ADVANCES IN SERIAL BLOCK FACE DUALBEAM ELECTRON MICROSCOPY FOR THE EXPLORATION OF CORTICAL CIRCUITS

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The mammalian brain comprises interconnected neurons that communicate via synapses to form a bewilderingly complex network. In every microliter of cortical grey matter there is approximately one billion synapses arranged along several kilometers of axons. Visualizing these circuits and all their connections is paramount to being able to understand how the brain works. Synapses are specialized junctions whose sizes range from over a micron in diameter to less than 100nm. Their size means that they can only be identified using the electron microscope, which also allows them to be classified according to their function, ie. inhibitory or excitatory. However, to understand the connectivity between neurons in the cortex, large volumes of neural tissue needs to be imaged at 'synaptic resolution' and in three dimensions (3d).

Traditionally serial section transmission electron microscopy has been the only method available in which images are acquired from sections that are cut and mounted on grids. These image representing a 3d volume can be used to reconstruct and analyze the structure of the elements within. This method is very labor intensive, requiring a greater deal of manual dexterity, and when small mistakes occur, a continuous dataset is lost.

Recent studies have explored an alternative method for serial image acquisition in which the block face of resin-embedded neural tissue was imaged within a FEG SEM [1]. Sections were removed from the imaged face in the microscope using an ultra microtome and then immediately imaged by the SEM. This provides a series of aligned images of the tissue in the block face and has the clear advantage that image acquisition can be fully automated. However the quality of the sectioning is critically dependent on homogenous resin hardness which is difficult to maintain when the electron beam is being used at higher resolution and part of the block suffers from the differential heating effects.

We have continued [2] to explore an alternative approach that is seemingly unaffected by variations in resin quality that uses a focused ion beam directed perpendicular to the block face. This is used to mill the surface of the resin from which serial images can be acquired. We have used automated repetitive cutting and imaging on the DualBeam (Slice and View) with consistent sectioning intervals to as low as 40 nm. A resin embedded block of adult mouse neocortex was mounted in the microscope and images from the block face acquired with a backscatter detector.

Results show that this focused ion beam technique is capable of sectioning neural tissue, and using the back scattered electrons we were able to visualize the detailed ultrastructure. Synaptic contacts were clearly visible, including the pre and post synaptic densities and the synaptic vesicles. In a fully automated mode we were able to collect serial images that allowed us to make detailed morphological analyses of the neuronal elements in 3d.

References:

[1] Denk W, Horstmann H (2004) Serial Block-Face Scanning Electron Microscopy to Reconstruct Three-Dimensional Tissue Nanostructure. PLoS Biol 2(11): e329

[2] JJ L Mulders, G Knott and BH Lich, M&M proceedings 2006, DualBeam Slice & View: Practical Aspects for Collecting 3D Cortex Image Data

Figures: