

Imaging and Reconstructing Microglia in 3 Dimensions Using FIB-SEM

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Microglia are the brain's resident immune cells. They are incredibly dynamic cells which constantly survey the brain for any changes in homeostasis and respond quickly to pathogens, tissue damage, or foreign objects entering the brain [1]. Within the last decade, their role in the brain's development and healthy ageing has also begun to be uncovered. They interact with other glial cells, neurons, blood vessels within the brain, and make many transient contacts with synaptic structures including dendritic spines and axon terminals [2]. Electron microscopy (EM) studies have allowed researchers to investigate the ultrastructure of these cells, as well as define their interactions with the neuropil both in development and diseased brain tissue [3]. Until recently, these EM images have been limited to transmission EM on ultrathin sections, rarely allowing investigation of contiguous microglial processes and somas, since microglia are highly ramified and the processes are almost never contiguous with their cell bodies in healthy tissue. Serial sectioning on ultramicrotomes allowed very small regions (small parts of processes) to be reconstructed in 3 dimensions, but the protocols are very arduous and hand-stitching the images may not account for stretches within individual sections [4]. Recent advances in 3D super-resolution imaging such as serial block face scanning EM (SBFEM) and focused ion beam coupled with scanning EM (FIB-SEM) and improvements in image processing has allowed increased throughput in 3D studies [3,5]. Here we describe the methods used to process our biological samples and investigate microglial interactions with the neuropil in the rodent brain using FIB-SEM.

Animals are perfused with 3.5% acrolein followed by 4% paraformaldehyde in phosphate buffer, while human tissue are fixed with a mixture of paraformaldehyde and glutaraldehyde overnight [6]. Tissues are cut into 50 μm sections on a vibratome. Samples are prepared using immunohistochemistry (if required) and post-fixed for SEM using sequential incubations in osmium, thiohydracarbazine, and osmium to enhance membrane contrast. The tissue is dehydrated and the region of interest (e.g. striatum, Figure 1, ventral hippocampus, Figure 2) is excised, embedded in Durcupan resin, and mounted on top of a resin block. An ultramicrotome is used to create a smooth surface prior to gluing the tissue on top of a pin stub with carbon paint, followed by application of 30nm platinum coating using a sputter coater.

Once in the FIB-SEM, a region of interest is selected for concurrent FIB-milling and SEM imaging (Figure 1). Atlas Engine 5 is used to deposit a protective platinum surface, followed by the carving of a "wolverine claw" of fiducial markers to allow precise automated focus and drift correction. 125 μm^3 volumes were captured over subsequent 18-24 hour imaging sessions. After the images were aligned and exported, we performed semi-automated carving and modeling of microglial cell bodies and processes with Ilastik (Figure 2). These models allow investigation into extracellular encircled items (Figure 2) and verification of phagocytic processes [7].

References:

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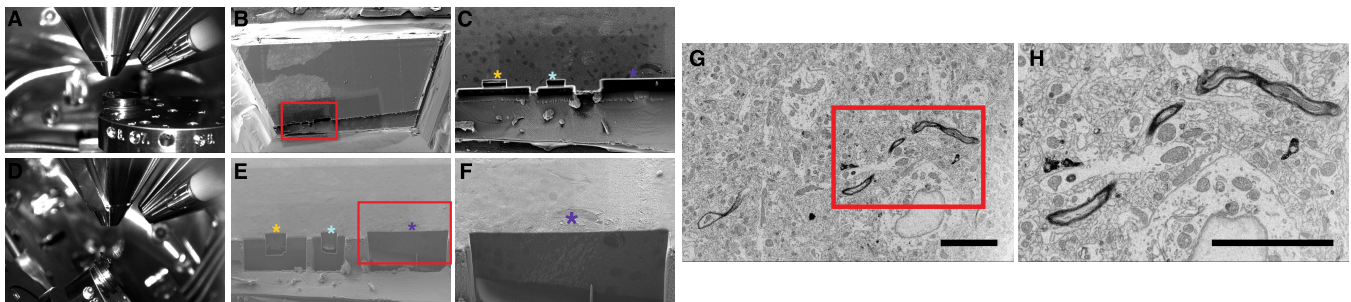


Figure 1. Example of tissue preparation for FIB-SEM imaging. Blocks are prepared on the ultramicrotome and placed into the FIB-SEM (a). A region of interest is identified on the block surface (b,c, asterisks), followed by tilting the specimen to 54° (d). The region of interest is then verified (e,f, asterisks), the surface is polished to reveal the biological sample (g, h). Purple asterisk indicates surface post polishing but prior to 3D imaging, yellow and blue asterisks indicate surfaces post imaging. Scale bars in g,h are 5 microns.

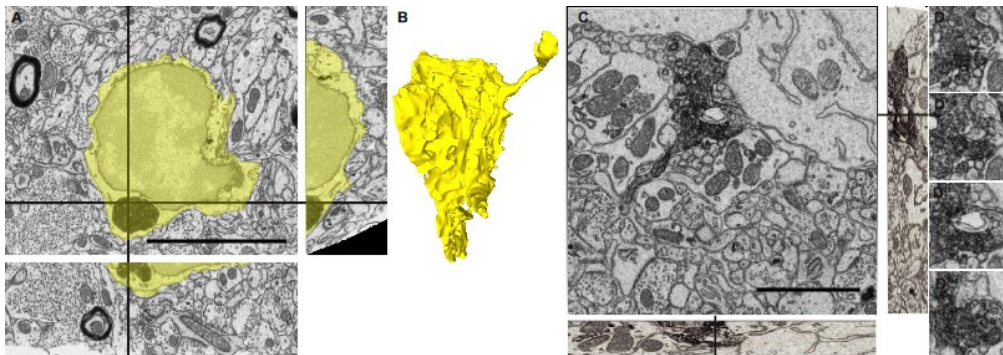


Figure 2. Example of microglia cell body (a) and processes (c, d) imaged using 3D FIB-SEM. Microglia is pseudocolored in yellow in a, b. A microglial process is marked by anti-Iba1 immunohistochemistry (c) and serial sections are used to prove the presence of a phagosome fully enclosed inside the process (d'-d'''). Scale bar in a is 5 microns, scale bar in c is 1 micron.