Helmholtz-Zentrum für Materialien und Energie Berlin (HZB), Germany

### 17:30 – 18:00
**Structural Analysis of Multicellular Organisms with CryoFIB and Cryoelectron Tomography**
Jan Harapin
University of Zurich, Switzerland

### 18:00 – 18:15
**Shaping the Plant Endoplasmatic Reticulum**
Maike Kittelmann
Oxford Brookes University, United Kingdom

### 18:15 – 18:30
**X-Ray Microscopy in the Life Sciences**
Rosy Manser, Carl Zeiss Microscopy GmbH, Germany
ATC Auditorium

### 18:30 – 20:30
**Conference Special Dinner**
EMBL Canteen
EMBL Workshop: From 3D Light to 3D Electron Microscopy

Programme

Wednesday 16 March 2016

09:00 – 16:00  Session 5
Diversity of Applications in Biology
Chair: Richard Webb, University of Queensland, Australia
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Richard Webb
University of Queensland, Australia

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University of Cambridge, United Kingdom

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Ben Giepmans
UMC Groningen, The Netherlands

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Mikael Simons
Max Planck Institute for Experimental Medicine, Germany

12:00 – 13:00  Lunch and Anonymous Feedback
ATC Foyer

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Various Locations* (s. page 13)

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FIB-SEM
Location: EMCF Room 309

Sample Preparation
Location: EMCF Room 305A

3View
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Array Tomography
Location: ATC Microscope Room 116

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**EMBL Workshop: From 3D Light to 3D Electron Microscopy**

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Abstracts of Papers Presented at the EMBL Workshop:
From 3D Light to 3D Electron Microscopy

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1

Analysing Neuronal Plasticity using Focused Ion Beam Scanning Electron Microscopy

Graham Knott1, Corrado Calì2, Bohumil Maco1, Anne Jorstad1
1 EPFL, Switzerland
2 KAUST, Saudi Arabia

Presenter: Graham Knott

In vivo imaging of mammalian brains has shown that neurons continually make small changes to their structure throughout their lifetime. Correlative electron microscopy of these imaged cells has revealed how this structural plasticity corresponds to alterations in synaptic connectivity. Focused ion beam scanning electron microscopy enables precise, targeted, serial imaging through the regions of interest. The quality of the resulting image stacks, with near isotropic voxels, allows computer vision algorithms to automatically segment and reconstruct different features ready for quantitative analysis. This approach to morphometric analysis takes a fraction of the time compared to any manual method and has been used recently to better understand how dendritic spines form and make new synaptic connections. It has also proved to be a efficient technique to densely reconstruct complete volumes of neuropil from adult and aged mice revealing the subtle changes that occur in the mammalian brain, at the level of synaptic connections, during the aging process.

2

No two CLEM Experiments are the same: A Diversity of Challenges

Jemima Burden
MRC Laboratory for Molecular Cell Biology, United Kingdom

Presenter: Jemima Burden

Correlative light and electron microscopy (CLEM) is an exceptionally powerful tool in any cell biologist’s tool kit. Bringing the best features of both techniques to a single sample, allows researchers to gain not only live, time resolved imaging of their protein of interest, but also its ultrastructural localisation and context within the cellular environment. The flexibility of this approach also allows the tailoring of the experimental technique to the specific research question. Whether you are interested in CLEM at the cellular, organelle or virus level in the context of cellular monolayers, 3D cultures, tissues or model organisms, there are multiple approaches available to you. If you need the speed of spinning disc light microscopy or the increased resolution of the super resolution light microscopy techniques to be combined with the high ultrastructural detail of transmission electron microscopy (TEM) or the larger volume data sets from serial block face imaging – almost anything is possible. However, despite being possible, challenges abound. Here we introduce some technically challenging CLEM projects involving a variety of light (widefield/spinning disc/super resolution) and electron (serial section TEM and serial block face) microscopy techniques and present some of the tricks we used to overcome these obstacles.

3

A 3D CLEM Workflow for Cultured Cells

Lucy Collinson
The Francis Crick Institute, United Kingdom

Presenter: Lucy Collinson

In cell biology, fluorescence microscopy reveals dynamic localisation of labelled proteins and infectious agents, and subsequent correlation to electron microscopy fills in the structural context. With novel 3D microscope technologies entering both the light and electron arenas, new approaches to correlation must be developed. Here, we describe a workflow for 3D light microscopy (confocal or super resolution) to 3D electron microscopy (Serial Block Face SEM) of cells cultured on coverslips. The workflow considers cell culture conditions, targeting of the cell of interest, resin embedding and relocation of the cell of interest at the block face, block mounting, imaging strategies in the SEM and alignment of the two 3D datasets for analysis. When required, additional resolution can be gained by integrating TEM of serial sections into the workflow. Recent examples of biological applications in the field of infection and immunity will be highlighted.

4

Caught in the Act: High Resolution 3D Imaging of Single Tumor Cells Invading Mouse Tissue

Matthia Karreman1, Luc Mercier1, Nicole L. Schieber1, Gergely Solecki1, Guillaume Allio1, Frank Winkler1, Bernhard Ruthensteiner1, Jacky G. Goetz2, Yannick Schwab1
1 EMBL Heidelberg, Germany
2 INSERM U1109, France
3 DFUZ, Germany
4 Zoologische Staatssammlung München, Germany

Presenter: Matthia Karreman

Metastasis is the main cause of cancer related mortality, but how tumor cells spread through the tissue in vivo is still largely unknown. Intravital microscopy (IVM) enables studying crucial steps of the metastatic process, but it is limited in resolution and it fails to reveal the structural context. Combining IVM to 3D Electron Microscopy (3DEM) enables to correlate functional and dynamic in vivo imaging to high resolution of the tumor cells and their microenvironment. However, keeping track of single tumor cells when moving from IVM to EM imaging is highly challenging in complex tissue samples. We have developed a method that exploits x ray microscopic computer tomography (microCT) to correlate IVM to EM. First, fluorescent tumor cells are xenotransplanted to living mice, and monitored by IVM. 3D datasets of the tumor cells and the surrounding fluorescently stained vasculature are obtained. Next, the region of interest is processed for EM and embedded in resin. The sample is then scanned with microCT, revealing the outlines of the resin block, the biopsy and the vasculature within. Using 3D imaging software, the IVM and microCT volumes are registered, enabling to map the position of the tumor cell within the resin embedded specimen. Finally, targeted trimming enables to quickly approach the tumor cell inside the resin block and expose it for 3D imaging with FIB SEM or electron tomography. The method will be demonstrated on capturing arrested tumor cells in the vasculature of mouse brain samples, and on migrating invasive tumor cells in mouse skin. Enabling to predict the position of the tumor cell within the resin block with an accuracy of ~5 µm significantly speeds up the process of correlating IVM to EM; from several months to ~2 weeks. The correlative approach uniquely enables multiple high resolution observations of rare metastatic events in tissue, allowing obtaining statistically relevant conclusions on the crucial steps in the dissemination of tumor cells.
Advancing Array Tomography for Mapping of 3D Subcellular Localization in C. elegans

Sebastian Markert, Sebastian Britz, Sven Proppert, Marietta Lang, Daniel Witvliet, Ben Mulcahy, Markus Sauer, Mei Zhen, JeanLouis Bessereau, Christian Stiglhofer

1 University of Wuerzburg, Germany
2 Lunenfeld-Tanenbaum Research Institute, Canada
3 eCGPhIMC Université, France

Presenter: Sebastian Markert

Array tomography (AT) makes it possible to obtain large volumes of correlated multi channel light and electron microscopic data. Long ribbons of serial ultrathin (50-200 nm) resin sections are applied to a glass surface and proteins of interest are labelled via immunohistochemistry. The sections are then imaged by fluorescence light microscopy (FLM), whereby super resolution in axial direction is achieved, since z resolution is determined by section thickness. After light microscopic imaging, the sections are processed for scanning electron microscopy (SEM). By using a detector for backscattered electrons at extremely low angles, ultrastructural features can be readily imaged at high resolution. FLM and SEM images are then correlated to put the molecular labelling in its full ultrastructural context. We advanced AT by combining SEM with super resolving Structured Illumination Microscopy (SIM) or direct Stochastic Optical Reconstruction Microscopy (dSTORM) on the same section to reach super resolution not only in z, but also in x and y. In addition, we established a method for easy, precise, and unbiased correlation. Together, these advances make it possible to map even small subcellular structures with high precision and confidence in 3D, what we exemplary show in C. elegans.

What’s Coming Through the Door now? Customizing Sample Preparation for Volume EM in a, Imaging Core Facility

Chris Guerin

VIIB, Belgium

Presenter: Chris Guerin

Volume EM began as a technique for neuroscience research, and it is still used heavily in connectomics. Brain is a very friendly sample to image in an SEM and good results can be obtained using a standard protocol (OTO/OTO). However, in the environment of a core facility there is no telling what someone will ask you to look at next, so customizing sample preparation to optimize imaging results is an important aspect of providing the best service to our users. In the VIIB Bio Imaging core in Ghent we have looked at samples from viruses to bacteria, trypanosomes to plasmodium as well as cells and tissues from mouse to human (in addition to several different species from the plant kingdom). The diversity of these samples provided many challenges in both staining and embedding. In many cases the problems revolved around generating contrast, minimizing charging and providing a proper block consistency for good sectioning. I will give examples of the problems we faced and some of the solutions we found in this challenging but exciting process.

The Acquisition of 3D Datasets at Ultrastructural Level following Light Microscopy Image Acquisition: Few Examples

Christel Genoud, Alexandra Graff Meyer

Friedrich Miescher Institute for Biomedical Research, Switzerland

Presenter: Christel Genoud

3D imaging by serial sections or serial block face scanning electron microscopy (SBEM) is a promising approach to reconstruct large volume in order to analyze their functions as well as to localize molecules in small organisms, tissues or cells in culture. In order to correlate light microscopy signals with ultrastructure, we will show some examples where we combine different EM techniques to solve different biological questions. In some cases, the SBEM is used as a screening tool to target a region that is then used in TEM to perform immunolabelling or tomography. In another approaches, overlay of fluorescence and SBEM images allow to identify a cell compartment or better understand the 3D structure of organeloids. In combination with Apex2 labeling, we used a correlative approach to visualize the uptake of vesicles. In summary, we did not develop a common workflow for 3DCELM but we have to find a unique solution for each request we receive as we are facing a diversity of sample and scientific questions. Each time, we have to adapt the sample preparation, the imaging modalities and the following quantitative analysis.

3D EM in Multiuser Environment: From Cultured Cells to Plant Roots and Human Carotid Arteries

Eija Jokitalo, Helena Vihinen, Ilya Belevich

1 Institute of Biotechnology, Finland
2 University of Helsinki, Finland

Presenter: Eija Jokitalo

Serial block face imaging using SEM (SB EM) has demonstrated its power in imaging large tissue specimens, such as sieve elements of Arabidopsis thaliana roots and carotid arteries of human patients. However, it has high potential for cell biology, too. We have used SB EM to analyze large extended organelle networks (e.g. ER and mitochondria), to quantitatively map the occurrence and distribution of different cell structures (e.g. autophagosomes and lipid droplets) in large numbers of cells, and to reveal the organelles in close proximity to each other. On the other hand, in projects focusing on organelle contacts or cytoskeleton, high pressure freezing and freeze substitution are used to ensure the best morphological preservation at the expense of high contrast, and thereby electron tomography will be better suitable option for imaging. As all of the methods have their pros and cons, it is important to discuss how different methods are chosen to best answer specific biological questions, and how much the chosen imaging method guides specimen preparation and vice versa.
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New Tools for Correlated Microscopies and Serial Blockface EM

Thomas Deerinck
The National Center for Microscopy and Imaging Research, University of California, San Diego.

Presenter: Thomas Deerinck

Serial blockface scanning electron microscopy (SBEM) is revolutionizing ultrastructural studies by allowing rapid and simple acquisition of large 3D datasets at EM resolution. One of the prerequisites for labeling proteins and organelles for correlated light and SBEM is the introduction of labels prior to embedding while maintaining the integrity of cellular architecture. Several new molecular-genetic labels have been recently introduced, including MiniSOG and APEX2 tags (Shu et al., 2011, Lam et al., 2015) that meet the needs for genetic labeling of fusion proteins without the need for permeabilizing detergents or compromised primary chemical fixation. Here we present two new tools for multiscale imaging. The first is a split HRP developed as a biocomplementation tool to study intercellular protein-protein interactions (Martell et al., in press). The second is a powerful and versatile new labeling approach based on click chemistry we named “Click-EM” which allows for the selective contrasting of a wide variety of cellular constituents and non-proteinaceous targets in both cells and tissues (Ngo et al., 2016). Click-EM utilizes copper mediated click chemistry labeling of metabolically incorporated precursor molecules with a wide range of functionalized fluorescent dyes in order label proteins, carbohydrates, lipids and nucleic acids. As with MiniSOG and APEX2, Click-EM allows for highly correlated LM and EM using fluorescence imaging followed by photooxidation of diaminobenzidine and provides uniform high resolution 3D labeling and simultaneous excellent preservation of cell ultrastructure. Additionally, incorporation of metabolic labels is time-dependant, opening the possibility of dynamic synthesis and turnover studies of cellular constituents at nanometer-scale resolution.

References:


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Correlated Serial Section Light and Electron Microscopy of the Cell-Cell Recognition Process during Zipping in Drosophila melanogaster

Mandy Börmel, Ohad Medalia, Damian Brunner
University of Zurich, Switzerland

Presenter: Mandy Börmel

Dorsal closure is a morphogenetic process, occurring in developing Drosophila melanogaster embryos, during which an epithelial gap is closed. A process called zipping completes dorsal closure. Cellular protrusions from the opposing epithelial leading edges sample the open space until the gap is sufficiently closed for them to engage with their accurate counterparts. The leading edge cells match according to their positional identity along the anterior posterior axis. How this positional information is conferred remains elusive. New data from our lab employing large volume electron tomography revealed large lamellar overlaps, with which leading edge cells from one side engage with those from the other side – thereby producing a significant single contact surface. We speculate whether the nature and 3D arrangements of these overlapping lamella confer any positional cue. In particular, we wonder whether the left/right up/down organization of the overlapping lamellae is determined by anterior/posterior identities and could therefore contain positional information. To study this hypothesis, we investigate the organization of the overlapping lamellae at the zipping site using 2D electron microscopy, combined with visualizing the posterior or anterior identity of the respective cells by using fluorescence light microscopy. Therefore, I established Correlated Serial Section Light and Electron Microscopy. The chosen pipeline, combining work from the Briggs and Rubin lab, preserves the endogenous GFP signal in cells with posterior identity up to 6 weeks after sample preparation while simultaneously achieving a subcellular preservation of membranes. First data of three different zipping sites, each covering approximately 30 um with serial sections, suggest a possible role of left right asymmetry for instructing positional information on opposing zipping cells.
3D Subcellular Imaging of the Hepatitis A Virus (HAV) Induced Replication Factories
Inés Romero Brey¹, Nicole Schiebel², Katharina Esser Nobis¹, Yannick Schwab¹, Ralf Bartenschlager¹, Volker Lohmann¹
¹ University of Heidelberg, Germany
² EMBL Heidelberg, Germany
Presenter: Inés Romero Brey

Electron tomography (ET) has enormously contributed to increase our knowledge about how viruses interact with their cellular host and, as a result of such an interaction, how the cell landscape and their membranes are being extensively remodeled. In this study we have applied ET to elucidate the cytoarchitecture of the replication organelles induced by Hepatitis A Virus (HAV), a member of the family Picornaviridae. Our tomographic analysis of cells expressing the HAV replicase proteins revealed that the HAV induced structures have a rather tubular morphology. These structures are found tightly apposed to each other, forming well defined cytosolic clusters. However, only a few of these tubular compartments seem to be interconnected. Furthermore, we have also observed connections of these HAV induced tubules to the surrounding endoplasmic reticulum (ER) membranes. In addition we have also imaged by Focus Ion Beam Scanning Electron Microscopy (FIB SEM), HAV infected cells that were previously selected by means of fluorescence microscopy. This correlative analysis provided us with detailed ultrastructural information, not only about the architecture of the HAV induced replication factories, but also about other cell organelles.

EM Image Processing: ilastik News and Algorithms with Priors
Anna Kreshuk
University of Heidelberg, Germany
Presenter: Anna Kreshuk

Machine learning is the cornerstone technique of modern image analysis. The ilastik toolkit brings learning based image analysis to the lab bench by allowing application domain experts to train segmentation, tracking and counting algorithms interactively. I will show the latest developments in ilastik, which make it faster, easier to use and better adapted to very large scale data processing. Complementary to the generic methods of ilastik, I will talk about specialized algorithms for EM neural data processing. In particular, I will show how high level biological priors can be exploited to improve neuron reconstruction for mammalian data and synaptic partner detection for insect data.

3D Electron Microscopy of Biological Samples
Minh Huynh, Patrick Trimby, Matthew Foley, Filip Braet
Australian Centre for Microscopy & Microanalysis, The University of Sydney, Australia
Presenter: Minh Huynh

Developments in serial block face scanning electron microscopy (SBF SEM) over the past few years have enabled biologists to image large volumes of various biological tissue in a fully automated and efficient way at nanometer resolution. In this presentation we will showcase some examples of the many different biological samples we have imaged on our 3view SBF SEM. Current projects include the large volume imaging of: ultra structural changes in the brains of malaria infected mice, ultra structural characterisation of liver tissue in rats and zebra fish, remodelling of the uterine epithelium during pregnancy, distribution and frequency of plasmodesmata in plants, distribution of anti cancer drugs in tumour spheres and white matter degeneration in brain tissue collected from chronic alcoholics. We will also discuss the advantages and disadvantages of this technique, along with some of the challenges facing the application of SBF SEM in the context of a centralised core facility serving more than 300 users per annum, focusing on issues such as user training, sample preparation, large data handling, 3D image visualisation and analysis.

Microscopy Image Browser: An Open Source Platform for Segmentation and Analysis of Multidimensional Datasets
Ilya Belevich, Merja Joensuu Joensuu, Darshan Kumar, Helena Vihinen, Eija Jokitalo
University of Helsinki, Finland
Presenter: Ilya Belevich

Understanding the structure – function relationship of cells and cell organelles in their natural context requires multidimensional imaging. Recent technical advances especially in multimodal 3D imaging techniques have enabled a new insight into the morphology of tissues, cells and organelles. As the performance and access to such techniques are improving, the amounts of collected data are growing exponentially posing a question about effective processing, visualization, and analysis of these large datasets. Quite often the detailed analysis of multidimensional data is impossible without segmentation of objects of interest out of the volume (creating of a model). Usually the segmentation is the most time consuming and challenging part of the image analysis routine. For example, it may take up to a month to properly segment a single electron tomogram. The slowness of the process is caused by two main factors: limited number of good software tools (even commercial ones) and segmentation algorithms that can be applied to facilitate the modeling. As a result, the real potential of the collected data is not completely realized. Here, we present a free user friendly software package (Microscopy Image Browser, MIB, http://mib.helsinki.fi) for effective segmentation and image processing of multidimensional datasets that improves and facilitates the full utilization of acquired data and enables quantitative analysis of morphological features. MIB is written in Matlab language which is familiar to many researchers and available for main common operating systems (Windows, Mac and Linux); alternatively MIB is also distributed as a standalone package for both Windows and Mac OS. The access to the code and the open source environment enables fine tuning and possibility of adding new plug ins to customize MIB for specific needs of any research project. Even though the focus of the program is 3D segmentation of electron microscopy datasets, MIB is rather universal and can be used to perform segmentation, analysis and visualization of 2D 4D datasets obtained by light microscopy. We already utilized MIB in more than 10 scientific research projects, where it allowed us to facilitating the segmentation part of the image processing workflow significantly.
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EcCLEM: An Open Source Plugin for CLEM Image and Volume Registration

Perrine Paul-Gilloteaux¹, Jean Salamero², Graca Raposo², Xavier Heilgenstein²

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² CNRS Institut Curie/ France Bio Imaging, France

Presenter: Perrine Paul-Gilloteaux

Image registration is one of the tedious tasks in a CLEM workflow due to the difference of scales and appearance of the EM and LM images. After reviewing the main difficulties in image and volume registration for CLEM, I will present here a simple plugin, dedicated to image registration based on manual landmarks identification by the operator, for 2D 2D image alignment, 3D 3D but also 3D 2D. The image transformation is calculated as a similarity transform, (2D or 3D rotation, translation, scaling and flip) and simultaneously displayed to guide the user and improve the landmarks pairing process, in speed and accuracy. A pyramidal approach in the case of large volume data sets is also proposed to reduce the computational load. From the landmarks pairing process, we display a statistical rigid registration error map to guide the positioning of landmarks in order to assign precisely a fluorescent signal to its corresponding EM ultrastructure. This error map is then compared to the actual discrepancy in position of landmarks and images and an automatic non rigid registration is then proposed to deal with local deformation which can be due to sample distortions for instance. The purpose is to reduce morphing artefacts by assessing first a rigid alignment before switching to non rigid registration.

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From a Biological Sample to High Quality Digital Image Representation: Avoiding and Restoring Image Artifacts through proper Acquisition and Image Restoration

Joris Roels¹, Jan Aelterman², Jonas De Vylder², Hiep Luong², Saskia Lippens³, Chris Guerin³, Yvan Saeys⁴, Wilfried Philips²

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Presenter: Joris Roels

The objective of modern microscopy is to acquire high quality image based data sets. A typical microscopy workflow is set up in order to address a specific biological question and involves different steps: sample preparation, image acquisition, image storage, restoration (if necessary) and analysis. In order to analyze the images and draw scientific conclusions, it is crucial to obtain image data that reflects reality as close as possible. We provide an overview of the fundamental artifacts and degradations that affect many micrographs during the acquisition and storage phase: non uniform illumination, blur, noise, digitization and compression. Image restoration techniques such as flat field correction, deconvolution and denoising can manipulate the acquired data in an effort to reduce the impact of artifacts due to physical and technical limitations, resulting in a better representation (i.e. a more accurate correspondence to reality) of the object of interest. However, precise usage of these algorithms is necessary in order to not introduce further artifacts that might influence the data analysis and bias the conclusions. For example, high JPEG compression rates may result into small file sizes, but typically introduce blocking artifacts that might affect consecutive image segmentation. It is essential to understand image acquisition and storage, and how it introduces artifacts and degradations in the acquired data, so that their effects on subsequent analysis can be minimized. We describe why artifacts appear, in what sense they impact overall image quality and how to mitigate them by first improving the acquisition parameters and then applying proper image restoration techniques.
Making Light Work of 3D Bioimaging

Peter O’Toole
University of York, United Kingdom

Presenter: Peter O’Toole

3D Bioimaging arguably came to life with the confocal microscope era and we continue to develop microscopy to help address more complex questions. Advances include these OS examples: The standard confocal microscope that readily enables optical sectioning of fluorescently stained samples. The advent of GFP which permits 3D imaging of more complex live samples and studies of individual proteins and multicellular dynamics. Multiphoton microscopy that takes us deeper into in vivo samples. Increases in detector sensitivity which has helped lower toxicity for live cell experiments and increases the available speed to vitiave temporal issues. Light Sheet microscopy that gives us the ability to image whole organisms over time with minimal perturbation. Together, we now have a huge arsenal of techniques in which to help visualise and inform our fundamental understanding of biology and how cells work. During this presentation, I will touch on the need to become less invasive for primary studies. The need to study the individual in real time as well as the population. The need to understand more about the individual through more complex microscopy techniques with high multiplex assays, higher resolution through to genotyping the same cell and making these technologies united. We are currently involved with development of multiple new imaging technologies. There is an increasing realisation that we need to vastly improve and exploit label free techniques. Ptychography is a quantitative label free, high contrast, live cell imaging technique. We have been exploring the capabilities of this technique and have demonstrated its ability to be used in the studies of cell cycle (1), apoptosis, differentiation, and now acting as a spur for novel experiments with groups focussed cancer research, immunology, stem cell and neurobiology. We are also developing novel correlative/integrated light and electron microscopy techniques. Recent advances in biological imaging have seen new techniques developed in an attempt to improve resolution using light microscopy (PALM/STORM/STED etc) and using Correlative Light and Electron Microscopy (CLEM). Despite these intricate and exciting developments, they are still not ideal for many biological studies and the methods are far from simple to apply by non specialists to help make crystal clear our fundamental understanding. Whilst the new techniques are bringing us closer to this goal, there still remains a critical resolution gap between electron and light microscopy. Our approach in collaboration with Lucy Collinson at the CRICK is to use a novel electron Super Resolution Microscopy (eSRM) technique, based on the novel ClairScope (2), that will seamlessly couple the technologies of electron and light microscopy together to achieve address key functional questions of protein-protein interactions. These techniques are at the two extremes of imaging modalities and I will give a nice speedy overview of some of the latest approaches that we have been involved with that bridge the gaps. This will cover AiryScan imaging, through to multiphoton and light sheet microscopy using example data from our own labs.

References:
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Structural Analysis of Multicellular Organisms with Cryo FIB and Cryo Electron Tomography

Jan Harapin
University of Zurich, Switzerland

Presenter: Jan Harapin

The direct application of cryo electron tomography (CET) is restricted to thin specimens such as peripheral areas of intact eukaryotic cells. The analysis of whole mount biological samples, however, requires chemical fixation and application of sectioning procedures prior to imaging with CET. We developed a method for visualizing tissues from multicellular organisms using cryo electron tomography. Our protocol involves vitrifying samples with high pressure freezing, thinning them with cryo FIB SEM (focused ion beam scanning electron microscopy) and applying fiducial gold markers under cryogenic conditions to the lamellae post milling. We applied this protocol to acquire tomograms of vitrified Caenorhabditis elegans embryos and worms, which showed the intracellular organization of selected tissues at particular developmental stages in otherwise intact specimens. The method described here offers a general solution for acquiring tomograms on thick samples with CET and can now be implemented to resolve macromolecular structures contained therein.

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Shaping the Plant Endoplasmatic Reticulum

Maike Kittelmann, Verena Kriechbaumer, Petra Kiviniemi
Oxford Brookes University, United Kingdom

Presenter: Maike Kittelmann

In plants the endoplasmic reticulum (ER) is the essential organelle for protein and lipid biosynthesis and storage and thus for plant yield. It has a very dynamic architecture consisting of tubules and sheets to allow for different biosynthetic functions; however the exact structure/function relationship is still unclear. In our lab we are specifically interested in how the ER network is formed and maintained and how cell plate formation after mitosis is affected by ER shaping proteins. Mutations in the plant atlastin homologue RHD3 and interaction partners such as reticulon 13 (RTN13) have been shown to alter the shape of the ER in Arabidopsis and tobacco leaves to more a more tubular form. Expression of non functional forms of other members of the reticulon family such as RTN3, induce defects in cell plate formation. As part of a wider collaboration I am analysing the ER morphology in wild type and mutant Arabidopsis root cells using our 3View system. Serial images of entire cells and cell plates with the ER specifically stained using the zinc iodide/osmium protocol are collected and then semi automatically rendered for analysis. Additionally I use optical tweezers to manipulate Golgi bodies that are connected to the ER to test if mechanical characteristics of the ER change in these mutants. With the lipid and protein analysis by our collaborators this will help us understand how ER morphology affects protein storage and lipid biosynthesis.

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X-Ray Microscopy in the Life Sciences

Rosy Manser, Carl Zeiss Microscopy GmbH, Germany

X-ray microscopy (XRM) provides non-destructive 3D imaging capabilities on specimens across a range of length scales, observing features with sizes spanning from nanometres to millimetres. Recent developments, inspired by results from dedicated synchrotron instruments, have incorporated a number of X-ray optical elements that have driven resolution and contrast to levels previously unachievable by conventional X-ray computed tomography (CT) instrumentation. As ZEISS continues its effort to develop laboratory X-ray microscopes, building on the technology developed from the company’s synchrotron heritage, we investigate the emerging breadth of applications coming from this new range of instruments. This includes the latest innovations in correlative microscopy, with developed workflows between XRM and Crossbeam (FIB SEM).

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New Sample Processing for Serial Block Face Scanning Electron Microscopy

Richard Webb, Robyn Chapman
University of Queensland, Australia

Presenter: Richard Webb

Serial block face scanning electron microscopy (SBF SEM) makes it possible to obtain three dimensional electron microscopy data over a large area of a biological sample in a semi automated fashion. However, as an SEM technique it suffers from problems of charging and signal generation. Sample processing protocols have been developed that deposit substantial amounts of metal into the sample in an attempt to overcome these issues. The processing though is long, taking several days. By using a sample processing microwave oven and quick polymerisation the full process can now be done in under 5 hours. The addition of chemicals such as imidazole can improve the electron density produced by this processing. Up to now these protocols have involved samples being processed at room temperature. It has long been known that rapid freezing followed by freeze substitution gives superior preservation of morphology to processing chemically at room temperature. New protocols will be presented that utilise quick freeze substitution processing and that produce samples that work well for SBF SEM even when imaged in high vacuum mode. Chemicals such as lanthanum chloride or imidazole can be included into the freeze substitution medium with osmium tetroxide and these impart a high electron density to the sample. Subsequently a double osmium method can be performed by using tannic acid or thioarcbhydrodiazide. Once warmed to room temperature other metal containing solutions such as uranyl acetate and lead acetate are used to give the sample more electron density. Heating after freeze substitution also imparts better staining to the samples. By utilizing the quick freeze substitution and rapid embedding methods the entire process can be completed in well under a day.
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Julian Ng¹, Gregory Jeffries²
¹ University of Cambridge, United Kingdom
² Medical Research Council Laboratory of Molecular Biology, United Kingdom

Presenter: Julian Ng

Labelling probes are widely used in fluorescence light microscopy to localise molecules and sub cellular structures within cells, for cell type identification and morphological tracing studies. The equivalent approach using electron microscopy (EM) has the additional benefits of visualising these structures in context under which these labelled structures reside in within their cellular, tissue, anatomical and whole organism environment, all at nm scale resolutions. This is particularly important in cellular neurobiology as neurons have diverse functions, are highly interconnected and densely packed in the brain. Neurons have specific morphological and molecular features that function within axonal, dendritic and synaptic compartments that often can only be resolved at nanometer scale resolutions. Identifying and determining the structural relationship of these constituent parts helps to understand the role of each neuron plays within the nervous system. Here we assess the potential of the genetically encoded EM dense marker miniSOG as, 1) reporters of defined neuronal populations, for 2) their ability to label neuronal sub compartments (mitochondria, synapses and membranes) and, 3) their labelling detection under different EM image acquisition strategies, in Drosophila brain tissue. We also present an imaging strategy that uses non destructive X rays to screen for samples and identify target regions of interest for subsequent volume EM analysis.

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3D CLEM: Past, Present and Future of Correlative Microscopy

Roger Wepf, Miriam Lucas
ETH Zurich ScopeM, Switzerland

Presenter: Roger Wepf

Since more than 40 years scientists try to combine light and electron microscopy to gather detailed information from biological samples for a better understanding of structure function relations. In parallel, the largest attempt to get a full 3D map of a biological organism the Nematoode C.elegans on the nanometer scale was performed during the years 1970-1986 leading to one of the longest articles ever published in the field of structure research by J. G. White and S. Brenner in Phil. Trans. R. Soc. London B314;1986 p 1-340. Combining both methods seems to be the obvious way to go for, as most recent publications in 3D CLEM highlight with excellent findings and 3D structure models. Doubtless to say, we all enjoy what we are doing and achieving in the “3D CLEM” field, but we have to answer a few questions either between us, the experts, or for the future users. Notwithstanding the excellent 3D CLEM work demonstrated and performed so far, we have to ask ourselves: is it worth the effort? Do we have the right tools at hand? Can the workflow be streamlined in such a way that it will become a routine method for Life Science one day? What are the prerequisites for a meaningful 3D CLEM approach? What are the limitations? How can, and in which direction shall correlative imaging be expanded to help solving biological questions?

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Large Scale EM (“Nanotomy”) & Correlative Microscopy

Ben Giepmans
UMC Groningen, The Netherlands

Presenter: Ben Giepmans

Microscopy has gone hand in hand with the study of living systems since van Leeuwenhoek observed microorganisms and cells in 1674 using his light microscope. A spectrum of dyes and probes now enable the localization of molecules of interest within living cells by fluorescence microscopy. With electron microscopy (EM), cellular ultrastructure has been revealed. Bridging these two modalities, correlated light microscopy and EM (CLEM) opens new avenues. The first focus will be on recent developed labeling strategies for molecules that allow CLEM. These include particles (gold, quantum dots) to highlight endogenous proteins, but also genetically encoded probes, as well as traditionally used stains for light microscopy that aid in EM analysis of samples. Probes that can only be detected in a single modality, and require image overlay, as well as combinatiorial probes that can be visualized both at LM and EM will be discussed. In addition, new approaches for large scale EM (www.nanotomy.org), either TEM based or ST(EM) based, to visualize macromolecules and organelles in the context of organized cell systems and tissues will be highlighted. Matching the areas of acquisition in CLEM and EM will not only increase understanding of the molecules in the context, but also is a straight forward manner to combine the LM and EM image data. Covering a wide variety of probes and approaches for image overlay will help to enable (new) users to implement CLEM to better understand how molecules (mal)function in biology.

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Myelination in the CNS

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¹ Tü Munich, Germany
² Max Planck Institute for Experimental Medicine, Germany

Presenter: Mikael Simons

Information processing in complex organisms requires fast nerve transmission in both the peripheral and central nervous system (CNS). In vertebrates this is accomplished by the ensheathment of axons with myelin that confines the action potential to the node of Ranvier to allow saltatory nerve impulse transmission. Oligodendrocytes, the myelinating cells in the CNS, are specialized in synthesizing large amounts of plasma membrane. First, oligodendrocyte precursor cells (OPCs) differentiate to premyelinating oligodendrocytes with highly ramified processes. After oligodendrocyte to axon contact has been established, the cellular processes are converted into flat sheets that spread and wind along the axons, followed by the extrusion of the cytosol from the different myelin membrane layers. The result is an insulating multi layered stack of membranes that are tightly attached at their cytosolic and external surfaces. We are interested in the question of how myelin is formed in the central nervous system during normal development. To answer this question we use an array of different techniques and approaches to dissect the mechanisms of myelin sheath growth. We use in vivo live imaging of myelin sheath growth in zebrafish. We perform detailed ultrastructural reconstruction of myelin sheaths (including 3D) using both transmission electron microscopy (TEM) following high pressure freezing and scanning electron microscopy of slices generated by focused ion beam milling (FIB SEM) to obtain snap shots of myelina ultrastructure during development of the optic nerve. Using this approach we could elucidate the mechanism of how myelin is wrapped around the axons (Snaidero et al. Cell 2014).
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Dynamics of Golgi Apparatus Studied by Correlative Microscopy

Areej Albariri, Thomas Kuner, Markus Grabenbauer
Heidelberg University, Germany

Presenter: Areej Albariri

The Golgi apparatus is a highly dynamic organelle, which plays important roles in many cellular mechanisms like protein secretion, lipid metabolism, intracellular signaling, and regulation of cell division. The structure and function of the continuous and intricate network of the Golgi apparatus in neurons is not well delineated, therefore we are developing novel methodologies for correlative microscopy, which combines fluorescence microscopy of in vivo dynamics with the resolution power of electron microscopes. The green fluorescent protein (GFP) and its derivatives revolutionized live cell light microscopy. Correlative microscopy of GFP through the photo oxidation method allows for the direct ultrastructural visualization of fluorophores upon illumination. Oxygen radicals generated during the GFP bleaching process photo oxidize diaminobenzidine (DAB) into an electron dense precipitate that can be visualized both by routine EM of thin sections and by electron tomography for 3D analysis.

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α Synuclein Containing Exosomes Induce Neurotoxicity and Activate Microglia Cells

Giulia Bertì, Luigi Bubacco1, Nicoletta Plategher1, Isabella Russo1, Elisa Greggio1, Laura Civiero1
1 Università degli Studi di Padova, Italy
2 University of Padova, Italy

Presenter: Giulia Bertì

Synucleinopathies, which include Parkinson Disease (PD), are characterized by the presence of abundant neuronal inclusions. Pathogenic misfolding of the presynaptic protein, a synuclein (aS), subsequent aggregation and accumulation are recurrent at least in the progression of the diseases. Recent work highlighting the seeding effect of pathogenic aS has been largely focused on the extracellular secretion of the protein. In this project, we studied aS unconventional exocytosis through exosomes. Exosomes are able to protect their content from extracellular proteolytic enzymes and it has been observed that aS within vesicles is more prone to aggregation than cytosolic aS. The goals of this study is to purify and characterize aS containing exosome and investigate their role in PD propagation. In order to address these issues, we decided to introduce to our paradigm the DOPAL modification. DOPAL is a toxic dopamine metabolite which is typically processed to the harmless DOPAC and it has been reported to accumulate into dopaminergic neurons. It is a highly reactive aldehyde which can chemically modify presynaptic proteins including aS. Vesicles purified from HEK293T cells exhibit the typical hallmarks of exosomes and contain aS and DOPAL modified aS (DaS). When apply to neuronal primary cultures, exosomes containing DaS are able to alter the synaptic boutons and activate the apoptotic cascade. Considering that neuron degeneration occurred also for an over activated microglia, we incubate these vesicles with mouse immortalized microglia cells, mediating the increment of COX 2 and IL 1β cytokine production. Our results highlight a novel toxic mechanism by which exosomes contribute and enhance the toxicity of aS, in terms of propagation and neurotoxicity. The next aim will be to determine the aS structure inside exosomes, which could provide further insights on the pathological role of these vesicles, considering the strict connection between structure and toxicity in aS.
Correlative 3D in vivo 2 photon and Electron Microscopy using Natural Landmarks

Steffen Burgold, Manja Luckner, Severin Fülscher, Eric Hummel, Yilmaz Niyaz, Gerhard Wanner, Jochen Herm

1. German Center for Neurodegenerative Diseases, Germany
2. Carl Zeiss Microscopy GmbH, Germany
3. Ludwig Maximilians University, Germany

Presenter: Steffen Burgold

In neuroscience research there is an increasing demand in correlative imaging techniques because of the complexity of the brain with morphological scales ranging from nanometers to several millimeters in the rodent brain. In the past 10 years several protocols were developed to correlate in vivo 2 photon with electron microscopy data based either on DAB conversion of GFP or on near infrared branding landmarks (NIRB). Here, we present a new workflow using natural landmarks that overcomes the difficulties with DAB conversions and leaves the surrounding tissue next to the structure of interest intact in contrast to the NIRB marks. The usability of the workflow is demonstrated by correlating the lifetime of dendritic spines with the ultrastructure of the synapses they establish. For the measurement of the lifetime of dendritic spines we applied in vivo 2 photon microscopy in combination with a cranial window implantation to assess the formation and elimination of single spines over time. A mouse line expressing green fluorescent protein in a subset of cortical neurons enables for the visualization of dendritic arbors with their spines. After fixation of the mouse brain tissue the in vivo imaged regions are identified and mapped with their natural markers. The brain sections are stained for electron microscopy and embedded according to a newly developed method. Thin sections will be first observed with TEM and electron tomography to reconstruct the 3D organisation of the cell, and subsequently analyzed with SEM EDX, ToF SIMS and nanoSIMS in a correlative way to obtain a highly resolutive chemical cartography at different chemical and spatial resolutions. Visualization of the most diffusible elements like cations will be performed to assess if our method has preserved the in vivo chemistry of the cell.

The Challenging Route towards 3D Chemical Imaging of Single Cells from the Marine Plankton

Johan Decelle, Hryhoriy Stryhanyuk, Matthias Schmidt, Benoit Gallet, Niculina Musat

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2. Helmholtz Centre for Environmental Research UFZ, Germany
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Presenter: Johan Decelle

Recent methodological advances in single cell chemical imaging, such as Secondary Ion Mass Spectrometry (SIMS) offer the possibility to study the physiology and metabolic capacities of unicellular organisms at a nanometer resolution. However, sample preparation is one of the major challenging tasks associated with chemical imaging. For SIMS techniques (e.g. ToF SIMS and nanoSIMS), many preparative steps, such as chemical fixation, dehydration, resin embedding, greatly alter the in vivo chemistry of the cell by losing or redistributing elements and molecules within the cell. Complementary electron microscopy techniques must be also used from the same sample to obtain information about the ultrastructure and to correlate with the chemical mapping. Therefore, a specific preparation that conserves not only the original ultrastructure but also the native chemical composition of the living cell needs to be developed in order to visualize the natural distribution and quantity of molecules, elements and isotopes within different cellular compartments. Here, we present a methodological strategy to unveil both the ultrastructure and chemical composition of eukaryotic cells using correlative electron microscopy and SIMS techniques. Organisms will be cryo fixed with the High Pressure Freezing technology, and then subjected to freeze substitution using different solvents. They will be finally cryo embedded or embedded at room temperature with different resins before sectioning. Thin sections will be first observed with TEM and electron tomography to reconstruct the 3D organisation of the cell, and consequently analyzed with SEM EDX, ToF SIMS and nanoSIMS in a correlative way to obtain a highly resolutive chemical cartography at different chemical and spatial resolutions. Visualization of the most diffusible elements like cations will be performed to assess if our method has preserved the in vivo chemistry of the cell.
Connectomics ZEISS MultiSEM – the Fastest Scanning Electron Microscope in the World Just Got Even Faster

Anna Lena Eberle, Stephan Nickell
Carl Zeiss Microscopy GmbH, Carl-Zeiss-Str. 22, 73447 Oberkochen, Germany

Presenter: Anna Lena Eberle

Connectomics is a rather new discipline in the Neurosciences where complex synaptic networks assembled by billions of neurons are studied. One way to shed some light on the structural organization of these networks is serial array tomography. Blocks of tissue embedded in resin are sectioned to ultrathin slices, followed by imaging these series of sections using a scanning electron microscope. After merging all acquired section images into a 3D volume the fine structure of neurons can be segmented and visualized. The result is a detailed 3D map of the brain: the Connectome. With the advent of automated sample preparation robots [1] and the first multi-beam scanning electron microscope [2], dense reconstructions of larger (1 mm³) brain volumes at high resolution are now within reach. A second variant of the multi-beam SEM with even more beams, namely 91 instead of 61, and a higher current per beam [3] increases the throughput even further. A net acquisition speed of up to two Terapixel per hour is now achievable, therefore enabling large scale imaging experiments. However, the enormously increased data generation rate poses a huge challenge on the handling, processing and analysis of the data, which shifts the emphasis of most Connectomics’ research towards computational tasks.

References:

Unraveling the Contribution of Hemodynamics to the Efficacy of Tumor Cell Extravasation using Biophysical and High-Resolution Imaging Approaches

Jacky G. Goetz
INSERM U1109, France

Presenter: Jacky G. Goetz

Metastatic spread is a multistep process leading to the dissemination and growth of circulating tumor cells (CTCs) at distant sites from the primary tumor. How blood flow contributes to the arrest of CTCs has never been investigated before. Using a combination of metastasis assays in the zebrafish embryo and microfluidics, to which high speed imaging and optical tweezing technologies are applied, we recently unraveled a permissive hemodynamic profile that favors arrest of CTCs in vivo. Our results thus suggest that local hemodynamic profiles are very likely capable of tuning the last, and yet essential, steps of the metastasis cascade. More recently, we developed a multimodal correlative approach allowing us to rapidly and accurately combine functional imaging with high resolution ultrastructural analysis of tumor cells in vivo. Intravital imaging has opened the door to in vivo functional imaging of cancer, however it is limited in resolution. Ultrastructural analysis of tumor metastasis in vivo has so far been hindered by the limited field of view of the electron microscope, making it difficult to retrieve volumes of interest in complex tissues. This reliable, versatile, and fast workflow offers access to ultrastructural details of metastatic cells with an unprecedented throughput and will provide important and unexpected insights into the mechanisms of tumor invasion and metastasis in vivo.

Insights into the Role of COPII Vesicles in Procollagen Trafficking using 3D CLEM

Kent McDonald, Randy Schekman, Amita Gorur
1 Electron Microscopy Lab, UC Berkeley, United States of America
2 Molecular and Cell Biology, UC Berkeley, United States of America
3 Graduate Program in Comparative Biochemistry, UC Berkeley, United States of America

Presenter: Amita Gorur

The coat protein complex II (COPII) achieves cargo sorting and vesicle formation at the endoplasmic reticulum (ER) and aids in protein trafficking through the early secretory pathway. The flexibility of the coat machinery to accommodate diverse size and shaped cargo in order to meet the demands of the cell is not well understood. An example of a large cargo protein is collagen, which is synthesized as 300nm precursor fibrils of procollagen in the endoplasmic reticulum (ER). The molecular mechanism by which large procollagen fibrils gets packaged in small COPII vesicles of about 70nm remains unclear. Genetic evidence for the role of COPII in collagen trafficking has been established in a skeletal disorder, Cranio Lenticulo Sutural Dysplasia (CLSD) where in a mutation in Sec23A (COPII subunit) results in defective collagen transport. Previous work in collaboration with the Rape Lab has shown that mono ubiquitination of Sec31 by the ubiquitin ligase complex Cul3 Klhl12 leads to the formation of enlarged COPII vesicles that drive the secretion of collagen (1). Using a combination of light (confocal, STORM, SIM) and electron microscopy techniques (Serial sectioning, FIBSEM) we have recently been able to demonstrate that COPII vesicles indeed play the role of transport carriers of intracellular procollagen.

Reference:
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Correlative Microscopy of GFP to Study Neurons in the CNS

Areej Albariri, Thomas Kuner, Markus Grabenbauer
University of Heidelberg, Germany

Presenter: Areej Albariri

The Golgi apparatus is a highly dynamic organelle, which plays important roles in many cellular mechanisms like protein secretion, lipid metabolism, intracellular signaling, and regulation of cell division. The structure and function of the continuous and intricate network of the Golgi apparatus in neurons is not well delineated, therefore we are developing novel methodologies for correlative microscopy, which combines fluorescence microscopy of in vivo dynamics with the resolution power of electron microscopes. The green fluorescent protein (GFP) and its derivatives revolutionized live cell light microscopy. Correlative microscopy of GFP through the photo oxidation method allows for the direct ultrastructural visualization of fluorophores upon illumination. Oxygen radicals generated during the GFP bleaching process photo oxidize diaminobenzidine (DAB) into an electron dense precipitate that can be visualized both by routine EM of thin sections and by electron tomography for 3D analysis. The application of these photo chemical methods in the central nerve system (CNS) would allow us to understand the spatial organization and dynamics of secretory organelles like Golgi apparatus and their role in the definition of neuronal polarity and the composition and maintenance of synapses.

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Co-Localization of two PIII Snake Venom Metalloproteinases with Type IV Collagen from Basement Membrane: A Model using Confocal Microscopy

Cristina Herrera¹, Teresa Escalante², Alexandra Rucavado¹, José María Gutiérrez³
1 Facultad de Farmacia, Universidad de Costa Rica, San José Costa Rica, Costa Rica
2 Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José Costa Rica, Costa Rica

Presenter: Cristina Herrera

Snake venom metalloproteinases (SVMP) are classified in three groups according to their domain composition (PII, PII, PIII). They have different hemorrhagic activity and some are not able to induce haemorrhage. The main targets of hemorrhagic SVMPs are components of basement membrane (BM). However, the key binding component has not been yet identified. Tissue localization on muscle microvasculature of three different class of hemorrhagic SVMPs revealed that PII and PIII SVMPs co-localized with type IV collagen in BM of microvessels to a higher extent than PII SVMP, which showed a more widespread distribution in the tissue. This is likely to depend on the presence of exolites in the extra domains of PII and PIII SVMPs which allow them to bind to microvessels. In the present study, we compared the tissue distribution of equimolar amounts of CnI, a hemorrhagic PIII SVMP isolated from Crotalus simus snake venom, and Basparin A, a prothrombin activating and non hemorrhagic PIII SVMP isolated from Bothrops asper snake venom, on cremaster muscle microvasculature. Labelled toxins with Alexa 647 were applied on the isolated tissue for 15 min. The whole tissue was fixed and immunostained to visualize the type IV collagen from BM. At least four images of the three vessels type per tissue (n=3) were taken by confocal microscopy. The images were analyzed for co-localization of the SVMPs with type IV collagen. Results were expressed as percentage of material co-localized and Pearson’s correlation coefficient. We found that non hemorrhagic PIII showed a widespread distribution in the tissue with lower percentage of co-localization with type IV collagen from BM as compared to hemorrhagic PII. However, hemorrhagic PII and PIII could be binding to other components since confocal microscopy is not able to discern between BM components. Thus, further studies with high resolution techniques, such as CLEM, are necessary in order to identify the binding component of these toxins.

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New Insights into Neutrophil Pathogen Interactions by Multiscale Imaging

Inge Herrmann
Swiss Federal Laboratories for Materials Science and Technology, Switzerland

Presenter: Inge Herrmann

Sepsis is a severe medical condition. Differentiation between infectious systemic inflammation (sepsis) and non infectious systemic inflammation is notoriously challenging. Recently it has been discovered that neutrophils release microvesicles in response to bacterial stimuli. Here, we will show how we employed a combination of imaging techniques including confocal light and electron microscopy, nanotomography and spectroscopy to detect phenotypic differences in microvesicle populations released by neutrophils in response to different stimuli. This study may have wide ranging implications as phenotypic shifts in microvesicle populations could be utilized to diagnose sepsis at an early stage. We will present first pilot study results where we exploited the potential of microvesicle based diagnosis in a set of clinical samples.

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Invasion Pattern Analysis of Oral Cancer Cells in vitro

Ehsanul Hoque Apu¹, Meeri Sutinen¹, Saad Ullah Akram², Fumi Suomi³, Lauri Ekklund³, Tuula Salo³
¹ University of Oulu, Finland
² University of Helsinki, Finland
³ University of Heidelberg, Germany

Presenter: Ehsanul Hoque Apu

Background: Cancer cell invasion has been traditionally studied in three dimensional (3D) models composed of rat or mouse extracellular matrix (ECM) proteins such as type I collagen and Matrigel. In order to study in vitro 3D oral squamous cell carcinoma (SCC) invasion, our research group developed a human derived myoma organotypic model using uterine leiomyoma tissue (Nurmenniemi et al. 2009). We have also developed a processed, gelatinous leiomyoma matrix named Myogel, according to the method for the preparation of mouse Engelbreth Holm Swarm (EHS) tumor derived Matrigel (Kibbey 1994). Our results suggest that the novel Myogel is practical and even superior to the commercial Matrigel. Objective: In this study we aimed to validate the quality of our new product Myogel and to explore its suitability for fast and reproducible testing of cancer cell TME interaction via in vitro assays. Methods: The hanging drop technique was used to observe the oral tongue SCC (HSC 3) cell movement under ECM culture conditions. Images were taken with a ZEISS Yokogawa CSU X1 spinning Disc Confocal Microscope with a Hamamatsu Camera#2 controlled by ZEISS Zen Blue software for 20 h. We analyzed the hanging drop experiments to study the average count, speed, size and shape (eccentricity/roundness) of the HSC 3 cells and their nuclei in three different conditions. An algorithm based mathematical method was developed to quantify the movement of HSC 3 cells. Results: The average oral tongue SCC cell speed in Myogel was more than their average speed in collagen and Matrigel. The count, size B shape (eccentricity/roundness) were also different. The nuclei and cellular shape were decreased in Myogel, which suggests there were more cell movement than in Matrigel. Conclusion: We found that our new product, Myogel mimics the native human TME better. It is suitable and even better than Matrigel or collagen for fast and reproducible testing of cancer cell TME interaction in a hanging drop assay.

Poster Abstracts
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**Shedding Light on the Candidate Phylum Poribacteria by Fluorescence in situ Hybridization Correlative Light and Electron Microscopy (FISH CLEM)**


1) GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany
2) University of Wuerzburg, Germany
3) King Abdullah University of Science and Technology, Saudi Arabia
4) University of New South Wales, Australia

**Presenter: Martin Jahn**

The majority of environmental microorganisms remains uncultivated and are commonly referred to as “microbial dark matter”. Here we apply Correlative Light and Electron Microscopy (CLEM) in combination with an RNA Seq approach to investigate the candidate phylum Poribacteria, a prominent and widespread uncultivated bacterial symbiont of marine sponges. We established a protocol for fluorescence in situ hybridization correlative light and electron microscopy (FISH CLEM) that enabled the identification of poribacterial cells in sponge tissue at electron microscopy resolution. Cellular 3D reconstructions revealed bipolar, spherical structures of low electron density, which are likely carbon reserves. Highly expressed proteins related to cellular compartmentation, were localized in poribacterial cells by combining, to our knowledge, for the first time FISH CLEM with immunohistochemistry (FISH IHC CLEM). Based on our findings, we hypothesize that Poribacteria carry out propanediol degradation, atypically, poribacterial cells by combining, to our knowledge, for the first time FISH CLEM with immunohistochemistry (FISH IHC CLEM). Based on our findings, we hypothesize that Poribacteria carry out propanediol degradation, atypically.

In the future, this system can be applied to a series of thin sections to analyze a volume correlative.
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Correlative Light and Electron Microscopy to Elucidate the Spatial Pattern of the PD Related Genes Pink1 and Parkin during Mitochondrial Fission

Felix Kleine Borgmann, Alexander Skupin
Uni Luxembourg, Luxembourg

Presenter: Felix Kleine Borgmann

We performed CLEM to address the question of whether or not proteins involved in mitophagy are accumulating on mitochondria undergoing fission in an irregular, possibly polarized pattern. This could indicate the existence of a sorting mechanism by which mitochondria selectively dispose of damaged material by selective fission and subsequent mitophagy. Two proteins involved in mitochondrial quality control and degradation are Pink1 and Parkin. Upon depolarization of the mitochondrial membrane, they accumulate at the OMM and mark the mitochondrion for mitophagy. We used overexpression of these proteins tagged with APEX2 and miniSOG in mouse primary cortical neurons to visualize their localization in EM. We acquired time lapse image series of live cells with the mitochondria stained by TMRM and mitoTracker. When subjected to conditions that mimicked stress and apoptosis, the cells were rapidly fixed and subjected to sample processing for EM. We identified the same mitochondria we observed live in EM and found Pink1 and Parkin to be localized at specific areas during onset and progression of fission.

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Correlative Microscopy using Automated Volume SEM

Anneke Kremer, Sonia Bartunkova, Saskia Lippens, Chris Guerin
VIIB, Belgium

Presenter: Anneke Kremer

Because life happens in 3D it is crucial to visualize biological structures within their context. Correlating high resolution light microscopy and automated scanning electron microscopy is a giant step forward in the process of obtaining 3D volume information at nanometer resolution. The instrumentation at the VIIB Bio Imaging Core in Gent spans a broad range: from micron scale magnification LM to nanometer range EM. Imaging in 3 dimensions on our confocal systems and also on our 2 different volume EM systems: a 3view SBF SEM and a FIB SEM allows us to incorporate information from LM and EM seamlessly and efficiently. To this end, we are developing workflows for 3D CLEM, which can be easily adapted to any specific sample or project. At the present, we have already developed several methods for 3D CLEM, using either gridded coverslips (for cellular monolayers) or NIRB (for tissue). In addition, we make use of the advantages of both our 3D SEM systems by incorporating our 3view system to image large areas and find a region of interest that will subsequently be imaged with FIB SEM to produce nm resolution reconstructions at high 3D resolution. For many biological questions it is important to combine the advantages of both live cell functional imaging and EM to correlate mechanisms and link structure to function in 3D at the nanoscale.

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Interplay between Autophagy and ERAD: An Ultrastructural Approach

Leticia Lemus; Veit Goder
University of Seville, Spain

Presenter: Leticia Lemus

Protein quality control mechanisms inside the endoplasmic reticulum (ER) aid in protein folding and select terminally misfolded proteins to be targeted to the cytosolic proteasome for degradation by a process called ER associated protein degradation (ERAD). Some misfolded ER proteins are not efficiently routed to ERAD and instead are targeted to the vacuole/lysosome for degradation, a process which in some cases also involves cellular components needed for autophagy. Why specific misfolded proteins are targeted to the vacuole/lysosome and how autophagy connects to these transport events is largely unknown. By investigating the degradation of the misfolded glycosylphosphatidylinositol (GPI) anchored protein Gas1* in the yeast Saccharomyces cerevisiae, we observed that a minor fraction is targeted to ERAD whereas the majority of the protein is routed to the vacuole. Interestingly, the degradation of GFP Gas1* was slowed significantly in cells depleted of Atg8, a key component for the formation of autophagosomes. Moreover, live cell fluorescence microscopy in these cells revealed a significant decrease of GFP inside the vacuole and an accumulation of GFP Gas1* in dot like structures suggesting that autophagy or autophagy related processes are involved in the targeting of this misfolded protein from the ER to the vacuole. A major goal of our work is to gain insight into the mechanism of where in the cell the autophagy machinery connects to the ER or ER exit material in order to promote targeting of particular misfolded proteins to the vacuole. In one approach we will use electron microscopy in combination with immunogold labeling (Tokuyasu technique) in order to identify subcellular structures with accumulated substrate in autophagy mutants. In another approach to address these questions we attempt to use correlative light electron microscopy (CLEM) techniques.

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Methodology Development at NYULMC Microscopy Core Correlative Light and Electron Microscopy Applications

Fengxia Liang
New York University Langone Medical Center, United States of America

Presenter: Fengxia Liang

Project variety is the nature of the core facility. Experimental design of complex procedures, such as CLEM for each individual project by utilizing current available instruments and reagents, is a challenge. Several CLEM strategies were established at the Core, such as: neighboring sections, hybrid labeling, correlative overlay and Tokuyasu cryosections. To study cortical inhibitory neuron maturation and synaptic development, we use hybrid labeling strategy to double immunolabel GFP and Somatostatin (SST, a growth hormone inhibiting hormone) of braun vibrotome sections of the Lmx6 GFP transgenic mouse; to detect GFP positive cells under fluorescent microscope following correlated ultrastructural immunolabeling using Nanogold as marker; and SST positive cells using HRP/DAB as marker. Double positive cells and single positive cells were clearly distinguished by silver enhanced gold particles and DAB darkened cells at ultrastructure level. To study melanoma invasion, we used the frozen section correlative overlay strategy to localize the melanocyte migration in the skin of Braf and P10 knock out mouse. The gridded bottom dish was adopted to study centrosome mutation and HIV infection using correlative overlay strategy, and Tokuyasu cryosections combined with super resolution microscopy were used to study the protein localization in intercalated disc of mouse heart.
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Activities of the DFG in the Area of (CL)EM

Gunter Merdes
Deutsche Forschungsgemeinschaft (DFG), Germany

Presenter: Gunter Merdes

As a major German research funding organization, it is the main task of the German Research Foundation (DFG) to fund the best research in a competitive process at research and higher education institutions. To this end we are also funding, on behalf of the federal government and states, investments into research equipment. Whereas in the Major Research Instrumentation funding schemes proposals can be submitted at any time, the Major Equipment Initiatives are special and time limited calls. These calls are designed by the DFG to fund the application and further development of new technologies e.g., X-ray microscopy, Magnetic Particle Imaging, etc., and to make these technologies available to the scientific community as exemplary installations. In all these programs, the funding of electron microscopy equipment has always played an important role. Therefore, we are keen to keep ourselves up to date with the newest technological developments and the needs of scientists by participating in community activities, and – vice versa to inform the community about the possibilities of our funding schemes. Accordingly, I can offer to present our current activities and ongoing discussions aiming to improve the scientific infrastructure in Germany, with an emphasis on (CL)EM.

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Spit and Glue: A Role for Actin and Myosin II

Dana Meyen, Tal Rousso, Eyal D. Schejter, Ben Zion Shilo
Molecular Genetics, Israel

Presenter: Dana Meyen

Regulated exocytosis is a key cellular process of specialised glands, in which membrane components or compounds destined for the extracellular space are wrapped into vesicles and transported to the surface of the cell. Upon arrival rapid content expulsion and vesicle integration into the plasma membrane clears the way for newly incoming vesicles. However, the release is especially challenging for large (3-8 µm) vesicles that secrete viscous cargo, such as surfactant proteins in the lungs or digestive enzymes in the exocrine pancreas. These types of vesicles require a contractile force that is facilitated by the interplay of actin and non muscle Myosin II. How the actin based machinery is assembled, structurally organised and how precisely it contributes to exocytosis is thus far not fully understood. To address these questions we utilise Drosophila salivary glands as a powerful model system for studying vesicle exocytosis. In the larva the salivary gland produces huge amounts of viscous “glue” proteins that are packed in µm scale vesicles to provide adhesion of the pupa to external surfaces during the process of metamorphosis. We employ genetic manipulations and high resolution live imaging of isolated salivary glands to study the mechanistic details of the secretion process. Amazingly, vesicle fusion to the cell membrane triggers the recruitment of the entire acto myosin machinery and furthermore, its components are organised in a very specific spatiotemporal manner. Currently we are focusing on the regulation of Myosin II, which forms a unique pattern on the vesicle surface.

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The Mind of a Larval C. elegans – how do Neuronal Circuits Function and Remodel throughout Development?

Daniel Berger\(^1\), Andrew Chisholm\(^2\), Steven Cook\(^2\), Scott Emmons\(^2\), David Hall\(^2\), Douglas Holmyard\(^2\), David Kersen\(^1\), Valeriya Laskova\(^1\), Jeff Lichtman\(^2\), James Mitchell\(^2\), Ben Mulcahy\(^1\), Marianna Neubauer\(^1\), Angie Qu\(^1\), Aravi Samuel\(^1\), Richard Schalek\(^1\), Manusnan Suriyalaksh\(^1\), Daniel Witvliet\(^1\), Mei Zhen\(^1\)

\(^1\) Authors in alphabetical order.

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\(^3\) University of Toronto and Lumenfeld-Tanenbaum Institute.
\(^4\) Division of Biological Sciences, Section of Cell and Developmental Biology, University of California, San Diego.

Presenter: Ben Mulcahy

When humans and worms are born, they have neuronal circuits in place to integrate sensory cues and effect appropriate behavioural or homeostatic responses. During the course of development, a substantial amount of remodelling occurs in the nervous system. New neurons are born and integrated into existing circuits, and existing neurons and circuits are remodelled. The rules of this remodelling are poorly characterised, because in order to investigate neuronal circuit development one needs a connectome level analysis of the circuit across multiple time points, in multiple animals. This strategy requires serial-section electron microscopy, which has been very low-throughput and labour-intensive. C. elegans represents an ideal organism to perform these studies on, because of their small size and stereotypical development – some of the reasons they were chosen for the original adult serial-section EM reconstruction that culminated in the acquisition of the adult connectome, dubbed ‘The mind of a worm’. Since this achievement, new technologies have been developed that improve the fidelity and throughput of the connectome reconstruction, including better fixation and sectioning methods, increased computational power and more advanced microscopes. We are reconstructing the entire nervous system of multiple larval animals at different time points using serial-section transmission and scanning electron microscopy. This larval (L1) animal is of special interest because of the major remodelling that occurs at the end of L1, including the birth of new major classes of motor neurons. We are finding both conserved and unreported structure and connectivity from the published adult wiring diagram. This dataset will be used as a framework to functionally interrogate the interplay between developing circuits and sensorimotor behaviours, and the mechanisms that allow this dialogue to take place.
Investigation of Myelin Maintenance and Turnover by Ultrastructural Analysis of an Inducible MBP Knock-Out in Adult Mice

Martin Meschkat, Wiebke Möbius
Max Planck Institute for Experimental Medicine, Germany

Presenter: Martin Meschkat

Myelin is composed of multiple membrane layers tightly wrapped around axons facilitating rapid action potential propagation and providing metabolic support. In the mouse central nervous system myelin is formed during development by early differentiated oligodendrocytes. Since myelin proteins are long lived, we ask the question how after the completion of myelination the myelin sheath is maintained in adulthood and turned over. This could be achieved by replacement of aged oligodendrocytes by newly differentiated oligodendrocyte precursor cells, or by cell based turnover of individual myelin components. To investigate this we have chosen a strategy to knock out myelin basic protein (MBP) by using a conditional and inducible Cre LoxP approach targeting adult differentiated oligodendrocytes. MBP is essential for the compaction of myelin in the CNS. By knocking out MBP after completion of myelination at the age of 8 weeks, any changes within the myelin sheath ultrastructure and compaction indicative of reduction of MBP by turnover are detectable only by electron microscopy. We analyzed mice at the ultrastructural, histological and biochemical level at the time points 2, 4, 6 and 12 months after KO induction. We observed that the myelin sheaths of recombined oligodendrocytes are progressively transformed into non compact membrane processes within 4 to 6 months. These structural changes first appear at the innermost myelin layers and result in complex membrane structures along the axon. By application of histological staining and volume imaging using focused ion beam scanning electron microscopy we observed a micro and astrogliosis and additional changes in the axons such as enlarged mitochondria and axonal swellings. Our results indicate that the myelin sheath although it appears as a very stable structure is slowly but continuously turned over. Moreover, we show that the loss of compact myelin induces a micro and astrogial response and affects axonal integrity.

Elucidation of Borrelia Attachment to Red Blood Cells using Correlative Methods

Björn Morén, Annelie Olofsson, Johan Normark, Sven Bergström, Linda Sandblad
Umeå University, Sweden

Presenter: Björn Morén

Borrelia is a pathogenic bacteria known to cause disease in humans. Different species of borrelia can form aggregates together with Red Blood Cells (RBC) which increases tissue invasiveness and infectiveness. It is therefore imperative to understand how this process works. The group of Sven Bergström at Umeå University has shown that different species of borrelia binds in a so called rosetta shape and that this binding depends on blood cell glycans (Burman et al., 1998; Guo et al., 2009). Current theories postulate that this binding depends on complement factor C3b and C4b to bind to complement receptor CR1 in an attempt to avoid detection by the immune system (Kraiczy et al., 2001; Kurtenbach et al., 2002; Sandholm et al., 2014). Unpublished results have shown that this is a direct binding to the RBC membrane, and that this binding can be examined in great detail in SEM if the sample preparation is performed carefully. It is our belief that examining the binding mechanisms of the bacteria borrelia can yield important insights into the mechanisms of complement binding during infection and persistence of the bacteria during infection. We plan to use correlative methods to examine the binding of borrelia to complement factor site on RBC in detail, to confirm a possible binding to these complexes. We are interested in learning advanced correlative methods to enable fluorescence and SEM/TEM correlation of cell samples for the borrelia/RBC samples, but also to learn for future method development at the EM facility at Umeå University which will expand greatly within the year. New microscopes have been funded, which is going to include a 300 kV TEM microscope and a SEM with support for cryo EM and FIB SEM. Insights into methodology for these techniques would also be of great interest. Currently we have support for ZEISS Shuttle and Find (S&F) and the EM facility has also enabled publications using other correlation methods on embedded samples (Francis et al., 2015).
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Multi Scale in vivo XYZT Imaging of Single Cells by 1P, 2P, and on Chip Visualization Technique, and Post Processing
Satoshi Nishimura
Jichi Medical University and the University of Tokyo, Japan
Presenter: Satoshi Nishimura

We made fluorescence multi scale imager including 1P, 2P microscope, and macro on chip systems, and visualized XYZT single cell dynamics in vivo. We also analyzed bio physical reactions by post processing. First visualization system is high resolution imaging based on non linear optics, with X (resonance), Y (galvano), and Z (piezo) axis scanning. Real time, multi color XYZT multi photon imaging enabled us to visualize single blood cell behavior in vessels and stroma. Morphological changes including thrombus formation in cardiovascular events were identified. Second, macro imaging system for non anesthetized, and awake mice was developed using XY (spinning), Z(stage), and tilting two more alphabeta axes. Additionally, BK CMOS camera, image intensifier, and low power/high NA lens enabled us to visualize cellular dynamics during free moving behavior. Third, wearable and implantable devices for long time recording were developed using lens less and on chip technologies. By post process procedures, we automatically determined immune cell types of leukocytes in living animals by binarization, cell edge detections, cluster analysis of texture parameters, and self learning approaches. We obtained morphological and physical parameters of single cells including gravity center, texture, velocity, acceleration, and local stress. We summarized 300GB data of single experiment, into 1MB specific leukocyte movement data.
We used PIV method, as well as tracking, to obtain single cell dynamics. We combined these system with light manipulation technique, to induce thrombus or inflammation reactions, and multi modality imaging enabled us to cover from micro to macro scale information for space and time axis. In sum, we developed multi scale XYZT imaging system which can evaluate the therapeutic strategies against thrombotic and inflammatory processes in adult common disease, by combined use of fluorescence imaging and post processes.

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A Novel Factor HN1 Contributes to Centrosome Assembly and Control of Mitotic Progression
Bilge Esin Ozturk, Loftman Varisi, Gencer Kaan Akyuz, Güzem Gulevin Takir, Kemal Sami Korkmaz
Ege University, Turkey
Presenter: Bilge Esin Ozturk

Hematological and Neurological expressed 1 (HN1) is an evolutionary conserved gene in vertebrates with its ubiquitous biogenesis. The physiological similarities between NCLDV members and challenge the proposed models of cellular membrane assembly. Combination of various electron microscopy preparation and imaging methods will provide insights into the complexity of virus biogenesis. We therefore, interactions between astrocytes and synapses in a rat model of epilepsy has been investigated at the ultrastructural level. For this, 3D electron microscopy was implemented by using a Focused Ion Beam/Scanning Electron Microscope (FIB/SEM) to image volumes of more than 1000 cubic micrometers at 4 nm resolution in all directions within the cortical region generating seizures before and after the onset of epilepsy. Since the information available within the stacks obtained by FIB/SEM is of a high complexity, 3D segmentation has been done with liastik software to isolate synapses and astrocyte allowing a full 3D reconstruction so that the percentage of synapses covered by astrocytes could be measured. To facilitate the alignment and the preparation of the stacks, several macros have been written for ImageJ. Since it was difficult to handle the image stacks with our computer resource, each stacks were split in four, and the mesh of the cleaved segmented objects were then sewn with an ImageJ plugin written for this purpose: https://github.com/Tom TBT/StackObjectCombiner.

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Interactions between Astrocytes and Synapses in a Rat Animal Model of Epilepsy
Karin Pernet Gallay1, Tom Boissonnet2
1 Grenoble Institut des Neurosciences, France
2 INSERM, France
Presenter: Karin Pernet Gallay

Epilepsy is a neurological disorder characterized by recurrent spontaneous seizures due to hyperexcitability of neurons. Our preliminary data indicate a significant contribution of astrocytes in the pathophysiology of epilepsy. Therefore, interactions between astrocytes and synapses in a rat model of epilepsy has been investigated at the ultrastructural level. For this, 3D electron microscopy was implemented by using a Focused Ion Beam/Scanning Electron Microscope (FIB/SEM) to image volumes of more than 1000 cubic micrometers at 4 nm resolution in all directions within the cortical region generating seizures before and after the onset of epilepsy. Since the information available within the stacks obtained by FIB/SEM is of a high complexity, 3D segmentation has been done with liastik software to isolate synapses and astrocyte allowing a full 3D reconstruction so that the percentage of synapses covered by astrocytes could be measured. To facilitate the alignment and the preparation of the stacks, several macros have been written for ImageJ. Since it was difficult to handle the image stacks with our computer resource, each stacks were split in four, and the mesh of the cleaved segmented objects were then sewn with an ImageJ plugin written for this purpose: https://github.com/Tom TBT/StackObjectCombiner.

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Assembly and Membrane Biogenesis in Giant Viruses
Emmanuelle Quemin, Jacamine Krijnse Locker
Institut Pasteur, France
Presenter: Emmanuelle Quemin

Nucleocyttoplasmic large DNA viruses (NCLDV) include the poxviruses, asfarviruses, iridoviruses and phycodnaviruses together with the recently described giant viruses infecting amoeba. These viruses replicate in cytoplasmic inclusions called viral factories (VF) which enable spatiotemporal coordination of virion assembly and effective recruitment of host factors. Our interest concerns the structural complexity and tight organization of VF. They are made de novo early during infection leading to massive rearrangements of host cytoskeleton and membranes. Current research in the laboratory focuses on the biogenesis of the internal membrane present in all NCLDVs using various viruses as models: Vaccinia virus, African swine fever virus, Pandoraviruses and Mollivirus. Sophisticated techniques such as serial imaging and 3D reconstructions are required to decipher each of the single events occurring during this complex and rapid multi stage process. In particular, transfection of recombinant proteins in the host and correlative light and electron microscopy — CLEM — will help to determine the precise location and function of key molecular players involved in the formation, stabilization and internalization of single membrane sheets. These techniques will be part of an integrative approach which is required to provide detailed understanding of fundamental mechanism of virus assembly. Combination of various electron microscopy preparation and imaging methods will provide insights into the physiological similarities between NCLDV members and challenge the proposed models of cellular membrane biogenesis.
**Charaterization of Singlet Oxygen in Intact Synechosystis and Microalgae by Fluorescence Imaging and Histidine Mediated Chemical Trapping Techniques**

Ateeq ur Rehman  
Biological Research Center, Hungarian Academy of Sciences, Hungary  
Presenter: Ateeq ur Rehman

Singlet oxygen (1O2) has been implicated as an important mediator of light induced damage of the photosynthetic apparatus during photoinhibition. Although various methods such as luminescence at 1,270 nm, fluorescent probes method and electron paramagnetic resonance (EPR) are available for 1O2 detection in isolated photosynthetic systems, these sensor molecules are unable to penetrate the cell wall of intact cyanobacteria and microalgae, which seriously limits the possibility to study the role of 1O2 in photoinhibition in vivo. In order to overcome this difficulty, we have developed a method for detection of 1O2 in intact cyanobacteria. The method is based on chemical trapping of 1O2 by histidine (His), which leads to O2 uptake during illumination that can be detected and quantified by commercial oxygen electrodes. The O2 uptake method is applied for Synechosystis to understand the role of orange carotenoid protein (OCP) as 1O2 quencher and our results demonstrates that OCP is a very efficient singlet oxygen quencher. Recently we proposed that the inhibition of the Calvin Benson cycle by glycoaldehyde and potassium cyanide during thermal stress in Symbiodinium cells promotes 1O2 formation. We have applied His mediated O2 uptake method together with 1O2 imaging technique in cultured Symbiodinium cells to understand 1O2 formation. We will report on the latest results obtained by using microscopy imaging techniques on the characterization of intracellular and extracellular 1O2 detection and its involvement in triggering the expulsion of Symbiodinium cells from the coral host, which leads to coral bleaching in Symbiodinium cells.

**Ultrastructural and Functional Fate of Recycled Vesicles in Hippocampal Synapses**

Stephanie Rey¹, Catherine Smith¹, Milena Fowler¹, Freya Crawford¹, Jemima Burden², Kevin Staras¹  
¹ University of Sussex, United Kingdom  
² University College London, United Kingdom  
Presenter: Stephanie Rey

Efficient recycling of synaptic vesicles is thought to be critical for sustained information transfer at terminals in the central nervous system. The readily releasable pool (RRP) is, by definition, the subset of synaptic vesicles that are first to undergo fusion in response to stimulation. Thanks to the privileged release status of this pool, and therefore its central relevance in signalling, investigations of the RRP have been the subject of substantial research effort, but the long term fate of the vesicles recovered after endocytosis and their contribution to future RRP composition is not clearly established. Here, we exploit time stamped electron microscopy approaches in native hippocampal synapses, as well as novel fluorescence methods, to track the positional and functional destiny of vesicles retrieved after RRP stimulation. We show that most vesicles are endocytosed near the active zone but are subsequently inserted randomly in the cluster volume with time, losing their preferential re use status. These vesicles non selectively queue, advancing towards the release site with further stimulation in a process that depends on actin turnover. However, a subset of the retrieved vesicle population operates differently, selectively re clustering near the active zone and undergoing privileged re release as part of the future RRP. We use a variety of protocols to examine the rules that underpin this differential behaviour. Heterogeneity in the fate of vesicles retrieved after RRP stimulation provides new understanding of the mechanisms that govern vesicle recycling at small central synapses and the origins of future synaptic vesicle pool composition.
Early steps of Phagophore Formation in Aggrephagy
Sebastian Schultz¹, Coen Campsteijn², Steingrim Svenning³, Hallvard Olsvik³, Harald Stenmark³, Andreas Brech⁴
¹The Norwegian Radium Hospital, Norway
²Institute for Cancer Research, Oslo, Norway
³Institute for Medical Biology, Tromsø, Norway
⁴Presenter: Sebastian Schultz

Degradation of cytosolic components by autophagy is a crucial process for cellular and tissue homeostasis. Over the last decade this research field has tremendously expanded and a lot of effort has been made to dissect the signaling mechanisms that regulate autophagy initiation and to unravel the origin of membranes that contribute to the newly formed, sequestering phagophore. Initially these questions were mainly answered in light of non selective turnover of cytosol upon nutritional starvation. However, over the last years it has become clear that autophagy also can selectively target cargo, such as protein aggregates (aggrephagy) and diverse organelles (mitochondria, peroxisomes etc.). Even though several cargo receptor molecules (e.g. p62 and NBR1) have been identified that allow for a molecular link between selected cargo and the autophagic machinery via direct interaction with LC3, very little is known about how selective autophagy is induced (thereby giving rise to LC3 containing phagophores) and how autophagic membranes and cargo are brought into close proximity. We therefore have focused on the selective turnover of puromycin induced protein aggregates in order to understand the early events of phagophore formation and cargo delivery to the autophagic membrane in aggrephagy. In particular we are currently trying to characterize small (100 500 nm in diameter) LC3 positive puncta that arise upon puromycin treatment in an NBR1 dependent manner. In order to understand more about the origin of the membrane forming the phagophore and the potential role of vesicle fusion events contributing to the growing phagophore we are using CLEM. One major challenge of this approach is to obtain a accurate correlation in 3D.

Does Multiinnervated Dendritic Spines Matter?
Memory Formation in Old Age
Malgorzata Sliwinska, Anna Trabczynska, Magdalena Ziołkowska, Kasia Radwanska
Nencki Institute of Experimental Biology, Poland

Presenter: Malgorzata Sliwinska

In the old age the synaptic transmission in the hippocampus is impaired but spatial memory still can be formed. What is the difference in the mechanism of memory formation between young and old individuals? We try to answer this question in mice model. In present study animals undergo spatial memory training in the automated cages IntelliCages. This allows us for detailed analysis of the learning process and observation of the behavioral differences accompanying learning of young and old mice. After the training the structural changes in the hippocampal CA1 region of animals are analyzed using electron microscopy technique. Serial Block Face Scanning Electron Microscopy (SBEM). SBEM facilitates the analysis of large volume of tissue from many samples in relatively short time. The acquired data was used for 3D reconstruction and then for characteristic of dendritic spines e.g.: spine density, volume, shape as well as some synaptic features (post synaptic density (PSD) density, shape, type and volume). We proved, that old mice can learn to find reward corner in their cage and that this is accompanied by specific changes in brain region responsible for spatial learning. We test the hypothesis, that multiinnervated spines are responsible for memory formation in old age.

CLEM: Current Uses and Future Strategies
Nadine Saplop, Devrim Acehan, Kunihiro Uryu
The Rockefeller University, United States of America

Presenter: Nadine Saplop

Correlative Light and Electron Microscopy (CLEM) is a valuable tool as it bridges the resolution gap between the two modalities. It takes advantage of the wide applicability of fluorescently labeled molecules in light microscopy (LM) and applies it to the increase in resolution and information of the surrounding ultrastructural architecture provided by electron microscopy (EM). Herein we describe examples where CLEM was a particularly useful strategy to locate a specific molecule of interest and to reveal the associated ultrastructure. These include vesicles labeled with the fluorescent steryl dye, FM 4-64, introduced to naïve cells and examined with TEM. Additionally, we used fluorescent molecules to relocate a cell of interest for SBEM imaging using Gatan 3View in a FE SEM. In addition, we share a new methodology in CLEM applications. We are developing a unique dehydratation method where native structures are highly preserved to allow visualization of ultrastructure at high resolution using secondary electron detection in SEM. Since this method preserves fluorescent reporting molecules such as GFP and RFP it is ideally suited for CLEM.

Correlative Cryo FM and Cryo SEM is a straightforward Tool to Study Host Pathogen Interactions as Close as Possible to Native State
Martin Strnad, Marie Vancova, Jana Elsterova, Jana Nebesarova
Biology Centre of ASCR, v.v.i., Czech Republic

Presenter: Jana Nebesarova

Correlative light and electron microscopy (CLEM) is an imaging technique that enables identification and targeting of fluorescently labeled or tagged structures with subsequent imaging at close to nanometer resolution. Correlative cryo fluorescence microscopy (cryo FM) and cryo scanning electron microscopy (cryo SEM) is a cutting edge approach, which allows to observe the studied object of interest very close to its native state, devoid of artifacts caused by slow chemical fixation. We employed cryo FM and cryo SEM on vitrified pathogenic bacterium Borrelia burgdorferi genetically tagged with green fluorescent protein to study its interaction with human neuroblastoma cell line. Here, we describe the method and the essential optimization steps for streamlining the workflow of cryo FM and cryo SEM. This method appears to be an unprecedentedly fast (<3 hours), straightforward, and reliable solution to study the finer details of pathogen host cell interactions and provides important insights into the complex and dynamic relationship between a pathogen and a host. The study is supported by the Technology Agency of the Czech Republic (TE01020118).
**Integration of a Standard, Commercial Confocal Laser Scanning Microscope (CLSM) in a Scanning Electron Microscope**

Jacob Hoogenboom, Josey Sueters, Pieter Kruit
TU Delft, The Netherlands

**Presenter: Josey Sueters**

Correlative light and electron microscopy (CLEM) combines fluorescence microscopy (FM) with electron microscopy (EM) to obtain complementary information from a single sample. FM allows live cell imaging and molecular identification, whereas EM provides high resolution images of the cellular ultrastructure. To simplify the CLEM process, our group previously designed and built an integrated wide field (WF) FM and scanning electron microscope (SEM) system. In this system, a region of interest can, in principle simultaneously, be imaged by SEM from above and FM from below. For thin tissue sections, or thin cell regions (like cell extrusions) EM and WF FM give good correlation. However, for thick specimen, or, e.g., living cells in a liquid cell holder, correlation gets compromised since WF FM images through the entire sample volume, whereas SEM only reveals the upper few 100 nanometers (depending on electron beam energy). To this end, we are integrating a standard commercial confocal microscope (CM) onto an SEM. Using optical sectioning through the biological specimen, we want to achieve better FM SEM correlation. The CM and SEM have been axially aligned through electron beam induced fluorescence (cathodoluminescence) and first proof of principle images with the integrate system were acquired. In future, we plan to use this system in combination with an in home developed liquid cell holder to image live cells in the SEM at near native conditions.

**Current Status and Future Prospects of CLEM**

Katlijn Vints, Peter Baatsen, Natalia V. Gounko
Electron Microscopy platform; VIB Bio Imaging Core; Department of Human Genetics KU Leuven; VIB Center for the Biology of Disease, KU Leuven.

**Presenter: Katlijn Vints**

The electron microscopy facility at the VIB Bio Imaging Core at the KULeuven offers a range of services using transmission- (TEM) and scanning electron microscopy (SEM). Besides preparing and studying samples by routine procedures, we use and develop more state-of-the art EM-approaches to sample preparation and imaging. This presentation focuses on developing CLEM (Correlated Light and Electron Microscopy), using super-resolution fluorescence microscopy and 2D-TEM, as well as some initial results with 3D-EM.

**Electron Beam Induced Optical Superresolution in Correlative Light Electron Microscopy**

Lenard Voortman¹, Aaro Väkeväinen¹, Pieter Kruit², Jacob H. Hoogenboom²
¹ Delft University of Technology, The Netherlands
² Aalto University, Finland

**Presenter: Lenard Voortman**

We present a novel optical superresolution (SR) method using integrated correlative light and electron microscopy. The recent development of different SR techniques has revolutionized the field of optical microscopy by achieving image resolution well below the diffraction limit, the fundamental resolution limit of traditional optical microscopy. Current SR methods involve stochastic techniques, beam shaping in combination with confocal scanning, external control over excited state relaxation pathways, and/or structured illumination. Correlation of SR data with structural images obtained with electron microscopy (EM) has been demonstrated, but requirements for SR microscopy are often in conflict with those for EM. Moreover, the optical localization accuracy in the correlation image may be severely compromised compared to the SR resolution by the additional error introduced by aligning the separate SR and EM images. Here, we present a novel approach for correlative SR EM using the focused electron beam to locally modify the fluorescence signal of fluorophores, and detecting the change in fluorescence intensity with a wide field epifluorescence microscope. We use an integrated light electron microscope that is used for correlative light and electron microscopy (CLEM). The integrated light microscope allows us to record the fluorescence signal while scanning the electron beam through the fluorescence field of view. By correlating changes in the fluorescence decay with the instantaneous electron beam position and the other EM signals, we obtain a SR fluorescence image, that is in perfect registry with a simultaneously acquired EM image. We will present some first results of our electron beam induced SR method on labeled tissue sections.

**Ultrastructural Analysis of Microglia Synapse Interactions**

Laetitia Weinhard, Giulia Di Bartolomei, Pedro Machado, Giulia Bolasco, Yannick Schwab, Cornelius Gross
EMBL Monterotondo, Italy

**Presenter: Giulia Di Bartolomei**

Microglia are the macrophages of the brain and have received increasing attention over the past years for their role in synapse elimination in the healthy brain. Although it has been well established that microglia processes are continuously moving through the brain contacting synapses and that synaptic material is found inside microglia, until now it has been difficult to reconstruct the precise series of events that allows microglia to engulf or otherwise remodel synapses. Such an analysis requires an imaging technique that can resolve subcellular ultrastructure of brief microglia synapse interaction events in brain tissue. To address this need, we have developed a Correlative Light and Electron Microscopy (CLEM) technique that allows us to identify rare microglia synapse interaction events in fixed brain tissue and then find the identified event in serial EM reconstructions. We will present data using both FIB-SEM and SEM tomography to determine the ultrastructure of events identified by confocal fluorescence microscopy that begin to shed light on the architecture of microglia synapse interactions.
196 Beams in a SEM with Transmission and Secondary Electron Detection

W. Zuidema, Y. Ren, S. Rahangdale, P. Kruit, J. Hoogenboom, A. Mohammadi Gheidari
Delft University of Technology, The Netherlands

Presenter: W. Zuidema

A major bottleneck for large scale and volume EM is the imaging speed. The total acquisition time needed for a single sample, can easily take days, or even weeks using standard single beam SEM’s. Multi beam microscopes have been developed to increase imaging speed [1,2], but it remains a challenge to achieve electron detection similar to a regular SEM in terms of signal type, contrast and resolution. We have developed a SEM employing 196 electron beams using a standard column of a FEI Nova NanoSEM. The 196 electron beams are generated from a single high brightness Schottky electron source. Modified source optics allows focussing of all beams in the sample plane, with the same probe current and probe size as in a single beam SEM. Both secondary and transmission electron signals can be detected, using scintillator screens in conjunction with either a CCD or CMOS camera. We present proof of principle results showing that sub 10 nm resolution can be obtained for transmission imaging of stained rat pancreas tissue. We will discuss our efforts towards improving the detection methods, also including backscatter electron detection, and the data processing speed. Furthermore, work will be shown on quantifying and comparing the signals obtained from secondary, transmitted and backscattered electrons on stained tissue sections, as imaged by a conventional SEM.

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