

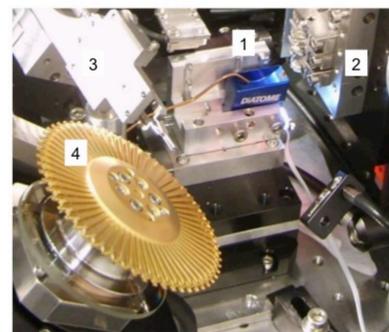
## Abstract

We are at the beginning of a project to image the entire brain of the fruit fly *Drosophila melanogaster* using serial section transmission electron microscopy (ssTEM). In the service of this goal and anticipated future projects, we are building custom hardware for high-throughput TEM imaging. These tools include:

- (1) an interferometric microtome ("iTome") for automated sectioning and pickup of serial 30 nm sections on conventional slot grids;
- (2) a next-generation Transmission EM Camera Array ("TEMCA2") for high throughput data acquisition (net ~50 megavoxels/second, 4x4x30 nm/voxel);
- (3) a fast piezo-driven TEM sample stage, capable of stepping and settling to nanometer stability in ~25-35 ms (versus ~3 seconds for a conventional stage); and
- (4) a multi-sample Autoloader, enabling automated sample exchange without breaking vacuum and 24/7 unattended image acquisition.

We recently acquired a pilot image data set from a manually cut series of 4000 ~35 nm whole-brain sections. 372 serial sections were imaged using TEMCA2 and the fast stage at 4x4 nm/pixel, resulting in a 3.8 TB dataset suitable for developing downstream software components (stitching, registration, intensity correction, automated segmentation). We are currently assessing traceability of fine neurites in the dataset; early results in the medial lobes of the mushroom body are promising.

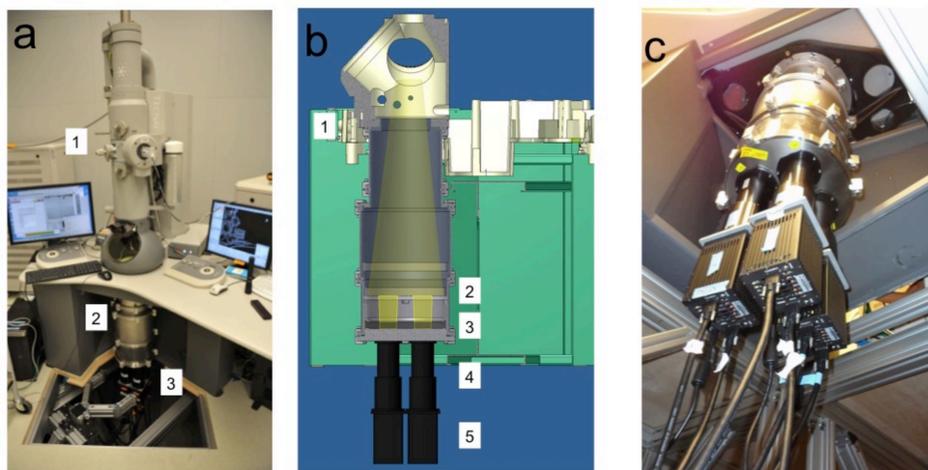
## (1) Interferometric microtome



"iTome" overview. 1) commercial diamond knife; 2) multiple sample holder; 3) gripper arm; 4) 60-grid "daisy" holder.

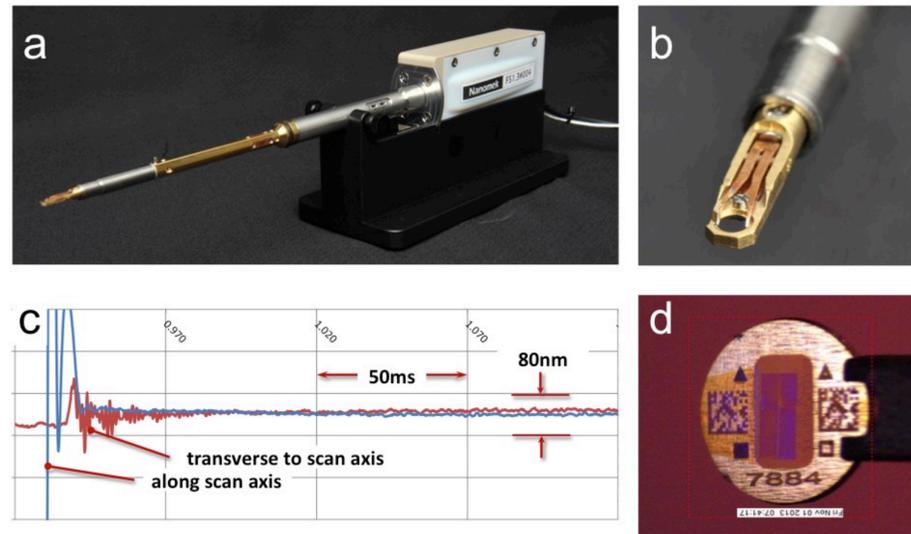
The longest run to date on the iTome with mammalian brain samples generated ~1100 sections cut at ~35 nm thickness. Each section was 1350 x 700 μm in size, with one section per 1 x 2 mm slot support grid. Work is ongoing to scale up the length and reliability of each iTome run.

## (2) Transmission Electron Microscope Camera Array 2



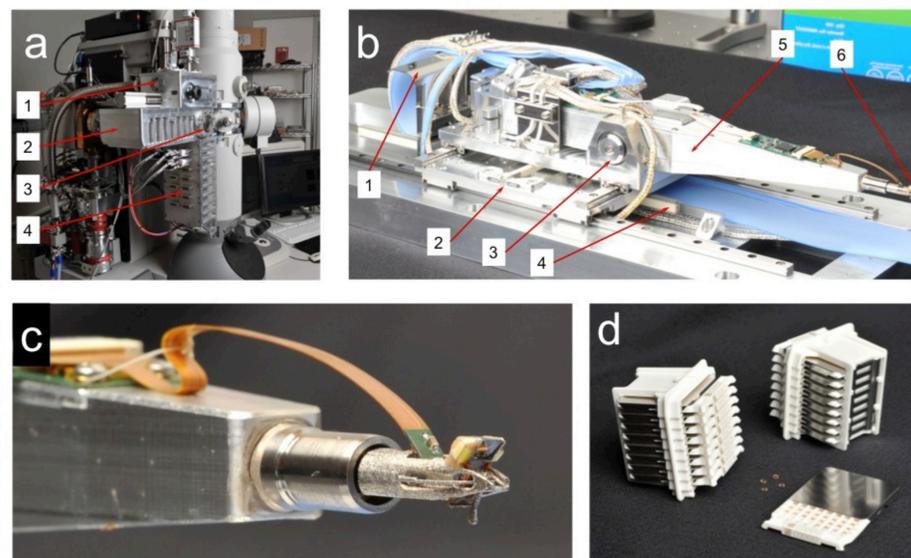
**Panel a.** TEMCA2 overview. 1) commercial FEI T12 BioTwin TEM; 2) custom vacuum extension; 3) camera array. **b.** Extension and array schematic. 1) custom base port; 2) scintillator; 3) leaded vacuum glass; 4) AMT C-lens; 5) BAE SciMos cameras. **c.** Extension and array bottom view.

## (3) High-speed piezo-driven TEM sample stage



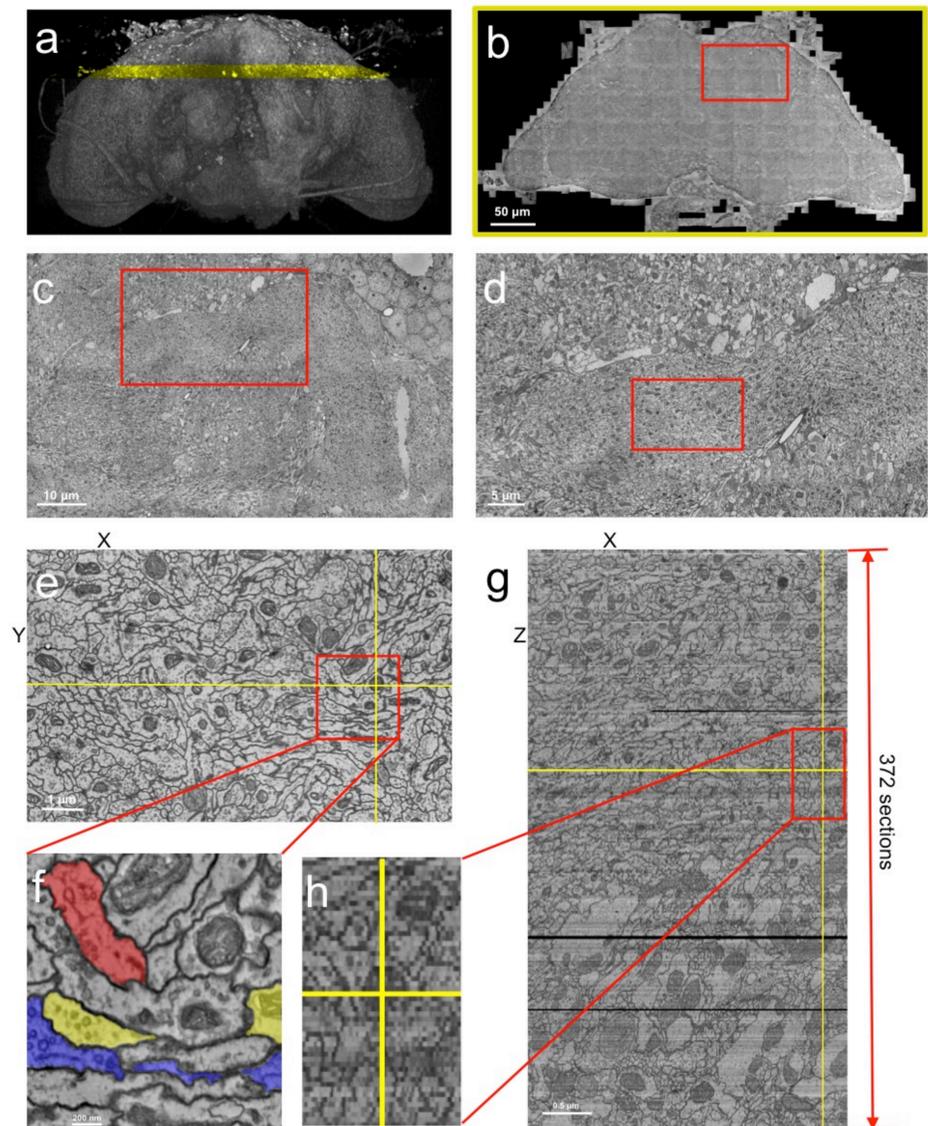
**Panel a.** Single-axis fast stage overview. Individual columns in a mosaic are acquired at high speed. Slower (~2 s) column-to-column moves are executed by the FEI microscope's Compustage™ motion system, into which the custom fast stage inserts. **b.** Fast stage grid holder (enlargement of red rectangle in **a**). **c.** Fast stage motion after an 8 μm step. **d.** Custom 2D barcoded sample grid with showing two serial sections cut at 40 nm nominal thickness on the iTome.

## (4) Autoloader



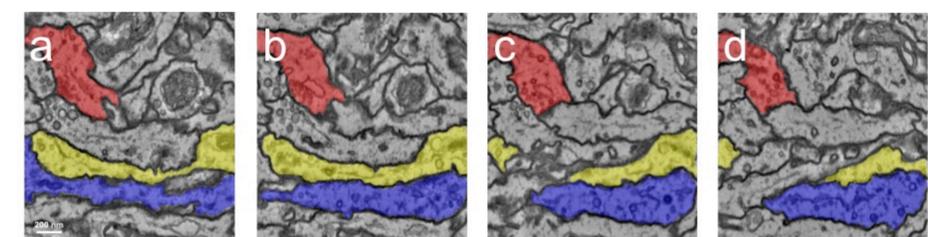
**Panel a.** Autoloader overview. 1) loadlock chamber; 2) grid positioning system (GPS) chamber; 3) view port; 4) cassette shuttle chamber. **b.** Grid positioning system (GPS). 1) wire harness; 2) transverse axis drive rod; 3) Pitch axis; 4) GPS shuttle drive rod; 5) scan/roll axis housing; 6) end effector and miniature camera. **c.** GPS end effector and miniature camera. **d.** TEM support grid cassettes and magazines. Each cassette holds 64 grids; each magazine holds 8 cassettes. At 20 minutes' imaging time per grid, a magazine will support about 7 days of uninterrupted imaging.

## Pilot data set: zoom to medial lobe of the mushroom body



**Panel a.** X-ray tomography volume of embedded brain prior to serial sectioning. TEMCA2-imaged volume false-colored in yellow. **b-f.** Zoom series into medial lobe of mushroom body from whole-brain horizontal section. **g.** An XZ plane image of the volume. The orthogonal cutting planes indicated in yellow lines. **h.** A zoom-in of **g**.

## Pilot data set: preliminary assessment of traceability



**Panels a-d.** Continuation from panel **f** from zoom series, above. Serial sections with false coloring of selected neurites. Additional work will be done using the entire pilot dataset to assess traceability of fine dendritic processes and identification of synapses. Traceability across the gaps resulting from the 2 lost sections in the series (1 during sectioning, 1 during imaging, at separated positions in the series) will also be examined.