

Coollest FIB in the world

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In this talk we will present the latest achievements in room temperature and cryo sample preparation, using a technique first pioneered by Rigort (2012, p. 4449) and characterization experiments, using a set-up that was recently obtained by Monash University. It consists of Thermo Fisher Helios G4 UX DualBeam accompanied by a Leica cryo tool-set consisting of customized EM VCT500 sample cryo transfer system, EM ACE600 cryo coater and EM VCM vacuum cryo manipulation system. This set of tools enables us to reach cryo temperatures below -160 degC for most demanding vitrified ice life sciences samples as well as materials samples such as emulsions, polymers or perovskites that can be easily damaged by ion beam at room temperature conditions or even would be impossible to be analysed, in case of emulsions. The newly obtained Thermo Fisher Talos Arctica 200keV cryo TEM, allowed us to rapidly screen the cryo-FIB prepared samples for further cryo tomography experiments.

We will present the sample preparation workflow including suggestions from Hayles (2007, p. 263) and challenges associated with it. Also an overview of the latest cryo tomography experiments and volumetric reconstruction work of nanoscale features obtained from consequent ultra-thin FIB-cut slices as seen in Fig. 1. Preliminary results of further planned research in a field of correlative light and electron microscopy following the steps of a group colleague Oorschot (2014, p. 241) will also be briefly presented.

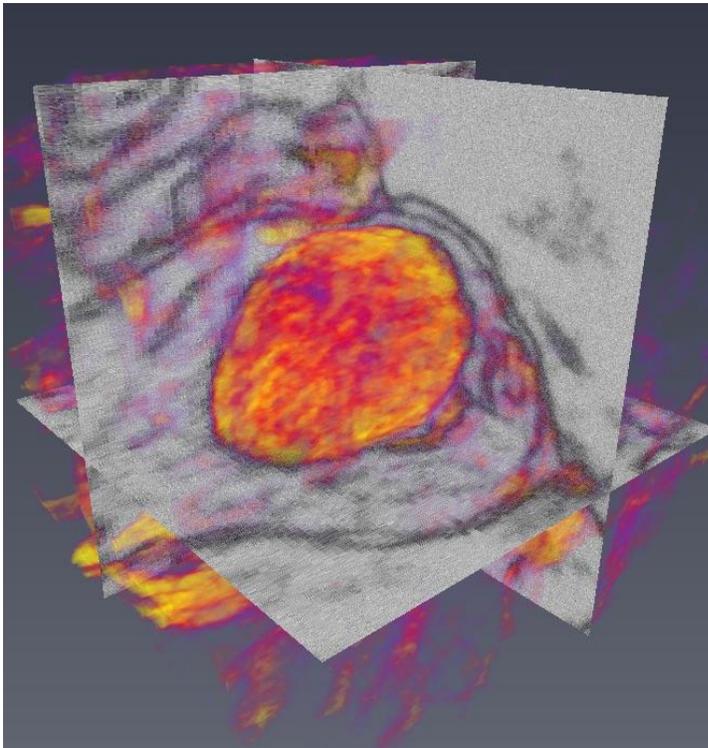


Fig. 1. Composite false-color image of a reconstructed mitochondrion.

Citations:

1. Rigort, A., Bäuerlein, F., Villa, E., Eibauer, M., Laugks, T., Baumeister, W. and Plitzko, J. (2012). Focused ion beam micromachining of eukaryotic cells for cryoelectron tomography. *PNAS*, 109 (12), p.4449-4454.

<https://doi.org/10.1073/pnas.1201333109>.

2. Hayles, M., Stokes, D., Phifer, D. and Findlay, L. (2007). A technique for improved focused ion beam milling of cryo-prepared life science specimens. *Journal of Microscopy*, 2007226 (Pt 3), p.263-9.

<https://doi.org/10.1111/j.1365-2818.2007.01775.x>.

3. Oorschot, V., Sztal, T., Bryson-Richardson, R. and Ramm, G. (2014). Immuno correlative light and electron microscopy on Tokuyasu cryosections. *Methods in Cell Biology*, 11 (14), p.241-258.

<https://doi.org/10.1016/B978-0-12-801075-4.00011-2>.