From 3D Light to 3D Electron Microscopy
Joint Workshop on Correlative Microscopy for the Life Sciences

9th - 11th March 2014 | Ghent
Expand Your Lab Capabilities with Latest Products from ZEISS

ZIESS 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.

From 3D Light to 3D Electron Microscopy

Life happens in 3D – and understanding biological context in the three dimensions has been a major driver in light microscopy in the past decades. In the process 3D imaging technologies such as laser scanning microscopy have become standard in every modern biosciences lab.

ZEISS in collaboration with the BioImaging Core Facility at the VIB Ghent are proud to host this joint workshop and scientific meeting on Correlative Microscopy and 3D SEM. The meeting is centered around scientific sessions covering a broad range of correlative microscopy and SEM methods and extending to data management approaches. In addition participants will have the opportunity to join instrument workshops and round table discussions.

VIB
VIB is a non-profit research institute in life sciences. Almost 1,300 scientists conduct strategic basic research on the molecular mechanisms that are responsible for the functioning of the human body, plants, and microorganisms. Through a close partnership with four Flemish universities – UGent, KU Leuven, University of Antwerp, and Vrije Universiteit Brussel – and a solid funding program, VIB unites the forces of 83 research groups in a single institute. The goal of the research is to extend the boundaries of our knowledge of life. Through its technology transfer activities, VIB translates research results into products for the benefit of consumers and patients and contributes to new economic activity. VIB develops and disseminates a wide range of scientifically substantiated information about all aspects of biotechnology. More information: www.vib.be

VIB Bio Imaging Cores
The VIB Bio Imaging Cores are located in Ghent and Leuven and act as resource centers for advanced light and electron microscopy to the VIB as well as both academic and industrial laboratories in Flanders and beyond. The cores were established in 2012 and have a structure that is complementary and synergistic. Advanced microscopy services such as multi-photon confocal, long term cell and tissue imaging, and high content screening are available at both locations. Specialized services requiring high end equipment and expertise are available in both Ghent and Leuven with Ghent specializing in very sensitive functional confocal microscopy (FRET/FLIM) and serial block face imaging 3D electron microscopy. Leuven has equipment and expertise in fast acquisition confocal imaging and super-resolution microscopy as well as TEM electron tomography. For more information go to www.bioimagingcore.be

Core for Life
Core for Life was established in 2012 as an Excellence Alliance of Life Science Core Facilities in Europe. The mission of Core for Life is to explore the potential of coordinating and bundling core facility expertise and resources across institutes and countries in order to advance knowledge and to benefit the entire scientific and technological community. For further information go to http://www.coreforlife.eu
Agenda

**Day 1 | Sunday, March 9th 2014**
Conference Center Het Pand, Onderbergen 1

14:00 – 15:30       Arrival and Registration
15:30 – 16:00       Welcome Speech from VIB & ZEISS
16:00 – 17:00       Correlative PALM and EM: Options and Tradeoffs (Harald Hess/Ashburn)
17:00 – 18:00       Toward Making the Invisible and Complicated Understandable: Microscopy Across Scales and Modalities (Mark H. Ellisman/San Diego)
18:00 – 19:30       Reception

**Day 2 | Monday, March 10th 2014**
VIB, Technologiepark 927

08:30 – 09:00       Transfer from PickUp Point in the City to the VIB
09:00 – 09:30       Registration FSVM Building Zwijnaarde
09:30 – 09:45       Welcome to VIB (Chris Guérin/Ghent)

**Session I: FIB-SEM I**
09:45 – 10:20       FIB-Nanotomography for biomedical applications: high-resolution analysis of cells and tissues (Marco Cantoni/Lausanne)
10:20 – 10:40       Which Direction to Take for 3D CLEM: CLSM Combined with FIB-SEM, or Array Tomography? (Miriam Lucas/Zürich)
10:40 – 10:55       Use of different methods of correlative light-electron microscopy and three-dimensional reconstruction of ultra-structures for the study of intracellular transport (Galina V. Beznoussenko/Milan)
10:55 – 11:20       Coffee Break
11:20 – 11:35       FLIPPER, A combinatorial probe for 3D-LM/(ST)EM analysis of large areas (Ben Giepmans/Groningen)
11:35 – 11:55       Genetically encoded probes for light and EM imaging in brain tissue (Julian Ng/Cambridge)
11:55 – 12:10       Achievements of the correlative light-electron microscopy in combination with the three-dimensional electron microscopy (A.A. Mironov/Milan)
12:10 – 13:10       Lunch & Poster

**Session II: FIB-SEM II**
13:10 – 13:30       Correlative Light and Electron Microscopy: taking snapshots of the living at the ultrastructural level (Yannick Schwab/Heidelberg)
13:30 – 14:05       Three-dimensional Imaging of Adherent Cells: a comparison of FIB-SEM, STEM and serial sectioning (Paul Walther/Ulm)
14:05 – 14:30       Cryo-FIB-SEM: 3D imaging of cellular ultrastructure in native frozen samples (Wiebke Möbius/Göttingen)

**Workshops & Round Table Discussion**
14:45 – 17:15       Workshops (CAT, 3View, FIB-SEM, WetLab, Computing)
Round Tables
- 3D microscopy - taming the data tsunami
- 3D SEM - can we replace TEM?
- The best way to CLEM, is there one?

**Session III: Data Handling & Segmentation**
17:30 – 18:05       Modeling Brain Circuitry over a Wide Range of Scales (Pascal Fua/Lausanne)
18:05 – 18:25       Interactive analysis of 3D EM data with ilastik (Anna Kreshuk/Heidelberg)
18:25 – 18:40       Cell-Specific Visualization and Manipulation of Synapses In Vivo through Correlated Light and Electron Microscopy Combined with Reverse Genetics (Olivier Urwyler/Leuven)
18:40 – 18:55       Comparative Connectomics: Analysis of anatomically derived connectivity data based on 3D electron microscopy (Dan Bumbarger/Tübingen)
19:00                  Evening Dinner Event – F.S.V.M Atrium
21:30                  Bus Transfer to City
Day 3 | Tuesday, March 11th 2014
VIB, Technologiepark 927

08:30 – 09:00  Transfer from PickUp Point in the City to the VIB

Session IV: Other Roads to CLEM I

09:30 – 10:00  Preservation of Fluorescence During Quick Freeze Substitution (Richard Webb/Queensland)
10:00 – 10:30  Linking structure to function in cells: Correlation to integration, electrons to X-rays
              (Lucy Collinson/London)
10:30 – 10:45  Correlative microscopy using SIMS: High-sensitivity high-resolution elemental mapping for
              biological and materials science applications (S. K. Eswara Moorthy/Luxembourg)
10:45 – 11:00  Cryo correlative light microscopy and electron tomography illuminates lipids in Streptomyces
              cell division (Roman I.Koning/Leiden)

11:00-11:15  Coffee Break

Session V: Other Roads to CLEM II

11:15 – 11:50  Acquisition of large datasets in the olfactory bulb of the zebrafish larvae with serial-block face
               scanning electron microscopy (Christel Genoud/Basel)
11:50 – 12:25  Correlating fluorescence microscopy and electron tomography to visualize dynamic membrane
               ultrastructures (Wanda Kukulski/Heidelberg)
12:25 – 12:45  X-ray Microscopy: Illuminating the path from LM to 3D EM (Arno Merkle/Thornwood)
12:45 – 13:00  Wrap-Up

13:00  Lunch
13:30  Departure Bus Transfer to Station or City

Workshops

14:00 – 15:40  Workshops (CAT, 3View, FIB-SEM, WetLab, Computing)
15:50  Departure Bus Transfer to Station or City

Speaker Abstracts

Sunday, March 9th 2014

Correlative PALM and EM: Options and Tradeoffs
Presented by: Harald Hess | Janelia Farms Research Campus, Howard Hughes Medical Institute, Ashburn

PhotoActivated Localization Microscopy, PALM, images protein distributions in cellular structures by sequential localization
of thousands of individual fluorescently labeled proteins with ~20 nm resolution, however, the context might be unclear.
Electron microscopy, EM, provides such context and a combined correlative technique enables one to colorize those protein
locations on a black and white EM image. Combining the two modalities is highly compelling dream but inevitably
involves compromise in the quality of either the protein labeling in optical imaging or the quality of the ultra-structure in EM
imaging. As a result a variety of correlative approaches are evolving each with its own advantages and disadvantages for a
particular biological quest. I will discuss several different protocols using plastic embedded sections, cryo-sections,
membrane replicas combined with a diversity of EM imaging in both 2D and 3D with SEM, TEM, and FIB-SEM and offer a
few hopes and dreams for the future of correlating super-resolution microscopy with EM.

Toward Making the Invisible and Complicated Understandable:
Microscopy Across Scales and Modalities
Presented by: Mark H. Ellisman | National Center for Microscopy and Imaging Research (NCMIR), San Diego

A grand goal in cell biology is to understand how the interplay of structural, chemical and electrical signals in and between
gells gives rise to tissue properties, especially for complex tissues like nervous systems. New technologies are hastening
progress as biologists make use of an increasingly powerful arsenal of tools and technologies for obtaining data, from
the level of molecules to whole organs, and at the same time engage in the arduous and challenging process of adapting
and assembling data at all scales of resolution and across disciplines into computerized databases. This talk will highlight
projects in which development and application of new contrasting methods and imaging tools have allowed us to observe
otherwise hidden relationships between cellular, subcellular and molecular constituents of cells, including those of nervous
systems. New chemistries for carrying out correlated light and electron microscopy will be described, as well as recent
advances in large-scale high-resolution 3D reconstruction with LM, TEM and SEM based methods.
Examples of next generation cell-centric image libraries and web-based multiscale information exploration environments for
sharing and exploring these data will also be described.
We are thankful to K. Dittmar for providing the cell samples and C. Genoud for help in the reconstruction of the brain structure.


Near the track of the ion beam, electron density can be measured and compared to the density of the substrate. The volume reconstructed in this case is 20 x 15 x 12 micrometer. In Fig. 2 a region of conventionally fixed and resin embedded rat cortex was imaged with a voxel size of 10 x 10 x 10 nm. This results in images with a contrast and resolution close TEM images of ultramicrotome cut thin sections. The FIB can automatically acquire large volumes of images with a thickness that is considerably smaller than slices prepared for TEM by an ultramicrotome [1]. Fig. 1 shows the result of a biocompatibility test. Cells have been grown on medical steel covered by a ceramic coating. The ion beam of the FIB cuts uniformly through the resin embedded cell structure as well as the ceramic layer and the steel substrate. A series of 1200 images with a voxel size of 10 x 10 x 10 nm allowed the reconstruction of almost a complete cell as well as the underlying coating and the grain structure in the substrate. The volume reconstructed in this case is 20 x 15 x 12 micrometer. In Fig. 2 a region of conventionally fixed and resin embedded rat cortex was imaged with a voxel size of 5 x 5 x 5 nm. Within the datasets, all stained structure can be visualized from any angle. This includes every membrane, organelle, and cytoskeletal elements such as microtubules. This quality of serial imaging allows for detailed structural analysis of many synaptic connections (about 1000 in this case) within a single volume [2]. Despite their structural diversity and random orientation within the volume, every synaptic vesicle can be drawn as well as the details of each pre- and post-synaptic density (Fig. 3). This study shows that FIB-SEM now provides a means of imaging cell and tissue ultrastructure within large volumes that are unachievable with such imaging devices. In combination with electron microscopy [3].

References
[3] We are thankful to K. Dittmar for providing the cell samples and C. Genoud for help in the reconstruction of the brain structure.

Speaker Abstracts

FIB-Nanotomography for biomedical applications: high-resolution analysis of cells and tissues

Presented by: Marco Cantoni | EPFL, Electron Microscopy Center, Lausanne

Detailed analysis of cell structure typically requires electron tomography of thick resin embedded sections. This provides high resolution serial images, but only through a limited volume, and with certain details obscured by the ‘missing wedge’. In this study we have explored how the FIB/SEM can be used to provide a similar quality of data, yet through far larger volumes. Resin embedded samples of cells and brain tissue stained with Osmium-tetroxide were found to be ideal for FIB Nanotomography. They are stable under the electron beam and the excellent contrast observed allows the application of all the different techniques of reconstruction and analysis already widely used in materials science. Optimising the conditions of image acquisition with the electron beam limits the penetration depth of the electrons to a few nanometers. This results in images with a contrast and resolution close TEM images of ultramicrotome cut thin sections. The FIB can automatically acquire thousands of images with a thickness that is considerably smaller than slices prepared for TEM by an ultramicrotome [1]. Fig. 1 shows the result of a biocompatibility test. Cells have been grown on medical steel covered by a ceramic coating. The ion beam of the FIB cuts uniformly through the resin embedded cell structure as well as the ceramic layer and the steel substrate. A series of 1200 images with a voxel size of 10 x 10 x 10 nm allowed the reconstruction of almost a complete cell as well as the underlying coating and the grain structure in the substrate. The volume reconstructed in this case is 20 x 15 x 12 micrometer. In Fig. 2 a region of conventionally fixed and resin embedded rat cortex was imaged with a voxel size of 5 x 5 x 5 nm. Within the datasets, all stained structure can be visualized from any angle. This includes every membrane, organelle, and cytoskeletal elements such as microtubules. This quality of serial imaging allows for detailed structural analysis of many synaptic connections (about 1000 in this case) within a single volume [2]. Despite their structural diversity and random orientation within the volume, every synaptic vesicle can be drawn as well as the details of each pre- and post-synaptic density (Fig. 3). This study shows that FIB-SEM now provides a means of imaging cell and tissue ultrastructure within large volumes that are unachievable with such imaging devices. In combination with electron microscopy [3].

References
[3] We are thankful to K. Dittmar for providing the cell samples and C. Genoud for help in the reconstruction of the brain structure.

CLEM has become a powerful tool in life science to combine large-scale volume imaging of tissues by LM with a high-resolution description of their morphology using EM. The combination of 3D microscopy techniques such as CLSM and FIB-SEM, and recently array tomography (AT) [1] opens up new possibilities to expand morphological context description and analysis into the third dimension. These two alternative approaches to correlate 3D LM data with SEM volume data have been shown to provide a valid alternative to TEM-based approaches [2,3]. Modern SEM-platforms allow imaging with an x/y resolution of 2.3 nm, and offer the advantage of automation: e.g. routine overnight FIB-SEM volume imaging can cover cross-sections of up to 50x40 µm2. With a slice thickness of 5-10 nm, these stacks can expand to several 10 µm in the z-axis. FIB-SEM further enables precise, site-specific milling. In combination with preceding screening of the specimen with CLSM to identify the ROI, this allows targeted high-resolution 3D imaging of the structure of interest. This approach requires a sample preparation that facilitates the application of both LM and EM on a bulk specimen. Therefore we have adapted a protocol for freez-substitution after high-pressure-freezing, and subsequent embedding in HM20 to include fluorophores [4]. Thus fluorescently labeled, resin-embedded specimens can also be used for AT. Here, ribbons of serial sections are loaded onto glass-slides, enabling even larger ROIs to be investigated by wide-field LM and subsequently by SEM. With the introduction of special surface coatings such as ITO these glass supports remain transparent for LM, but become highly conductive for SEM investigation. Such ribbons can be stained for histology (e.g. Toluidine blue or H&E), or fluorescent immuno-labeling can be applied to specifically identify a ROI by LM prior to HR-SEM. If needed, the sections can also be post-stained with uranyl acetate for SEM-imaging.

Software solutions are available to facilitate the relocation of the ROI by a marker-based calibration of the sample in both LM and SEM. And automated recording of 3D volumes is possible for both FIB-SEM and AT. However, the FIB-SEM approach can achieve the better z-resolution, whereas the z-resolution for AT is limited by the sectioning process. FIB-SEM can therefore record isotropic voxels, which is an advantage when it comes to 3D reconstruction and modeling. AT on the other hand has a much larger field of view, and enables specific labeling to identify the ROI. Furthermore, FIB-SEM is a destructive method, while the AT-ribbons can be stored and re-investigated ad libitum [5]. Both approaches – CLSM combined with FIB-SEM, as well as AT – have the potential to bridge the gap between systems biology and high-resolution EM. The method of choice of will have to be determined anew for every project, according to the size of the volume of interest and the desired imaging resolution in x,y, and z.

References
Use of different methods of correlative light-electron microscopy and three-dimensional reconstruction of ultra-structures for the study of intracellular transport

Presented by: Galina V. Beznoussenko | Istituto FIRC di Oncologia Molecolare, Milan

In recent years, electron microscopy starts to revive. This revival is based on the wide use of two new applications of electron microscopy in the practice of biological research. This is largely due to the rapid development of the two directions of electronic microscopy, including 1) the combined use of the same sample methods of light and electron microscopy, which can be directed to identify rare patterns and rare events, and 2) development of new methods of three-dimensional reconstruction of ultra-structures, which includes analysis of samples with the help of scanning electron microscopy (pseudo 3D), stereo-pairs examined under TEM or SEM; tilting series obtaining during tilting of the sample; serial sections obtained outside a chamber of an EM and examined in TEM or SEM; serial bloc face SEM; focused ion beam SEM and finally, TEM or SEM tomography.

Here, we will demonstrate our experience in the examination of different aspects of intracellular transport with the help of CLEM combined with 3D reconstruction of the ultra-structure examined. We will demonstrate pitfalls and problems arising from the use of CLEM in combination with 3D EM.

We showed that procollagen and other cargos leave the ER again inside large pleiomorphic carriers rather than in COPII vesicles (Mironov et al., 2003). This observation is at the origin of a large literature on the mechanism of secretion of collagen. We opened the way to the visualization by EM of single microinjected cells and described the effects of antibody-mediated neutralization of several specific machinery proteins (Kweon et al., 2004; Marra et al., 2007). We described the ultrastructure of invadopodia in metastatic cells (Baldassarre et al., 2003); and we used CLEM in many other studies. Finally, using the deep 3D reconstruction of rat hepatocytes we demonstrated that mega-vesicles containing lipid particles in their lumen do not exist. At all steps of intracellular transport, namely, at ER-Golgi interface, intra-Golgi and at the level of post-Golgi transport, all membranous structures containing lipid particles inside their lumen are connected with other organelles.

References

FLIPPER, A combinatorial probe for 3D-LM/(ST)EM analysis of large areas

Presented by: Ben Giepmans | University Medical Center Groningen, Department of Cell Biology

Fluorescent proteins (FPs) have allowed major insight into biological processes as they occur in live cells. Despite recent advances in fluorescence imaging, EM is still superior to resolve structures. Genetically encoded fluorescent probes that can also be visualized at the electron microscopy (EM) level allow correlation of live-cell imaging and ultrastructural examination. These family of probes allows stringent fixation, while avoiding harsh permeabilization conditions damaging cell structure and protein localization.

We designed and applied a new genetically-encoded probe allowing visualization of proteins in live cells and by EM, named FLIPPER (Fluorescent Indicator and Peroxidase at EM Resolution). FLIPPER is based on a fluorescent protein coupled to horse radish peroxidase. FLIPPER allows 3D live-cell imaging as well as EM-analysis of different Z-planes and/or large X/Y areas. The 3D EM can potentially be done using serial sections, with the advantage over block face imaging is that the ultrastructural contrast is outstanding using STEM mode, even for low contrasted samples. This enables sensitive detection of FLIPPER, but is also compatible with more traditional immuno-EM labeling with gold or quantum dots. We also implement large scale STEM to visualize macromolecules and organelles in the context of unlabeled organized cell systems and (human) tissues, similar to what has been published with traditional TEM (www.nanotomy.nl). 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.

Genetically encoded probes for light and EM imaging in brain tissue

Presented by: Julian Ng | MRC-Laboratory of Molecular Biology, Division of Neurobiology, Cambridge

To understand how the nervous system functions, it is essential to identify the neural elements and determine how they are organized within a whole brain environment. Tracing individual neuronal and synaptic connections is key to unravelling the neural circuit principles, however imaging these structures come with particular challenges. One difficulty in doing so derives from the fact that imaging neurons is a multiscale process. While initial axon and dendrite projections can span across 100s of microns to centimetres (depending on the neuron and organism studied), they ultimately terminate at synapses; subcellular contact sites at nerve endings responsible for neural transmission. While light-based fluorescence confocal microscopy is suitable for axon and dendrite tracing, visualising terminal synapses and subcellular structures (organelles, vesicles and subsynaptic complexes) require sub-micron resolution, as synaptic elements range from 30-100 nm. Large volume electron microscopy (EM) is a key approach that can deliver unsurpassed data on the 3D organization of neuronal elements as large volume EM can identify long range neuronal projections over micron scales as well as provide ultrastructural data at the 10-100 nm range of the synaptic termini, all within in a complex 3D brain environment. However, deciphering their complex patterns from large volume EM data remains a challenge. In our work, we are investigating the feasibility of using the genetically encoded light and EM imaging probes together with 3D EM. The ultimate aim of our work is to generate volumetric EM data for discrete regions within the fly brain corresponding to the Drosophila olfactory neural network. We have recently been optimising the miniSOG technique [1] for tissue-based imaging under fluorescence light and TEM. To reconstruct the Drosophila olfactory circuit, we now would like to use SBF-SEM to generate large volume EM data and identify genetically labelled subsets of Drosophila olfactory neurons and decipher their projections and terminal connection patterns.

References
Correlative Light and Electron Microscopy: taking snapshots of the living at the ultrastructural level

Presented by: Yannick Schwab | European Molecular Biology Laboratory, Heidelberg

Our work is focused on the development of methods that enable high-resolution snapshots of dynamic events in cells and small model organisms. To achieve that, correlating light and electron microscopy is a powerful solution, because it combines functional imaging (fluorescent microscopy, time lapse imaging) with an enhanced readout of the sub-cellular organization (electron microscopy). By improving targeting strategies, we are successfully combining live imaging and EM on various sample types, allowing the correlation in 3D of rare events in cultured cells, nematodes, zebrafish embryos and mouse tissues.

Three-dimensional Imaging of Adherent Cells. A comparison of FIB-SEM, STEM and serial sectioning

Presented by: Paul Walther | University Ulm

FIB-SEM tomography challenges the traditional methods for 3D analysis in the electron microscope, serial sectioning transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) tomography. With all three methods, relatively large volumes of resin embedded biological structures can be analyzed at resolutions of a few nm within a reasonable expenditure of time. The traditional method is serial sectioning and imaging the same area in all sections. Another method is TEM tomography that involves tilting a section in the electron beam and then reconstruction of the volume by back projection of the images. When the scanning transmission (STEM) mode is used, thicker sections (up to 1 µm) can be analyzed. The newest approach is focused ion beam/scanning electron microscopy (FIB/SEM) tomography, in which, a sample is repeatedly milled with a focused ion beam (FIB) and each newly produced block face is imaged with the scanning electron microscope (SEM). This process can be repeated ad libitum in arbitrary small increments allowing 3D analysis of relatively large volumes such as eukaryotic cells. We show that resolution of this approach is considerably improved, when the secondary electron signal is used. However, the most important prerequisite for three-dimensional imaging is good specimen preparation. For all three imaging methods, cryo-fixed (high-pressure frozen) and freeze substituted samples have been used.
**Cryo-FIB-SEM: 3D imaging of cellular ultrastructure in native frozen samples**

Presented by: Wiebke Möbius | Max-Planck-Institute of Experimental Medicine, Göttingen

Focused ion beam (FIB) milling combined with serial block face imaging in the scanning electron microscope (SEM) is an efficient and fast method to generate volume data for 3D analysis. The next obvious step is the application of this technique for the purpose of directly imaging volumes of native frozen samples at cryo-conditions. In a pioneering study (Schertel et al., Journal of Structural Biology 184 (2013): 355-360) we applied cryo FIB-SEM to image fully hydrated frozen specimens of mouse optic nerves and Bacillus subtilis spores prepared by high-pressure freezing. We were able to establish imaging conditions for the direct visualization of the ultrastructure in the block face at −150°C by using an in-lens secondary electron (SE) detector. By serial FIB milling and block face imaging in the optic nerve we obtained within 3 h a volume of X=7.72 µm, Y=5.79 µm and Z=3.81 µm with a lateral pixel size of 7.5 nm and milling steps of 30 nm. The intrinsic contrast of membranes was sufficient enough to distinguish subcellular structures like Golgi cisternae, vesicles, endoplasmic reticulum and cristae within mitochondria. Serial images could be used for a three-dimensional reconstruction of different types of mitochondria within an oligodendrocyte and an astrocytic process. Applying this technique to dormant Bacillus subtilis spores we obtained volumes containing several spores and discovered a novel core structure, which was not visualized before by any other imaging technique. In summary, we describe here the use of cryo FIB-SEM as a tool for direct and fast 3D cryo-imaging of large native frozen samples. This technique is especially suited for specimens that are difficult to dehydrate such as bacterial spores or those that require remaining hydrated like skin samples.

**Modeling Brain Circuitry over a Wide Range of Scales**

Presented by: Pascal Fua | EPFL Lausanne

Electron microscopes (EM) can now provide the nanometer resolution that is needed to image synapses, and therefore connections, while Light Microscopes (LM) see at the micrometer resolution required to model the 3D structure of the dendritic network. Since both the arborescence and the connections are integral parts of the brain’s wiring diagram, combining these two modalities is critically important. In this talk, I will therefore present our approach to building the dendritic arborescence, to segmenting intra-neuronal structures from EM images, and to registering the resulting models. I will also argue that the techniques that are in wide usage in the Computer Vision and Machine Learning community are just as applicable in this context.

**Interactive analysis of 3D EM data with ilastik**

Presented by: Anna Kreshuk | University Heidelberg, IWR

The interactive learning and segmentation toolkit (ilastik) is a modular software framework, which allows for interactive visualization and analysis of very large datasets in up to 5 dimensions. Most analysis operations are performed lazily, which enables targeted interactive processing of data subvolumes, followed by complete volume analysis in offline batch mode. One of the main applications, driving the development of ilastik, is automated segmentation of serial EM stacks of neuronal tissue. I will present several such workflows along with the general architecture of ilastik. In particular, ilastik-based approaches for the following neural image processing tasks will be shown:

1. Membrane and synapse detection in 3D EM data by interactively trained classification. Classification can be based on pixel-, as well as object-, level annotations and features. As an example, I will demonstrate the application of this workflow to isotropic FIB/SEM and anisotropic ssTEM volume images.
2. Semi-automatic extraction of single processes in EM and LM volume images based on inside-outside user annotations and segmentation uncertainty feedback (carving). I will demonstrate an application of this workflow to FIB/SEM volume image segmentation.
3. Fully automatic segmentation of EM volume images into tentative neural processes based on an interactively learned classifier of superpixel affinities. I will show the application of this workflow to FIB/SEM data.
4. Myelinated axon tracing and detection of nodes of Ranvier in SBFSEM volume images.

ilastik is available as open source software at www.ilastik.org.
Speaker Abstracts

Monday, March 10th 2014

Cell-Specific Visualization and Manipulation of Synapses In Vivo through Correlated Light and Electron Microscopy Combined with Reverse Genetics

Presented by: Olivier Urwyler | VIB, Vesalius Research Center, Leuven

Regulation of synapse formation and elimination is essential for precise wiring of the nervous system during development, and its de-regulation may lead to neurodevelopmental disorders. However, particularly the molecular pathways controlling synaptogenesis in the central nervous system (CNS) are not well understood. Small genetic model systems act as a good starting point for identifying and characterizing these molecular pathways. To study CNS synapse formation and specificity, we developed new tools for efficient genetic labeling and manipulation of single sensory neurons in the Drosophila CNS. Our system allows analysis of panoptic of cellular markers in defined wild-type or mutant mechano-sensory (ms) neurons across multiple animals. The central projections of these neurons form a stereotypic branch pattern and are large, which greatly facilitates discrimination of sub-cellular structures with light microscopy techniques. We have conducted a pilot genetic screen to identify molecular pathways underlying synapse formation and selection, and will present some candidate regulators of pre-synapse development. For in-depth characterization of synaptic defects, we have established correlative light and 3D electron microscopy (CLEM) on single ms neurons. This allows analysis of the same defined neuron in different animals at the ultrastructural level. We use near-infrared branding marks in the tissue to 1) efficiently approach and find the region of interest (ROI) in the block-face scanning electron microscope; 2) determine the field of view to be imaged in the ROI; and 3) readily find the ms neuron in the 3D-EM dataset. The optimized protocol allows us to get correlated light and electron microscopy datasets within roughly two weeks. Here, we show CLEM at the level of single pre-synaptic active zones, and also use this approach to analyze number and distribution of mitochondria at axonal branch points. Our system opens up possibilities for future studies of synapse structure, development and plasticity in both wild-type and mutant animals in vivo.

Comparative Connectomics: Analysis of anatomically derived connectivity data based on 3D electron microscopy

Presented by: Dan Bumbarger | Max Planck Institute for Developmental Biology, Tübingen

Though technology for 3D reconstruction of large volumes with electron microscopy have advanced significantly, synapse-level wiring diagrams based on these methods are available only for a small number of animals. We have described the synaptic connectivity in the pharyngeal nervous system of the nematode Pristionchus pacificus to that of the well-known model organism Caenorhabditis elegans. Image data used for generating connectivity networks were acquired and analyzed with a graph theoretical approach, and revealed a surprising degree of divergence in connectivity between the two species. A dramatic difference in the proportion of motoneurons points towards fundamental changes in how information is processed. Some changes in wiring intuitively reflect known differences in feeding behavior, and some system-level patterns are conserved despite the wiring differences. Modified methods for centrality analysis identify candidate neurons for more complex regulation of feeding behavior in the predatory nematode P. pacificus.

Tuesday, March 11th 2014

Preservation of Fluorescence During Quick Freeze Substitution

Presented by: Richard Webb | Centre for Microscopy and Microanalysis University of Queensland Australia

For correlative light/ electron microscopy (CLEM) to work successfully a link is needed between these two imaging systems in order to give confirmation that the same cell/organelle/structure is being observed in both modes. Fluorescence imaging has been the labelling technology of choice, particularly with the use of genetic markers such as GFP, which allows localisation of the protein of interest in a section of the processed and embedded sample. This section may then be observed in the transmission electron microscope and the position showing the fluorescent labelling can again be located. Freeze substitution coupled with fast freezing has been shown to produce morphological preservation of a high standard with few artefacts. Combining these with only light fixation and staining using uranyl acetate and then embedding in Lowicryl HM20 resin we have shown that it is possible to preserve the fluorescence throughout sample processing so that it can still be observed in sections of the embedded samples. We have also shown that freeze substitution protocols, which have been notoriously long, often being up to a week in length, can be drastically shortened so that they can be as little as 90 minutes in the case of single celled samples, and three hours or less for larger samples. No drop off in the quality of the preservation is seen using these shorter protocols and, in fact, it can be better than when using longer procedures. Lowicryl resin routinely requires 4 days to polymerise, so when it is combined with standard freeze substitution protocols, the time for a complete process to achieve polymerised blocks can take such long times that they have major impacts on achieving results in realistic times. We have now modified the uranyl acetate fixation and Lowicryl embedding freeze substitution protocol so that it can take advantage of the quick processing methods, making it now possible to go from frozen sample to fully processed and embedded sample in less than 24 hours. This ability to produce processed samples in extremely short times has incredible advantages for enhancing workflow in the EM laboratory while producing samples with comparable or better preservation of morphology, antigenicity and fluorescence.

Correlated Light and Electron Microscopy Combined with Reverse Genetics

Presented by: Olivier Urwyler | VIB, Vesalius Research Center, Leuven

...
Correlative microscopy using SIMS: High-sensitivity high-resolution elemental mapping for biological and materials science applications

Presented by: S. K. Eswara Moorthy | Centre de Recherche (SAMI), Luxembourg

A characterization technique that is capable of simultaneously providing high chemical sensitivity and high spatial resolution is of paramount importance to gain deeper understanding in physical and biological sciences. To address this need we have combined in-situ the extraordinary chemical sensitivity (down to distinguishing isotopes) of the Secondary Ion Mass Spectrometry (SIMS) with the exceptional spatial resolution offered by techniques such as Transmission Electron Microscopy (TEM-SIMS), Helium-Ion Microscope (HIM-SIMS) and Scanning Probe Microscopy (SIMS-SPM).

To determine the feasibility and to demonstrate the applications of the aforementioned techniques, we have developed the following three prototype instruments for correlative microscopy:

(a) TEM-SIMS: A specially modified Tecnai F20 with enlarged gap between the pole-pieces, a special high-voltage sample holder, extraction optics for secondary ions and a compact high-performance mass spectrometer

(b) HIM-SIMS: A high-performance compact SIMS system attachable to a Zeiss HIM. [1, 2]

(c) SIMS-SPM: A Cameca NanoSIMS50 with attached SPM/AFM unit for combining chemical sensitivity and topographic imaging. [3-5]

To enhance the low intrinsic yield of secondary ions for non-reactive primary ion beams such as Ga+ for the TEM-SIMS or He+ or Ne+ beams in HIM-SIMS, we use reactive gas flooding [1, 6]. Specifically, the enhancement of negative secondary ion yields due to Cs flooding and the positive secondary ion yields with O2 flooding were found to be up to four orders-of-magnitude. This optimisation of secondary ion yields leads to detection limits varying from 10⁻³ to 10⁻⁶ for a lateral resolution between 10 nm and 100 nm.

References

Acquisition of large datasets in the olfactory bulb of the zebrafish larvae with serial-block face scanning electron microscopy

Presented by: Christel Genoud  |  FMI Basel

The study of biological systems together with the understanding of their fine molecular mechanisms requires new microscopy and image processing approaches. In particular, it is often necessary to image the same biological sample at different scales, ranging from a few millimeters down to a few nanometers. To obtain a 3D volume at the ultrastructural level that is covering a field of view compatible with the field of view obtained by light microscopy, the serial block-face scanning electron microscopy (SBEM) has been shown to be suitable. Following 2photon in-vivo imaging, zebrafish larvae are fixed and processed for EM following a protocol providing a high contrast (based on NMCD protocol). Using an SEM containing a microtome inside the vacuum chamber (Denk and Horstmann 2004; GATAN 3View; FEI Quanta 200F), the olfactory bulb of the larvae is imaged. The surface of the block is imaged using a montage strategy to cover the entire region of interest at 12 nm per pixel. 30 nm are then cut from the surface to have a new surface to image. This technique allowed getting an entire larvae olfactory bulb at the ultrastructure level. In order to extract the components to be analyzed, different image pre-processing tools have been developed in order to register the images in XY and Z, to homogenize the contrast in all dimensions and generate cubes suitable for analysis in Knossos (Denk et al). Image quality is sufficient to manually reconstruct the morphology of individual neurons, even though some processes are very thin. Such a technical workflow allows going from the live organism to the ultrastructural connectome allowing to link the structure to the function of the larvae olfactory bulb.

Correlating fluorescence microscopy and electron tomography to visualize dynamic membrane ultrastructures

Presented by: Wanda Kukulski  |  European Molecular Biology Laboratory, Heidelberg

The application of fluorescence and electron microscopy to the very same specimen has the potential to reveal ultrastructural details of dynamic and rare cellular events. We have developed a correlative approach combining high accuracy of correlation, high sensitivity as well as robustness to permit large dataset collections. Using a fiducial-based correlation, signals of fluorescent proteins can be mapped into 3D electron tomograms. The versatility of the approach was demonstrated by application to various cellular systems. More recently, we used it to describe how the plasma membrane is reshaped during endocytosis in a time-resolved manner. We systematically located 211 endocytic intermediates, assigned each of them to one of nine defined time windows during endocytosis, and reconstructed their ultrastructure in 3D. Combined with a quantitative analysis of the membrane shapes, correlative microscopy allowed us to produce a virtual 4D-movie of how protein-mediated shape changes occur during transition from a plane membrane into a tubular invagination and to scission of a vesicle. This study demonstrates the capability of our simple, robust correlative microscopy approach to answer structure-function related questions in cell biology.

X-ray Microscopy: Illuminating the path from LM to 3D EM

Presented by: Arno Merkle  |  ZEISS X-ray Microscopy

X-ray Microscopy (XRM) presents a new opportunity to efficiently bridge length scales between light and electron microscopy and ease the "needle in a (3D) haystack" navigation problem. One example where XRM is demonstrating its effectiveness as a correlative technique is in the field of Neuroscience. Great interest in creating complete neural network maps of the brain and the need for information across multiple length scales in 3D has scale spawned the development of high-throughput 3D electron microscopy (3DEM) techniques, based on TEM and SEM methods incorporating either physical sectioning via cutting (ultra-microtome) or ion abrasion (FIB-SEM). However, these 3DEM techniques can only prosper if long acquisition times, sensitivity to sample preparation and quality, and inefficiencies in locating regions of interest (buried subsurface features) can be averted. Recently, XRM techniques acting as a bridge between light- and electron- microscopy have acted as an efficiency multiplier in this field and others. In this talk we will present the current status and vision for future workflows that incorporate 3D volumetric imaging with x-rays between light and electron-based methods.
**Background:** The synovium is a distinct structure that is primarily formed by fibroblast-like synoviocytes (FLS). Its multicellularity requires precise coordination between cells to generate an organized tissue that confers specialized functions critical to joint homeostasis. Direct cell-to-cell interactions may provide a means for intercellular communication and, thus, may facilitate the concerted behavior of FLS within the synovial tissue. Using a simplified three-dimensional model of the synovium, we analyzed the capacity of FLS for the exchange of cytoplasmic content through intercellular connections.

**Methods:** Human FLS were prepared from synovial tissues obtained as discarded specimens following joint arthroplasty or synovectomy. Passaged FLS were cultured in spherical extracellular matrix with an average size of 3 mm in diameter. For live cell imaging, FLS were labeled with fluorescent membrane dyes or loaded with fluorescent non-degradable latex beads and synovial micromass cultures were scanned under a confocal microscope. Analysis of the resulting 3D live cell imaging movies was done with Imaris® Bitplane software. Results: To examine cytoplasmatic transfer from one cell to another, we labeled 50% of FLS with a red cell tracker membrane dye (CMRA®, Invitrogen) and loaded the other 50% with fluorescent microspheres. In an eight day long time series of scanning micromasses, we found that green microspheres do indeed appear in red labeled cells. First evidence for a green microsphere in a red cell was found on Day 3 and over the course of the following six days more and more green microspheres accumulated in red labeled cells. The transfer rate was found to be 10% of newly affected cells per day. Live cell imaging of a 2D FLS culture demonstrated microsphere movement within interconnecting membrane nanotubes.

**Conclusions:** These studies suggest transfer of cytoplasmic cargo between FLS and may provide insight into the communication system of these cells forming the synovial tissue. Further studies will demonstrate the significance of directed exchange through intercellular connections for cellular cooperation and the function of the normal as well as the diseased synovium.
Cellular Function and Biogenesis of Microsporidian Mitosomes

Christian Hacker and John M. Lucocq (University of St Andrews, School of Medicine, Medical & Biological Sciences Building, North Haugh, St Andrews, Fife, KY16 9TF)

Microsporidia are eukaryotic intracellular parasites that can infect a wide range of organisms and are clinically important. They possess extremely reduced genomes which are amongst the smallest of any eukaryote. However, due to their reductive life style, microsporidia rely strongly on their host cell and therefore parasite-host interactions play an essential role in the supply of metabolites and energy. Microsporidia and other human parasites were originally thought to lack mitochondria but interest in them was renewed when our group and others discovered relict mitochondria inherited throughout the life cycle of those organisms. A wide-scale approach involving 3D SEM of different cell stages of the microsporidium using correlative light and electron microscopy will be essential to investigate how the mitosomes are delivered of ATP from host cytoplasm to the growing and differentiating parasite.

Poster Abstracts

Molecular biology of neuronal wiring: A lesson from mental disorders

Pietro Fazzari (VIB11 and K.U.Leuven, Belgium)

The brain is by far the most complex structure of our body. Billions of neurons with very varied morphologies are wired with mesmerizing precision to assemble multiple circuitries. These different brain systems carry on robust fundamental functions as well as higher cognitive processes. The powerful tools of the postgenomic era provided us with an unprecedented knowledge of the genetic basis of mental disorders. However, the cellular and molecular biology of the assembly of neuronal circuits is still poorly understood. Even less is known about the relation between structure and function of brain: what specific connections are required for the various brains functions? What perturbations of neural circuitries underlie mental pathologies?

In our work, we investigated the role of Nrg1, a major schizophrenia risk gene, and of γ-secretase, a protease involved in Alzheimer’s Disease, during the formation of cortical circuitries. We recently showed Nrg1 forward signalling controls the formation of cortical inhibitory circuits via its specific receptor ErbB4 (Fazzari et al., Nature, 2010). In particular, ErbB4 is expressed by chandelier and basket interneurons that establish inhibitory connections respectively on axon and on the soma of pyramidal neurons. Ultrastructural analysis revealed that ErbB4 localizes to axon terminals and postsynaptic densities of interneurons. In addition, gain and loss of function experiments demonstrated that Nrg1/ErbB4 signalling promotes both the establishment of excitatory synapses formed by excitatory neurons onto interneurons and of inhibitory synapses onto the pyramidal cells.

On the other end, we found unexpectedly that deficient mice for Aph1BC, a crucial subunit of γ-secretase, displayed a behavioural phenotype similar to that of Nrg1 deficient mice. Moreover, Nrg1 processing was impaired in Aph1BC null mice and we showed that Schizophrenia associated cSNP V321L hampers γ-secretase (Dejaegere et al., PNAS 2008). Our current work is aimed at testing the hypothesis that Aph1BC-γ-secretase complexes control neural wiring via selective regulation of Nrg1.

Cellular Function and Biogenesis of Microsporidian Mitosomes

Microsporidia are eukaryotic intracellular parasites that can infect a wide range of organisms and are clinically important as emerging human pathogens which can cause disease in immune-suppressed individuals. These obligate organisms are related to fungi and appear highly adapted to an intracellular life style. They possess extremely reduced genomes which are amongst the smallest of any eukaryote.

However, due to their reductive life style, microsporidia rely strongly on their host cell and therefore parasite-host interactions play an essential role in the supply of metabolites and energy. Microsporidia and other human parasites were originally thought to lack mitochondria but interest in them was renewed when our group and others discovered relict mitochondria with unknown function. These tiny double-membrane bounded organelles, termed mitosomes, contain no genome and are stripped of most of the functions assigned to “classic” mitochondria. Our lab is trying to discover mitosome functions, to understand their life history and to analyse how these organisms obtain energy from their host. With a size of just 50 – 200 nm, electron microscopic techniques play a key role in analysing all aspects of mitosome biology.

Mitosomes, apart from the production of Fe-S-clusters, have lost canonical mitochondrial functions like that of ATP-production by aerobic respiration. To identify candidate mitosome functions we systematically study the localisation of proteins involved in possible pathways located to the mitosome compartment using quantitative immuno EM and we are currently investigating the presence of iron-sulphur cluster assembly proteins as well as mapping the distribution of nucleotide transporters. In order to understand how microsporidia manage to sustain their energy requirements we investigated the parasite-host interaction by analysing the spatial relationship between the parasitophorous vacuole of the microsporidium Encephalitozoon cuniculi and its host cell environment. We could show that mitochondria bind directly at the vacuole membrane via electron dense bridging structures (< 10 nm length) and that this interaction is sensitive to digestion by proteases. Interestingly, we also discovered that the ATP exporting voltage gated channels of the mitochondrial outer membrane (VDAC) are concentrated at the sites of mitochondrial binding and we therefore conclude that Encephalitozoon cuniculi induces direct protein-dependent binding of host mitochondria to the parasitophorous vacuole to facilitate the delivery of ATP from host cytoplasm to the growing and differentiating parasite.

Another important focus of our lab is the quantitative study of mitosome biogenesis. Mitosomes are present in limited numbers and our studies indicate that a specialized mitosome division process is coordinated with cell and mitosome growth in order to maintain mitosome function as the parasite population expands. Targeting specific stages during the cell cycle of the microsporidium using correlative light and electron microscopy will be essential to investigate how the mitosomes are inherited throughout the life cycle of those organisms. A wide-scale approach involving 3D SEM of different cell stages of the parasite will enable us to obtain large datasets which we can analyse in respect to mitosome biogenesis. Furthermore, the stereological analysis of serial sections and electron tomograms allows us to estimate mitosome volume, structure and distribution quantitatively.

The CryoCapsule: Simplifying correlative microscopy

Xavier Heiligenstein¹,², Jérôme Heiligenstein³, Cédric Delevoye¹,², Ilse Hurban³,⁴, Sabine Bardin³,⁴, Perrine Paul-Gilleteau⁵,⁶, Lucie Sengmanivong⁵,⁶, Gilles Régnier⁶, Jean Salamero⁶, Claude Antony⁷, Graca Raposo⁸,⁹

¹Institut Curie, Centre de Recherche, Paris 75248, France. ²Structure and Membrane Compartments, CNRS UMR144, Paris 75248, France. ³Molecular Mechanisms of Intracellular Transport, CNRS UMR144, Paris 75248, France. ⁴Spatio-temporal modeling Imaging and cellular dynamics, CNRS UMR144, Paris 75248, France. ⁵Cell and Tissue Imaging Facility (PICT-BI4A), CNRS UMR144, Paris 75248, France. ⁶Processes and Engineering in Mechanics and Materials, Centre National de la Recherche Scientifique (CNRS), UMR 8006, CER de Paris, Arts et Métiers ParisTech, Paris, France. ⁷Department of Structural Biology and Genomics, Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM, U964, CNRS, UMR7104, Illkirch BP10142, France. ⁸CryoCapCell, 75015 Paris, France.

Correlating light and electron microscopy is both technically challenging and scientifically highly informative. Hence, many approaches are simultaneously developed to address each individual biological question. One of them consists in imaging live a fluorescent specimen, immobilize it and then process it for electron microscopy imaging (Guizetti et al., 2011; King & Verkade, 2008). Another approach start from the already immobilized specimen where the fluorescence is preserved when processed for electron microscopy (Kükülski et al., 2011; Nixon et al., 2009). To achieve fluorescence preservation, it is established that cryo-immobilization is required. High Pressure Freezing (HPF) is the state of the art method for vitrification of biological specimen up to 300μm. But HPF is a complex and tedious technique involving high skills.

We invented a new tool, the CryoCapsule (Heiligenstein et al., 2014), compatible with HPFM type high pressure freezing machines that dramatically facilitate the sample manipulation and allows us to combine these two approaches into one simple workflow. The CryoCapsule embeds all the parts commonly used for live CLEM (Mcdonald et al., 2010) into one single “easy to handle” block. As the sample preparation steps are simplified, we can visualize highly dynamic endosomal compartments interacting with melanosomal structures.

We conducted 3D live cell fluorescence imaging followed by electron tomography of serial sections to analyze the interactions between these two sub-cellular compartments from a specific and dynamic to a resolutive and contextual scale.
**Poster Abstracts**

### Integrated CLEM: From image overlay at biomolecular precision to live-cell imaging

N. Liv, M. T. Haring, D. S. B. van Oosten Slingeland, P. Kruit, J. P. Hoogenboom  
(Imaging Physics, Faculty of Applied Sciences, Delft University of Technology, Delft, The Netherlands)

In the biological sciences, correlation between fluorescence (FM) and electron microscopy (EM) allows for ultrastructural analysis (i) with biomolecular identification through fluorescent labels, and (ii) after live-cell imaging [1]. Especially high-resolution, molecular-scale image overlay and correlation with live-cell dynamics are challenging. Integrated microscopes facilitate CLEM, but the integration often compromises the capabilities of either FM or EM as compared to stand-alone microscopes. Here, however, we will show that integrated microscopy can also lead to entirely novel imaging methodology addressing both above mentioned challenges, provided the integration is performed such that EM and FM can be performed simultaneously on the same area of a sample.

We have recently presented an integrated microscope that enables high-NA fluorescence microscopy inside a Scanning EM [2]. Here, the electron beam can be positioned anywhere within the FM field of view [3], which allows us to:

(i) Achieve image overlay with sub-5 nm EM-FM registration in a fully automated fashion and without fiducial markers. The position of the electron beam is monitored directly in the fluorescence image frame by detecting the electron-beam induced luminescence from the sample substrate. As the luminescence can be recorded at high signal-to-noise ratio, the electron beam positions can be retrieved with high accuracy in the fluorescence image. We use these retrieved coordinates to determine the full non-linear transformation between EM and FM images.

(ii) Record EM snapshots during live-cell FM. To this end, biological cells are contained in liquid in the integrated microscope using a holder with electron and light transparent windows. Based on the FM observations of cellular dynamics, we determine the locations for structural SEM snapshots. We show that the SEM can enhance fluorescence observations of the uptake of epidermal growth factor conjugated quantum dots by revealing positions of individual quantum dots in filopodia in living cells.

### Alzheimer’s Tools for Gene & Protein

Mohammed Iftekhar (BioCuration Labs, Bangalore, India)

Alzheimer's Disease (Dementia) is a brain disorder that seriously affects a person's ability to carry out daily activities. The most common form of dementia among older people is Alzheimer’s disease (AD), which initially involves the parts of the brain that control thought, memory, and language. Although scientists are learning more every day, right now complete information on the disease causes AD is not known, and there is no proper treatment is available. The disease information is spread in different databases, it make a user to get complete information on the disease in a single database difficult. The Alzheimer’s Disease database have complete information of disease, which is presented in user friendly and can be useful for students and researchers.

The project is developed for a Students, Researcher’s, Doctor’s to know about Alzheimer’s disease Information through database System. This project help every one to access all disease data directly so that he can get required information for their research. This Tools is for Data Collection of Alzheimer’s disease. Biocuration of Alzheimer’s disease Data curation With Hadoop Technology and backend Designing by MYSQL, PHP & Front end designing and connecting to the database.

### Correlative Photoactivated Localization and Scanning Electron Microscopy

Benjamin Kopek (Howard Hughes Medical Institute – Janelia Farm Research Campus, Ashburn, USA)

The ability to localize proteins precisely within subcellular space is crucial to understanding the functioning of biological systems. Recently, we described a protocol that correlates a precise map of fluorescent fusion proteins localized using three-dimensional super-resolution optical microscopy with the fine ultrastructural context of three-dimensional electron micrographs. While it achieved the difficult simultaneous objectives of high photoactivated fluorophore preservation and ultrastructure preservation, it required a super-resolution optical and specialized electron microscope that is not available to many researchers. We present here a faster and more practical protocol with the advantage of a simpler two-dimensional optical (Photoactivated Localization Microscopy (PALM)) and scanning electron microscope (SEM) system that retains the often mutually exclusive attributes of fluorophore preservation and ultrastructure preservation. As before, cryosections were prepared using the Tokuyasu protocol, but the staining protocol was modified to be amenable for use in a standard SEM without the need for focused ion beam ablation. We show the versatility of this technique by labeling different cellular compartments and structures including mitochondrial nucleoids, peroxisomes, and the nuclear lamina.

We also demonstrate simultaneous two-color PALM imaging with correlated electron micrographs. Lastly, this technique can be used with small-molecule dyes as demonstrated with actin labeling using phalloidin conjugated to a caged dye. By retaining the dense protein labeling expected for super-resolution microscopy combined with ultrastructural preservation, simplifying the tools required for correlutive microscopy, and expanding the number of useful labels we expect this method to be accessible and valuable to a wide variety of researchers.

### Foraminifera are unicellular organisms that are typically surrounded by an external shell made of calcium carbonate (CaCO3).

David Kirchenbüchler (Weizmann Institute of Science, Department of Materials and Interfaces, Rehovot, Israel)

With an intact shell it is difficult to observe the cytoplasm of Foraminifera. To overcome this we high pressure freeze the sample and fracture it without thawing. This procedure provides access to a single layer cut through the specimen. In the scanning electron microscope we are able to observe compartments of the cell, including the shell and membranated vesicles. The same sample is then observed, still frozen, in light microscopy. Here we are able to determine the locations of Ca2+ concentrations labeled with calcein. The correlative approach provides the opportunity to localize compartments involved in the processing of Ca2+ and to characterize them at high resolution under close to physiological conditions.
Quantifying ER-mitochondria contact points using 3D-EM imaging

Michiel Krol⁴, Bob Asselbergh¹, Anneke Kremer², Saskia Lippens³, Chris Geurin², Sophie Janssens⁵, Vincent Timmerman¹

¹VIB Molecular Genetics Department, University of Antwerp, Antwerpen, Belgium. ²VIB microscopy Core, Gent, Belgium. ⁴VIB Inflammation Research Center, Ghent University, Gent, Belgium

We study peripheral neuropathies such as Charcot-Marie-Tooth (CMT), a genetic disorder leading to progressive degeneration of the peripheral nerves, causing muscle weakness and atrophy amongst other symptoms. Despite over 60 different genes having been identified, little progress has been made in understanding the pathomechanism and the development of effective cures. Of these >60 genes, a disproportionate number plays a role in mitochondria or at the endoplasmic reticulum (ER)-mitochondria interface known as Mitochondria-Associated Membrane (MAM). We aim to develop a new method to quantify contact points between the ER and mitochondria, using a 3D electron (EM) microscopy approach. To this end, we are imaging fixed cells using the Zeiss Auriga Focused-Ion-Beam Scanning Electron Microscope (FIB-SEM). This method provides high-resolution image stacks of the cell, allowing precise 3D reconstruction and unbiased analysis of the organelles of interest. Besides optimising the imaging itself, we have experimented with several approaches to analyse these complex datasets, using both manual 3D reconstruction as well as automated 3D rendering with software such as Imaris. In addition to these approaches, we have also applied a stereologic method to estimate mitochondrial volume and surface within our image volume and determine the fraction of mitochondrial surface that is in contact with the ER membrane. We wish to use these methods to examine contact point formation in cells expressing WT and mutant genes in the context of peripheral neuropathy.
Poster Abstracts

**CLEM and 3D SEM Imaging in a Research Resource Facility**

Geoffrey Perumal, Benjamin Clark and Frank Macaluso
(Analytical Imaging Facility, Albert Einstein College of Medicine, Bronx, NY, USA)

The Analytical Imaging Facility (AIF) supports the microscopy needs of a large user group at Albert Einstein College of Medicine and provides a comprehensive light and electron microscope imaging facility dedicated to bringing state of the art methods in modern imaging to biomedical scientists with all levels of expertise. Correlative microscopy bridges the gap between light and electron microscopy. Functional information obtained with fluorescence probes in live and fixed samples is combined with nanometer scale structural information provided by electron microscopy of exactly the same region. The staff of the AIF has been developing sample preparation techniques to maintain fluorescence signal while preserving morphology for correlative fluorescence and scanning electron microscopy. Recent work in the facility is illustrated by visualization of primary cilia in fibroblasts and alphavirus budding in Vero cells. The primary cilium is a microtubule-based, solitary organelle to coordinate signaling pathways that critically regulate cellular processes during development and in tissue homeostasis. In this study using mouse embryo fibroblasts, cilia are detected by immunofluorescence microscopy with antibodies against acetylated tubulin to localize proteins along the cilium. Alfaphviruses are small enveloped RNA viruses that bud from the plasma membrane of host cells. Virus budding was studied by utilizing a fluorescent protein tag on one of the viral envelope proteins. CLEM experiments are facilitated by sequential imaging of the same field of view with a Zeiss AxiObserver and Zeiss Supra 40 FESEM both equipped with shuttle and find. Obtaining high resolution 3D images of large cell volumes is possible utilizing the Zeiss Supra 40 FESEM with ATLAS large area mapping. A number of methods are available to accomplish 3D SEM including serial block face sectioning, collecting serial sections on tape and FIB-SEM. The AIF has chosen the method of collecting serial thin sections on tape using an automated tape-collecting ultramicrotome, ATUM. A decided advantage is that the sections are archived and can be revisited if desired. A project presented here is 3D reconstruction of mouse kidney podocyte foot processes. The kidney glomerular filtration barrier has three layers: fenestrated endothelial cells, basement membrane, and the foot processes of glomerular epithelial cells. The highly organized podocyte foot process architecture is critical for maintaining the filtration barrier. A better understanding of cell to cell interaction in podocytes requires 3D visualization of individual cells and individual foot processes.

**A CLEM approach to investigate fine morphological changes associated with the overexpression of lamin B1 in a mammalian cell line.**

Roberto Marotta¹, Roberta Ruffilli¹, Andrea Falqui¹, Denise Ferrera², Caterina Giacomini², Laura Gasparini²
¹Electron Microscopy Lab, Nanochemistry Department, Istituto Italiano di Tecnologia, Genoa (Italy)
²Neuroscience and Brain Technologies/Neuro Technology, Istituto Italiano di Tecnologia, Genoa (Italy)

Lamins are intermediate filament proteins constituting the major structural component of the nuclear lamina, a fibrillar meshwork that lines the inner nuclear membrane in eukaryotic cells. The nuclear lamina plays various cellular functions, providing skeletal support for the nuclear envelope, mediating the attachment of the nuclear envelope to interphase chromatin, and allowing the proper organization and anchoring of the nuclear pore complexes. Vertebrates synthesize a variety of laminas, namely lamin A, B and C, which are encoded by different genes or generated by differential RNA splicing. Recently, gene duplication and protein overexpression of lamin B1 (LMNB1) have been reported in pedigrees with autosomal dominant leukodystrophy (ADLD). However, how the overexpression of LMNB1 affects nuclear ultrastructure remains unexplored. To investigate the morphological changes associated with the overexpression of LMNB1 we transiently transfected HEK293 cells with bicistronic expression vectors containing cDNA for both the LMNB1 protein and the enhanced green fluorescent protein (EGFP) reporter or the EGFP reporter alone. Coupling in vivo confocal fluorescent microscopy with transmission electron microscopy (TEM) and electron tomography (ET), we were able to selectively focus our ultrastructural investigation only on EGFP-positive transfected cells. The over-expressed LMNB1 was located to the nuclear lamina, as revealed by confocal fluorescent microscopy, TEM and ET observations on the LMNB1 over-expressing cells revealed the presence of membrane structures forming extensive arrays of stacked cisternae aligned with the nuclear envelope, or laying inside the nucleoplasm. These cisternae clearly expressed LMNB1 within their stacks, as revealed by immuno EM. Moreover, in the nuclear envelope of HEK293 overexpressing LMNB1 the nuclear pores complexes were clustered together. To minimize the artifacts due to chemical fixation and room temperature dehydration, the transfected HEK293, once analyzed by confocal fluorescent microscopy, were processed by high pressure freezing and freeze substitution, using a protocol having the aim to enhance the visibility of the nuclear membranes. Respect to conventional EM preparation, nuclear membranes appeared smoother, and the stacked cisternae were separated by a larger nucleoplasmic space. These morphological data clearly demonstrate that LMNB1 overexpression deeply alters the structural organization of the cell nucleus.

**Targeted 3D-CLEM workflow on cultured cells**

Anna Steyer (EMBL, Heidelberg, Germany)

Correlative light and electron microscopy (CLEM) experiments show high accuracy and can provide the link between the imaging of living cells and their high resolution 3D ultrastructure. But CLEM often suffers from a low throughput, due to difficulties in tracking the object, tedious procedures for sample preparation and the lack of automation in the data acquisition by electron microscopy. The expected outcome of this project is to be able to collect data from a significant number of cells, from a heterogeneous population, clearing the way to statistical analysis of important mechanisms in cell biology. This will first be developed on cultured cells and used in a cell biology project. We foresee applications in other biological areas such as pharmacology, developmental biology or virology.
**Poster Abstracts**

**Knossos - A tool to reconstruct neuronal morphology and connectivity from 3D electron microscopic data**

*Fabian Svara (§), Jörgen Kornfeld (§), My-Tien Nguyen, Andreas Knecht, Oren Shatz, Kevin Briggman, Moritz Helmstädter, Winfried Denk (§) equal contributions*

Max Planck Institute for Medical Research, Heidelberg, Germany

Large 3D microscopy data sets, such as those generated by volume electron microscopy (e.g. SBEM), are difficult to browse and annotate on consumer-grade hardware because their size usually far exceeds available random access memory. Specialized software is therefore required. We developed KNOSSOS to address this issue. The program dynamically loads only microscopy data from the surround of the current view point. This is complemented by switching between different resolution versions of the dataset when zooming. KNOSSOS allows seamless navigation, zooming and manual annotation ('skeletonization' by spatial graphs) of these data on standard computers. Data sets can be stored locally or on a remote file server (FTP). Good performance over low-bandwidth connections is ensured by optional JPEG2000 data compression. KNOSSOS is complemented by a suite of scripts written in common programming languages (Python, MATLAB) that help researchers in converting their microscopy data sets to the KNOSSOS 3D image pyramid and to analyze generated annotation-files further. It is openly developed under the GPL license and freely available.

**The choroid plexus epithelium: a unique communication interface between blood and brain**

*Roosmarijn Vandenbroucke (Ghent University, VIB, Ghent, Belgium)*

The choroid plexus epithelium (CPE) is a unique single layer of epithelial cells situated at the interface of the blood and the cerebrospinal fluid (CSF), forming the blood-CSF barrier (BCSFB). In recent years, the BCSFB has gained increasing attention, especially its role in inflammatory diseases. We identified the BCSFB as the first CNS barrier disrupted during systemic inflammation, which was linked with dramatic changes in mRNA expression in the CPE. Additionally, upon induction of systemic inflammation, we observed a fast decrease in CPE miRNA expression levels, which was inversely correlated with increased levels of miRNAs in the CSF. Interestingly, this was linked with the release of extracellular vesicles (EVs) into the CSF. These EVs are ~100 nm in size, are secreted from multi-vesicular bodies and carry specific miRNAs. Moreover, we observed that secreted EVs are taken up by brain cells and are able to transfer a pro-inflammatory message from the blood to the CNS. In short, we identified an important role for CPE-derived EVs as a new mechanism of blood-CNS communication during systemic inflammation by transferring a pro-inflammatory message from the CPE to recipient brain cells.

**Ciliary remnants in mitotic cells during mouse brain development**

*Michaela Wiltsch-Bräuninger, Judith TML Paridaen, Wieland B. Huttner*

(Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden, Germany)

During the embryonic development of the mouse brain highly polarized epithelial cells amplify and differentiate to form the complex adult brain structure of neurons and glial cells. The elongated progenitor cells in the neuroepithelium stretch between the basement membrane to the ventricular lumen. From there the cells can receive important signals for their fate or proliferation, which they pick up by a primary cilium protruding into the ventricle. For cell division, the apical progenitors round up and shift their cell body to the apical side next to the ventricle but still keep a long basal process. Upon mitosis cells have been assumed to loose their primary cilium in order to free the basal body/centriole for its function in organising the mitotic spindle. Visualization of the fine structure of the basal body/centrosome in a mitotic cell is challenging due to the small size of the two centrosomes relative to the large volume of the dividing cell. However, by the combination of confocal, correlative light and electron microscopy and serial block face scanning electron microscopy, we were able to show that during mitosis the ciliary membrane is not completely disassembled but remains associated with one centrosome. The high resolution 3D analysis of the neuroepithelium by serial block face SEM, revealed a heterogeneity of the ciliary remnants consisting of either a centriole attached membrane sack or of multiple small membrane vesicles in the vicinity of the spindle pole. The preservation of the ciliary membrane in some (daughter) cells but not in others can provide a temporal advantage for receiving signals after mitosis, important for the stem versus neuronal cell fate of the daughter cells.
ZEISS MERLIN and ZEISS SIGMA 3View
Perform Block-Face Imaging for Tissue Samples Using an Ultramicrotome

ZEISS MERLIN systems are high end field emission SEMs (FE-SEM). Expect outstanding performance in resolution, ultra-fast speed, and intuitive operation. Your MERLIN is optimized for high throughput and ease of use. With its modular design MERLIN serves as a host for application specific modules and a comprehensive nano characterization laboratory.

Combine your MERLIN or SIGMA VP with 3View technology from Gatan Inc. to acquire high resolution 3D data from resin embedded cell and tissue samples. Get results in the shortest possible time and the most convenient way. 3View is an ultra-microtome inside the SEM chamber.

ZEISS Atlas Array Tomography
Arrange Your Serial Sections Conveniently and Fast

Image large areas and large amounts of serial sections in the shortest possible time – it’s fully automated with Atlas Array Tomography. Use Atlas Array Tomography’s workflow-oriented graphical user interface to determine which areas of the sample you want to image automatically. Define unlimited regions of interest with an arbitrary shape and assign different acquisition protocols. Pre-defined protocols help you acquire images easily at optimum conditions – even for different resolutions and sample types. Use the Atlas Array Tomography Viewer to zoom seamlessly through your image data – from nanometers to centimeters – always at the most appropriate resolution. And it’s just as easy to import and align images from other sources, such as light optical images.