3D Electron Microscopy In Montpellier

Photo kindly provided, courtesy of Louise Hughes, Oxford Brookes

VOLUME EM

& the ZebraFish challenge

Invited Speakers

- Christel Genoud, FMI Basel Suisse
- Louise Hughes, Oxford Brookes UK
- Matthia Karreman, EMBL Heidelberg
- Allon Weiner, iBV Nice
- Karin Pernet-Gallay, Gin Grenoble

Organizers

- Laurence Berry, DIMNP
- Karima Kissa, DIMNP
- Erwan Oliviero, MEA
- JF Dubremetz, DIMNP
- Yvon Sterkers, MiVEGEC

December 14–15 2017

Faculté de Médecine campus Arnaud de Villeneuve

Thursday December 14th

13h30 - 14h Welcome of participants

14h00 - Introduction

14h10 - **Christel Genoud, FMI Basel** (Switzerland) -Volume-SEM techniques for 3D Imaging

14h50 - **Emine Korkmaz, FEI** - Large volume analysis with the Thermo Scientific Volumescope

15h15 - **Daniel Monville**, Gatan - 3 view by Gatan

15h40 - **Louise Hughes, will be replaced by Jiri Tyc Oxford Brooks (UK**) - What SBF-SEM can tell us about the *Trypanosoma brucei* - organelle ontogeny through a cell cycle revealed by whole-cell reconstructions

16h10 - Coffee break

16h25 - **Karin Pernet-Gallay, Institut des Neurosciences Grenoble** - How do the astrocytes interact with the synapses ? : A 3D study by using FIB/SEM microscopy

16h55 - **Andreas Schertel, Zeiss Oberkochen (Germany)** - Cryo FIB-SEM Volume Imaging of Cellular Ultrastructure in Vitrified Biological Samples

17h20 - **Jérémie Silvent, Tescan France** -

17h40 - General discussion

18h - End

Friday, December 15th

9h 9h30 welcome

9h30 - 10h00- **Matthia Karreman, EMBL Heidelberg (Germany)** – Find Your Way with X-Ray: Multimodal Correlative Microscopy to Study Tumor Cells at the Blood Brain Barrier

10h - **Allon Weiner, Institute of Biology Valrose, Nice (France**) - Correlative FIB/SEM tomography and dynamic microscopy reveal macropinosomes are key players in *Shigella* invasion

10h30 - Pause and discussions

11h - 13h **Zebra fish challenge and Conclusion**

Abstracts

Volume-SEM techniques for 3D EM imaging by Christel Genoud, FMI Basel

The main different techniques used in volume-SEM will be presented. The use of one of the technique is determined by the scientific question asked. Depending on the volume required, the resolution needed and the structure that need to be characterized, each technique has strengths and limitations. Examples of projects done at FMI will be shown.

Large volume analysis with the Thermo Scientific Volumescope, by Emine Korkmaz, FEI

Serial blockface SEM (SBF-SEM) is a particularly versatile and accessible technique for the collection of 3D EM data in life sciences research covering a broad range of applications such as the study of subcellular features, the analysis of cell-to-cell interactions and the investigation of tissue samples and small model organisms.

The Volumescope, Thermo Fisher Scientific's solution for large volume analysis, embodies the versatility and accessibility of the technique with its tight integration of a hard- and software.

Here, we will show how it enables straightforward, reliable and isotropic 3D volume acquisition, how its data integrates into analysis workflows using Amira and the platform's benefits beyond SBF-SEM (e.g. array tomography and STEM).

What SBF-SEM can tell us about the Trypanosoma brucei - organelle ontogeny through a cell cycle revealed by whole-cell reconstructions, by Jiri Tyc, Oxford Brookes

Trypanosoma brucei, a unicellular flagellate and a causative agent of sleeping sickness disease in Africa, has a complex cell cycle and multiplies rapidly. It is important to understand its cell biology including the process of cell division. Serial block face scanning electron microscopy (SBF-SEM) technique allows not only to quantify the volume of the cells and organelles, but also to observe the spatiotemporal changes and organelle division and inheritance during different stages of the eukaryotic cell cycle. The results highlighted a complex arrangement of organelles and revealed new spatiotemporal features of Golgi body position, mitochondrion biogenesis and mitotic spindle placement. In addition SBF-SEM 3D volume reconstructions can be used for characterization of mutant *T. brucei* cells and description of obtained phenotypes.

References:

Hughes L., et al. (2017), Patterns of organelle ontogeny through a cell cycle revealed by whole-cell reconstructions using 3D electron microscopy, J Cell Sci, 130, 637-647

Kaser S., et al. (2016), Outer membrane protein functions as integrator of protein import and DNA inheritance in mitochondria, PNAS, 113(31), E4467–E4475

How do the astrocytes interact with the synapses ? : A 3D study by using FIB/SEM microscopy by Karin Pernet-Gallay, Institut des Neurosciences Grenoble

Astrocytes are star-shaped cells whose processes extend in the neuropil. At least some of the processes from each cell form expansion that contact the surface of brain capillaries, they are involved in the uptake of nutriments from the blood vessel and their transport toward the neurons. Astrocytes are not only supporting cells, they are also active components of the neural network. Indeed, their numerous thin processes wrap the synapses and modulate synaptic transmission through the release and uptake of neurotransmitters or neuroactive substances near synapses. During brain development and maturation, astrocytic processes also contribute to regulate, either positively or negatively, synapses formation through secreted and contact-mediated signals. Therefore, a disruption of astrocytic supportive functions and/or of glio-transmission or uptake has the potential to alter synaptic transmission, synaptic plasticity and neuronal excitability.

What is the contribution of astrocytic signalling mechanisms to cognitive behaviour? How do they communicate with the neurons? What is the language of the astrocytes?

The goal of our work is to decipher "the language of astrocytes" in order to understand how they participate in memory and cognition. To this end we study the subcellular organization of astrocytes by using of 3D electron microscopy.

Cryo FIB-SEM Volume Imaging of Cellular Ultrastructure in Vitrified Biological Samples, by Andreas Schertel, **Carl Zeiss Microscopy GmbH, Customer Support Center Europe, Oberkochen, Germany**

Focused Ion Beam – Scanning Electron Microscopy (FIB/SEM) volume imaging of heavy-metal-stained biological specimens embedded in resin is a well-established technique to reconstruct and to analyse subcellular structures in all three dimensions, e.g. brain mapping. Cellular ultrastructure is visualized by detecting the low loss backscattered electrons generated by the interaction of the primary electrons with the stained resin-embedded tissue.

Classical resin embedding preparation technique involves dehydration and impregnation with heavy metals by either freeze substitution or chemical fixation. Any step of these preparation protocols involves the potential risk of introducing structural modifications or artefacts. In contrast, the investigation of vitrified biological specimens (i.e. samples that are plunge or high pressure frozen) enables the visualization of cellular ultrastructure in a near native fully hydrated state, unadulterated by harmful preparation methods.

A novel, exciting approach for FIB/SEM is block face imaging of vitrified biological samples omitting any staining, chemical fixation or dehydration. In our recent work, we applied serial FIB milling and block face imaging to acquire 3D data cubes of high pressure frozen mouse optic nerves and bacillus subtilis spores under cryo conditions [1]. By using Inlens secondary electron detection we succeeded to directly visualize the cellular ultrastructure in the freshly exposed serial FIB cross-sections. The observed contrast between lipid-rich membranes and water-rich areas allowed differentiating subcellular structures like the Golgi apparatus, nuclear envelope, vesicles, endoplasmic reticulum and cristae within the mitochondria. This new method is comparatively easy and fast. Only one step – the cryo-immobilization – is required for preparing the specimens for cryo FIB/SEM. Here recent cryo FIB/SEM results on high pressure frozen HeLa cells, yeast cells, zebrafish larvae, sea urchin embryos and Algae Emiliania huxleyi (EHUX) are presented [2,3,4]. The simultaneous detection of the directly back-

scattered electrons allows easy imaging of the electron dense mineralized structures for investigating bio-mineralisation.

In addition, this novel technique offers extended possibilities for correlative workflows between light, electron and x-ray microscopy. The correlation between cryo light and electron microscopy data will greatly benefit from an increase in resolution in fluorescence imaging. Cryo Airyscan is a first step in increasing the resolution in that direction and can deliver three dimensional optical sectioning data that can be used to reliably target cellular structures in a FIB/SEM microscope, before the structural context is explored by cryo FIB/SEM tomography.

References

[1] A. Schertel et al., *J Struct Biol.* 2013 184(2):355-360. DOI: 10.1016/j.jsb.2013.09.024.
[2] S. Sviben et al., *Nat. Commun.* 7:11228 DOI: 10.1038/ncomms11228 (2016).
[3] N. Vidavsky et al., *J. Struct Biol.* 2016 196(3):487-495. DOI: 10.1016/j.jsb.2016.09.016.
[4] N. Vidavsky et al., *PNAS* 113:12637-12642. DOI: 10.1073/pnas.1612017113.

The plasma FIB: a Swiss army knife for life sciences, by Jérémie Silvent, TESCAN Orsay Physics, Fuveau, France

Biological samples are difficult to observe in electron microscopy (EM) due to their high-water content, their sensitivity and their non-conductivity. In order to observe them, first they can be fixed and then prepared for the EM observation, or they can directly be observed using cryo-conditions. To obtain the 3D information of the sample, the dual FIB /SEM (Focused Ion Beam/ Scanning Electron Microscope) system is the perfect tool. Currently, two kinds of technology are mainly used: one based on a metal source (mainly a gallium source) and another one based on a plasma source (mainly a xenon source). This last technology, developed by Orsay Physics (France), allows to work with higher current (2 µA vs 100 nA) allowing to mill and cut larger areas, i.e. more than 50 times faster than with the gallium source. Moreover, even the use of lower currents with plasma FIB shows better sputtering due to the fact the xenon ions are heavier than the gallium one. The use of the plasma FIB decreases the artifacts due to the work on the sample. Indeed, contrary to the gallium, the xenon penetrates twice less deep in the sample allowing a decrease of the amorphous phase that could be found on the surface of the sample, which is essential for a TEM lamella preparation. In addition, the xenon does not form intermetallic compounds that might interfere with the analysis.

This dual beam can be used for many applications and this presentation will give an overview of this polyvalent tool. In addition to image cells and tissues in their natural uncoated and hydrated state at low and ultra-low beam energies for maximum ultra-structural information and surface sensitivity, the plasma FIB allows the preparation of high-quality TEM lamellae of large sections of biological samples. Besides, this technology allows the cross-sectioning of whole cells and tissues, allowing the sublayer analysis in large and hard-to-cut samples such as teeth. Another application is to achieve FIB-SEM tomography of a large section of resin-embedded tissue and cells, allowing the analysis of 3D ultra-and nanostructures. Finally, the plasma FIB allows the use of a TOF-SIMS (Time Of Flight-Secondary Ion Mass Spectrometry). It is a label-free technique that not only reveals the chemical composition of a biological sample providing an excellent metabolite and lipid detection, but also cell phenotype discrimination.

Find Your Way with X-Ray: Multimodal Correlative Microscopy to Study Tumor Cells at the Blood Brain Barrier by Matthia Karreman, DKFZ Heidelberg, Germany

Correlative microscopy combines the advantages of multiple imaging tools, enabling to capture an object or event of interest in a living sample with fluorescence microscopy (FM) and imaging it at high resolution with electron microscopy (EM). The main challenge, however, is to keep track of the region of interest (ROI) when moving from one imaging modality to the next. We have developed Multimodal Correlative Microscopy, where microCT is exploited to accurately and quickly retrieve the ROI with EM. Significantly speeding up the correlative workflow, this method allows to move from in vivo imaging to 3D EM within a period of ~2 weeks.

We employ our newly developed approach to study extravasation, a crucial step in cancer metastasis. Hereto, we performed in vivo FM imaging of intracardially injected fluorescent tumor cells, arrested in the brain vasculature of a living mouse. Brain biopsies containing the tumor cells were processed for EM and embedded in a resin block. Next, microCT scans were obtained and correlated to the FM volumes, based on structural features of the sample visible in both datasets. 3D registration of both datasets enabled to predict the position of the tumor cell inside the resin block, allowing to accurately approach this area and studying it at high resolution with EM. This approach enables us to visualize, at high resolution and 3D, a significantly relevant number of tumor cells at the blood-brain-barrier.

Correlative FIB/SEM tomography and dynamic microscopy reveal macropinosomes are key players in *Shigella* invasion by Allon Weiner, iBV Nice

The intracellular bacterium *Shigella flexneri*, the causative agent of bacillary dysentery, invades host cells in a vacuole that is subsequently ruptured to allow growth of the pathogen within the host cytoplasm. We applied dynamic imaging and advanced large volume correlative light electron microscopy (CLEM) in the form of focused ion beam/ scanning electron tomography (c-FIB/SET) to study the highly transient events of *S. flexneri*'s early invasion into host epithelial cells. The results obtained provide the basis for a new model of the early steps of *S. flexneri* invasion, establishing a different view of the enigmatic process of cytoplasmic access by invasive bacterial pathogens.

Reference: Weiner A et al. (2016) Macropinosomes are Key Players in Early Shigella Invasion and Vacuolar Escape in Epithelial Cells. PLoS Pathog 12(5): e1005602

Venue

The conference will take place at the "nouvelle fac de médecine" of Montpellier, campus Arnaud de Villeneuve,

Address : 642, avenue doyen Gaston Giraud, 34093 Montpellier cedex 5

This is a brand new building, designed by the architect Francois Fontes, and opened in October 2017.
The building is readily accessible via the Tram line 1, stop Occitanie. The tram line 1 has also a stop in front of the train station ("Montpellier Saint-Roch"), it takes 20 minutes from there. For those coming by car, there is a large car park adjacent to the tram station "Occitanie" (not for free).

For further information write an E-mail at volumeem@umontpellier.fr

Registration is free, but you must fill in the registration form here .