Dear Colleagues,

The Electron Microscopy Core Imaging Facility (EMCIF) is preparing to submit a NIH high-end shared instrument grant. Below I provide a short description of the instrument sought, special features and new applications that will be available to UMB researchers once we acquire it. I have set up a shared folder with a few references in pdf for you to access: (https://www.dropbox.com/sh/u4eqorpsfp69vtz/AACMB_AeGAAZUeWWNugpBndHa?dl=0)

Please let me know if you are interested to support this instrument grant application and be listed as one of the users. It would also be extremely helpful if you could send me your updated biosketch listing your current funding. I will follow up with more guidelines about a description of your research that is needed for the grant application. Please feel free to email me if you have any questions. Thank you for your help. Ru-ching (rhsia@umaryland.edu)

Short description of instrument

The instrument we are requesting is a Thermo Fisher Scios 2 field emission scanning electron microscope (SEM) bundled with interchangeable cryo SEM stage, *in situ* ultramicrotome stage (volume scope), retractable transmission scanning electron microscope (STEM) detector and navigation/tiling/stitching/correlation software (MAPS 3). This multifunctional instrument will not only enhance our existing capabilities and workflow but also enable EMCIF to perform a wide range of advanced EM techniques that are not available on the UMB campus currently. Moreover, this instrument will further expand and complement the current live cell and super resolution light microscopy imaging, animal imaging and cryo TEM imaging capabilities on the UMB campus and will allow researchers to achieve a range of biomedical imaging from the molecular to the whole animal or human scales.

Special features of the requested instrument:

- Advanced detector with high sensitivity allowing low voltage imaging of sensitive biological specimens/materials at the highest resolution without metal coating;
- Multiple charge mitigation strategies and drift correction enabling imaging of resin embedded cells and tissue specimens and producing TEM-like images;
- Automated stage movement, tiling, stitching allowing automated acquisition of large overviews of complex tissue sections at high resolution;
- *In situ* ultramicrotome allowing automated serial sectioning and imaging to collect large 3-D volume image datasets;
- Cryo SEM stage allowing freeze fracture and imaging of frozen hydrated biological samples allowing imaging of ultrastructural features that are labile or sensitive to dehydration. These include biofilms, hydrogels, emulsions, and scaffolding materials etc.

Advanced techniques that are difficult or impossible to perform with EMCIF current capabilities:

Montage capability: One of the major drawback of high resolution imaging of complex tissue specimens using TEM is the limits on sample size and field of view. It is often difficult to correlate the region of interest (ROI) to the relative location of the original tissue specimen. Currently, a series of overlapping images have to be taken manually and aligned using an offline image analysis software to generate a whole mount reference image. It is a tedious and time consuming process. With the "Maps 3" software and autostage function, Scios 2 will enable taking overlapping images of ultrathin sections of a resin-embedded tissue, stitching and producing a montage image automatically. This will allow a user to navigate, zoom in and view individual images in high magnification, without ever losing sight of the relative location of his/her ROI.

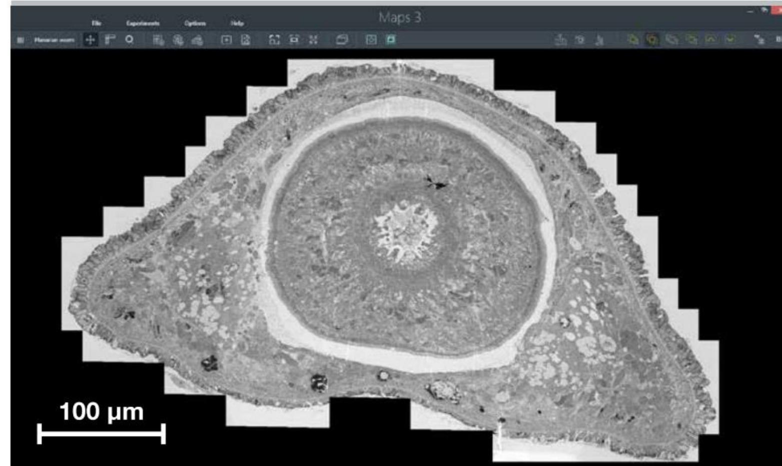
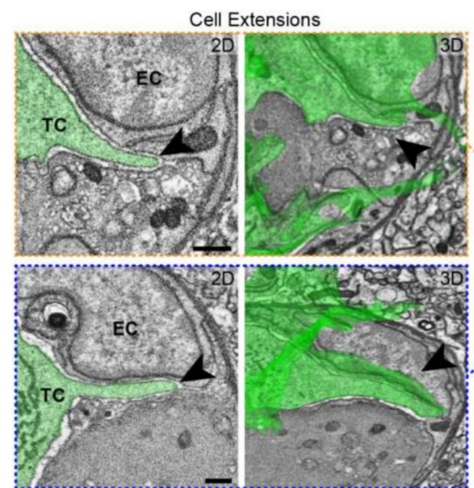


Figure 1. Maps software automates the acquisition of large overviews at high resolution and stitches the resulting tile set. Users can zoom between an overview and the native image resolution seamlessly. Section of a planarian worm, imaged on a Verios SEM in STEM mode. Sample courtesy of Melainia McClain, Stowers Institute for Medical Research.

Correlative Light and Electron Microscopy (CLEM): CLEM allows researchers to locate cellular structures using specific markers in a large field of view using a light microscope and then to zoom in for a closer look using EM. This dual examination combines the advantages of both LM and EM techniques and provides valuable, complementary and often unique information. EMCIF has been performing CLEM techniques using manual relocation, a technically challenging, labor intensive, inaccurate and time consuming method. The "**Map**s" program bundled with the SCios 2 will allow users to import light microscopy images derived <u>from any modality</u>, perform 1, 2, or 3-point alignment using fiducial markers of other specific features, enable the location of ROI, and reveal the ultrastructural details in high magnification. CLEM can be further combined with serial block face sections (see below) to generate correlated 3-D volume information.



3-D volume analysis using serial block face (SBF) imaging technique.

Conventional high resolution 3-D volume analysis of complex tissues relies on collecting images of serial sections or a tilt series of 2D projections (electron tomography). Both techniques are time consuming, limiting with volume information and require a lot of post processing time to align the images. The *in situ* ultramicrotome stage installed in the Scios 2 can be driven by the automation software. This allows the instrument to continuously acquire serial images of the exposed block face of a resin embedded tissue/cell specimen after the removal of each thin sections (20 to 50nm) using the built-in diamond knife. X Y resolution of 5–10 nm in the block-face plane can be easily achieved. Z-resolution is dependent on the section thickness at 20 to 50 nm but can be further improved by sub-slice imaging and deconvolution to 5 to 10 nm. This automated process can be repeated thousands of times to collect volume information to 10 to 50 micron depth . See example images below and references in the shared folder.

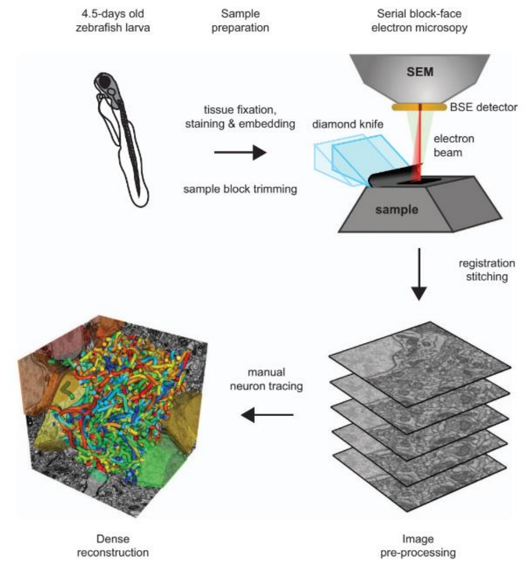
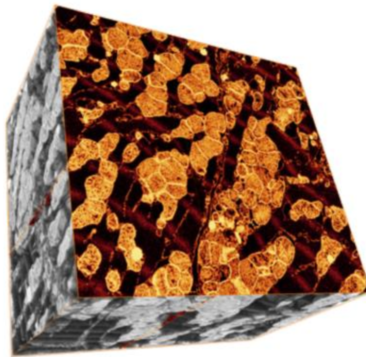
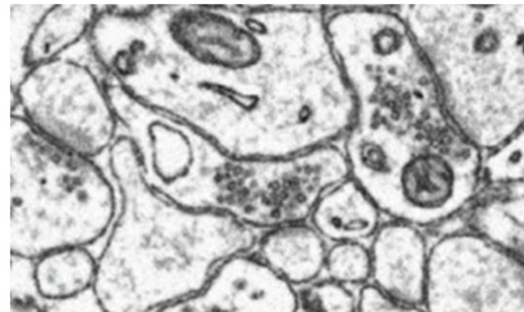


Figure 1. Schematic illustration of workflow for sample preparation, SBEM imaging and neuron reconstruction.



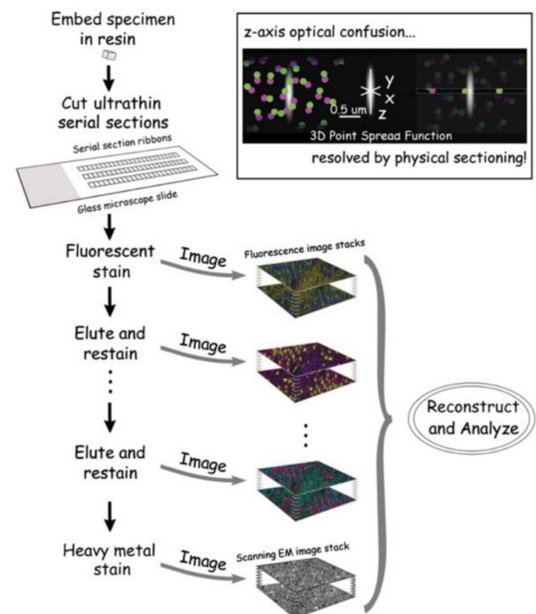
Volume reconstruction of a mouse heart muscle. Data acquired by a combination of physical and virtual slicing in HiVac mode. The data were acquired in BSE mode with optical sectioning. Nominal resolution of 5 x 5 x 10 nm (x, y, z), 1102 slices @ 40 nm (physical cut), volume of 16 x 15 x 11 µm3. *Specimen courtesy of Dr. Madesh Muniswamy, Temple University, USA.*



Mouse brain sample imaged on the Volumescope 2 SEM. Fine features such as vesicles and synaptic clefts can be observed. Image courtesy of Dr. Gabor Nyiri, Institute of Experimental Medicine, Budapest, Hungary.

Array tomography:

Array tomography can be performed at light microscopy or electron microscopy levels. Fluorescence array tomography achieves much higher resolution and molecular multiplexing than most other fluorescence microscopy methods, while electron array tomography can capture three-dimensional ultrastructure much more easily and rapidly than traditional serial-section electron microscopy methods. A correlative fluorescence/electron microscopy mode of array tomography further offers the unparalleled opportunity to merge multichannel molecular imaging information with three-dimensional cellular architectures in high resolution.



References: pdf in shared folder

Russell, Matthew R.G., Thomas R. Lerner, Jemima J. Burden, David O. Nkwe, Annegret Pelchen-Matthews, Marie Charlotte Domart, Joanne Durgan, et al. 2017. "3D Correlative Light and Electron Microscopy of Cultured Cells Using Serial Blockface Scanning Electron Microscopy." *Journal of Cell Science* 130 (1): 278–91. https://doi.org/10.1242/jcs.188433.

Burel, Agnes, Marie-Thérèse Lavault, Clément Chevalier, Helmut Gnaegi, Sylvain Prigent, Antonio Mucciolo, Stéphanie Dutertre, Bruno M. Humbel, Thierry Guillaudeux, and Irina Kolotuev. 2018. "A Targeted 3D EM and Correlative Microscopy Method Using SEM Array Tomography." *Development* 145 (12). https://doi.org/10.1242/dev.160879.

Hughes, Louise, Chris Hawes, Sandy Monteith, and Sue Vaughan. 2014. "Serial Block Face Scanning Electron Microscopy-the Future of Cell Ultrastructure Imaging." *Protoplasma*. https://doi.org/10.1007/s00709-013-0580-1.

Micheva, Kristina D., and Stephen J. Smith. 2007. "Array Tomography: A New Tool for Imaging the Molecular Architecture and Ultrastructure of Neural Circuits." *Neuron* 55 (1): 25–36. https://doi.org/10.1016/j.neuron.2007.06.014.

Peddie, Christopher J., and Lucy M. Collinson. 2014. "Exploring the Third Dimension: Volume Electron Microscopy Comes of Age." *Micron*. Elsevier Ltd. https://doi.org/10.1016/j.micron.2014.01.009.