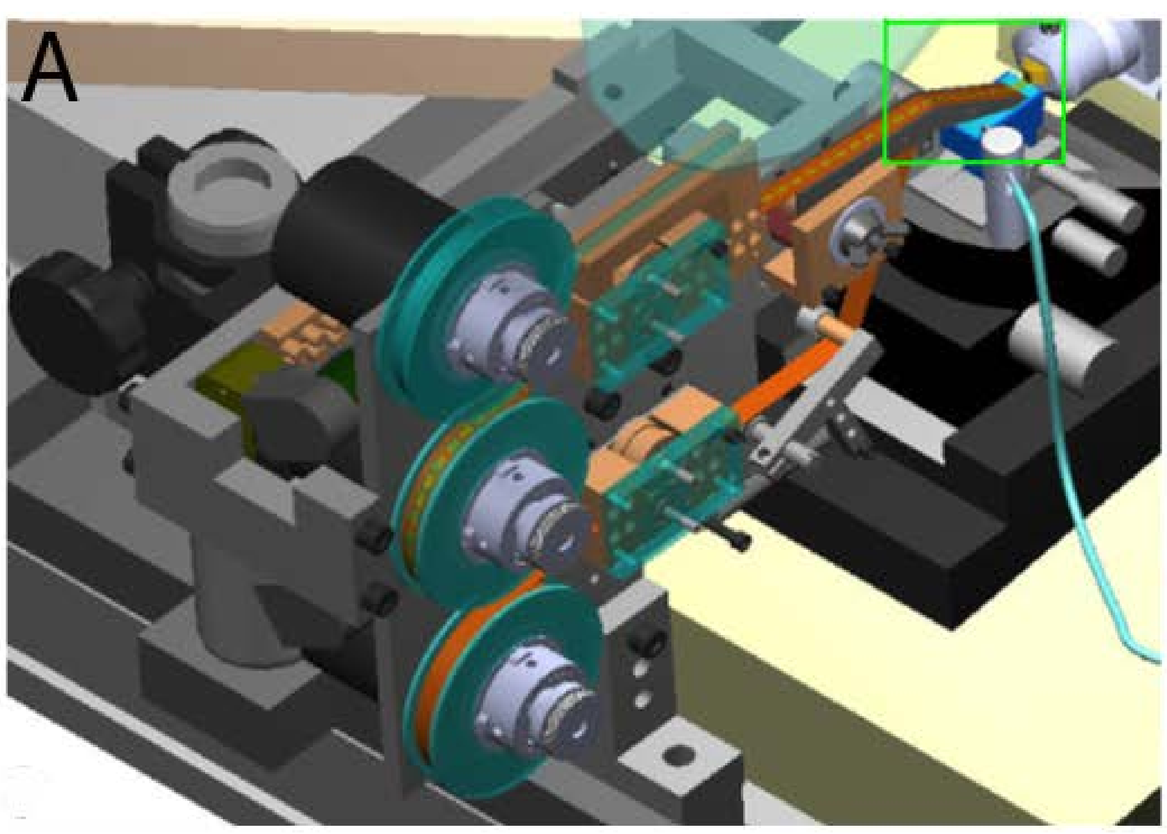
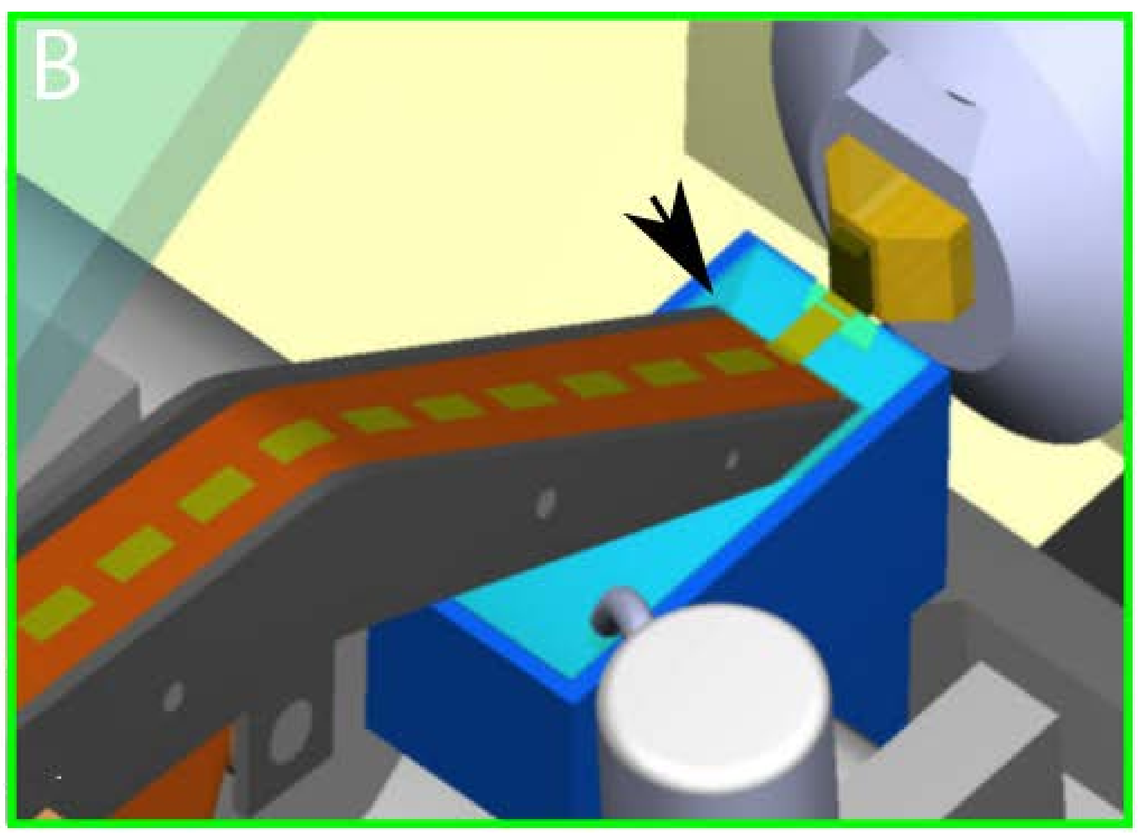
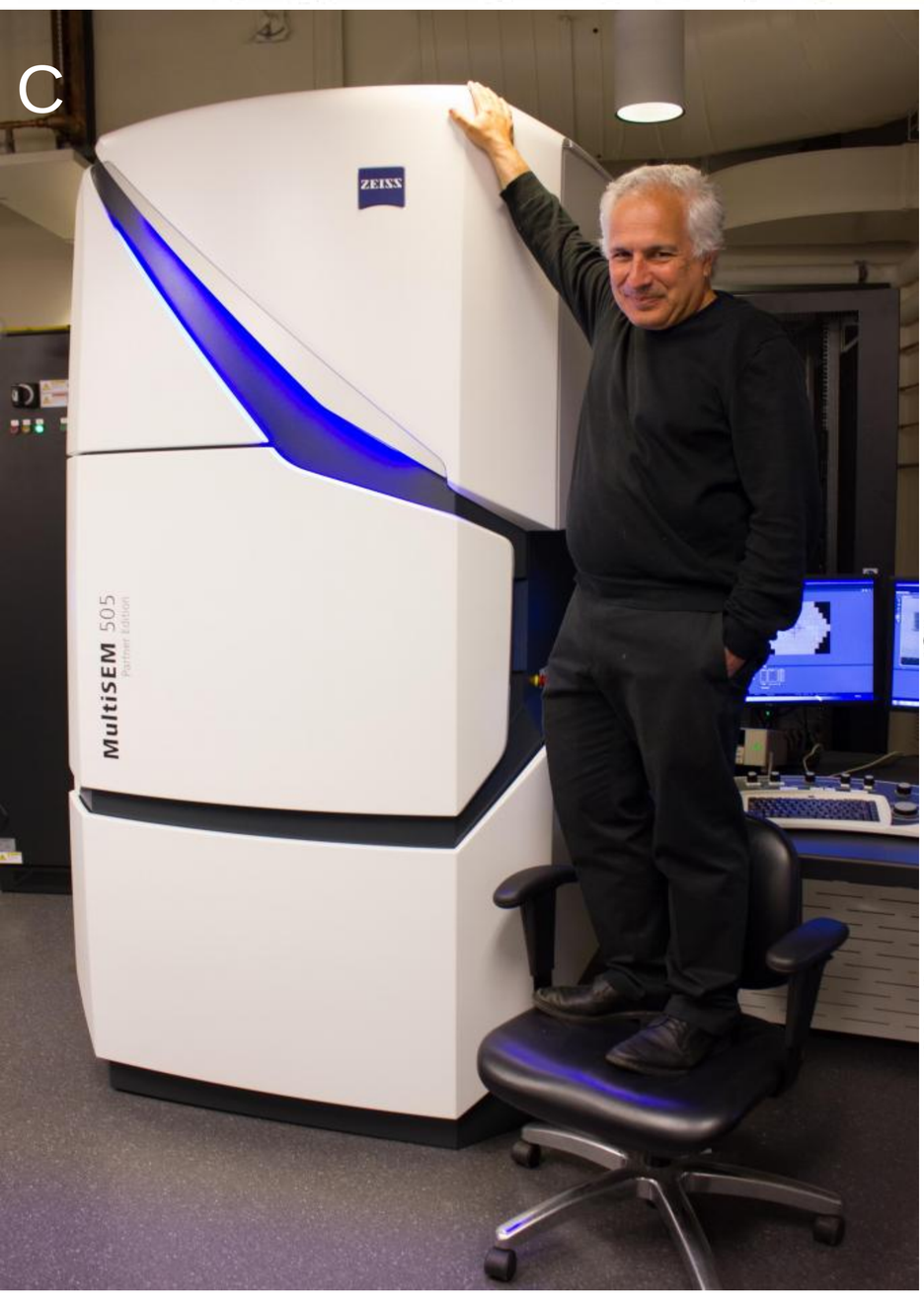
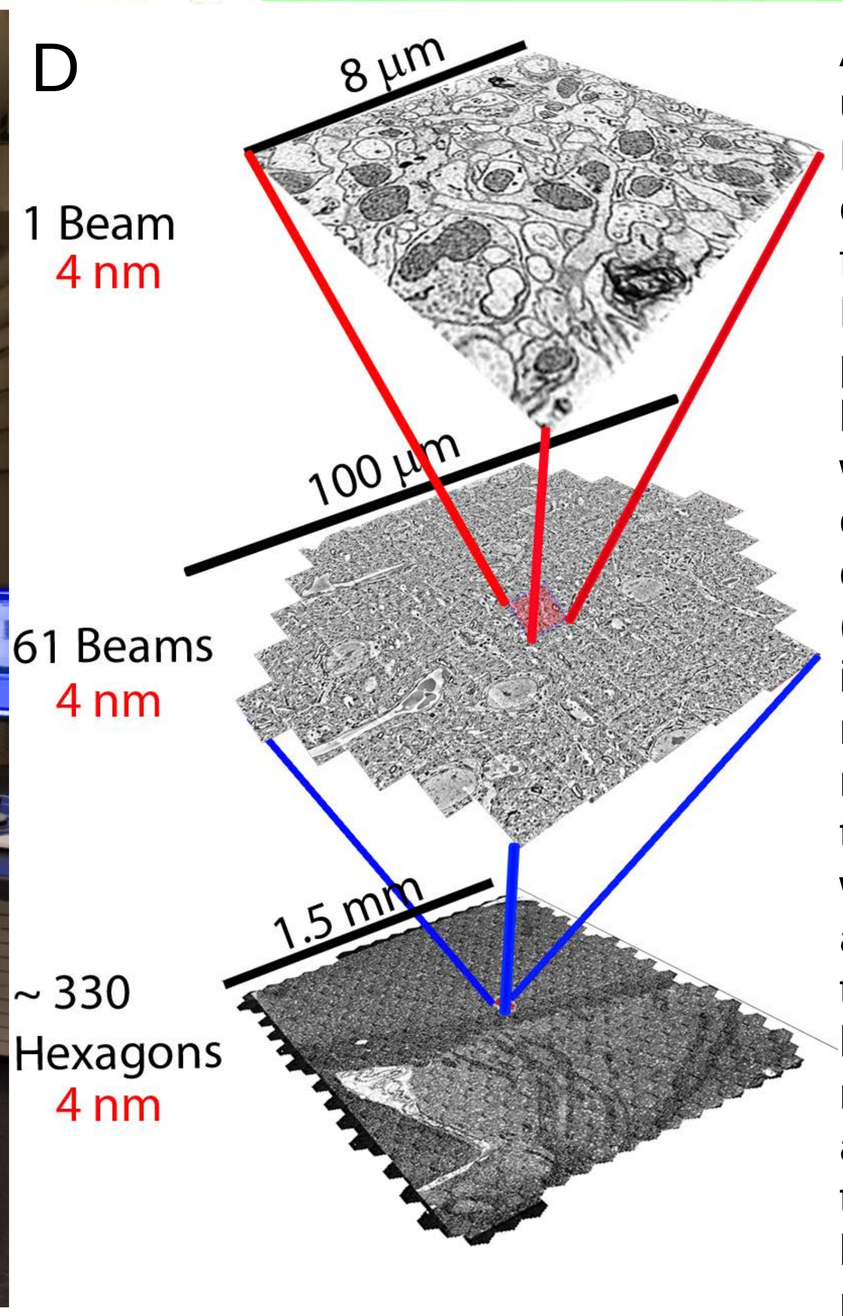
Harvard University & Boston University School of Medicine

In order to generate large volumetric datasets of brain tissue, we have developed an automated pipeline to section, and image neural circuits in brain samples. We prepare standard resin embedded blocks of brain samples that are stained with heavy metals. These blocks are sectioned using a diamond knife ultramicrotome that is modified so that it automatically picks of the samples and puts them on a conductive tape substrate ("ATUM"; see panels A and B below). These samples are sectioned below 30nm in thickness and are each typically more than a square millimeter in area. The tape pickup allows up to ~12,000 sections to be collected per day. The tape is then cut into strips, adhered to a flat silicon wafer, for further enhancement staining and mounting on the stage of a scanning electron microscope. To automate image acquisition we developed software to locate the positions of sections in the scanning electron microscope and make montaged images of each section (a program called "Wafermapper", Hayworth, Morgan et al., 2014). To speed further speed image acquisition throughput, we participated in the development of a multibeam scanning electron microscope (Carl Zeiss Inc.;) optimized for imaging brain samples. This device, now resident in the Harvard lab (see panel C below), uses multiple scanning beams arranged in a hexagon to image in parallel at a rate that may approach 1 billion pixels per second. The device uses 61 low (several kilovolts) electron beams each scanned at 10-20 M pixels per second to generate secondary electrons from each site that are detected without cross talk. Lateral image resolution is 4nm per pixel (see panel D below). This suite of methods and tools may be useful for the acquisition of large brain samples at high resolution.









A: Automatic tape collecting ultramicrotome (ATUM) developed by K Hayworth and R Schalek at Harvard collects sectioned tissues samples on a firm plastic tape for subsequent viewing. B Inset of green box in A showing the process of sectioning with a diamond knife, floating the section in a water boat where it is picked up by a conveyor belt of tape. The reel of tape is subsequently cut in strips and placed on silican wafers (~250 sections per wafer, not shown) for imaging in a scanning electron microscope. C. The Zeiss MSEM-multibeam scanning electron microscope that uses 61 beams to image in parallel which speeds up imaging by approximately that rate. D Imaging with the MSEM allows a montage of heagagons (bottom) by stage movements. Each hexagon covers an area of ~100 X 100 µms (middle) one of the panes of the hexagon (from one beam) is shown at the top At 4nm resolution synaptic vesicles, and the fines neural processes are visible.

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