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Correlative Microscopy Application in Spinal Cord Injury Research

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Spinal cord injury (SCI) affects approximately 250,000 people in the United States, with about 11,000 new injuries each year. Neuron regeneration, a critical process to restore the communication between the body and the brain after SCI, is an import topic in both basic science and clinical research. After a severe SCI, surviving neurons repair and develop functional synapses to re-establish axonal pathways. Glia cells are believed to regulate many signaling mechanisms that control the neural repair ability before and after SCI [1, 2]. Increased numbers of glia cells gather around motor neurons after SCI. The interaction between motor neurons and surrounding glia cells may play critical roles in reconnecting neural circuits. Three-dimensional (3D) structural information about the interaction between motor neuron and surrounding glia cells at synapses would help researchers understand the process of neuron repair. However, such 3D structures are currently unavailable.

Based on the dimension of motor neuron cell body (20-30μm), glia cell (~10μm) and synapses (~1μm), FIB/SEM tomography (Figure 1) is an ideal tool to investigate the interactions between motor neuron and surrounding glia cells. The challenge we encountered in FIB/SEM application was that the exact location of motor neurons and glia cells in the sample block (~1mm^3) was unknown, so FIB/SEM initiated at a random location cannot guarantee to capture images of the area of interest at a reasonable resolution to recognize the cell-cell interactions.

To solve the problem, we proposed a correlative microscopy method, which combined X-ray Computed Tomography (X-ray CT), FIB/SEM tomography and Scanning Transmission Electron Microscopy (STEM) tomography methods. Firstly, X-ray CT was applied on a mice spinal cord sample (~1mm^3) to obtain a low to medium resolution 3D map [3], which was used to locate the area of interest (Figure2a). Because X-ray CT is a non-destructive 3D imaging method, the same sample can be used for additional electron microscopy investigation after X-ray CT scanning. Once the areas of interest were recognized and labeled in the 3D maps, excessive material was removed by using plasma FIB, ultramicrotomy or a razor blade. The trimmed specimen will be examined using FIB/SEM tomography at intermedium resolution (Figure 2b). If even higher resolution images are needed, FIB Lift-out method or ultramicrotomy can be utilized to make EM grids which are suitable for STEM tomography study (Figure 2c). Our 3D results disclosed that glia cells are closely associated with motor neurons after SCI (Figure 2b). Interaction of motor neuron and microglia was identified (Figure 2c). These results confirmed former studies that that direct interactions between microglia and neurons many play a crucial role in neural repair.

In summary, correlative microscopy overcomes the barrier of different microcopy techniques. By combining techniques that have overlapping resolving powers, we are able to accurately locate and pick out areas of interest from sample blocks. The area of interest will be investigated at higher resolution;

while we have the overview of the whole specimen.

Methods: Lumbar spinal cord of mice was cut to ~500um blocks. Samples were then chemically fixed and *en bloc* stained. After dehydration and infiltration, tissue sections were embedded in durcupan resin and incubated at 60°C for 2 days. X-ray CT data were collected on Zeiss Xradia 520 Versa system. FIB/SEM tomography data were collected on the Helios Nanolab 600 DualBeam (FIB/SEM). STEM tomography data were collected on the FEI probe-corrected Titan^{3}™ 80-300 S/TEM. [5]

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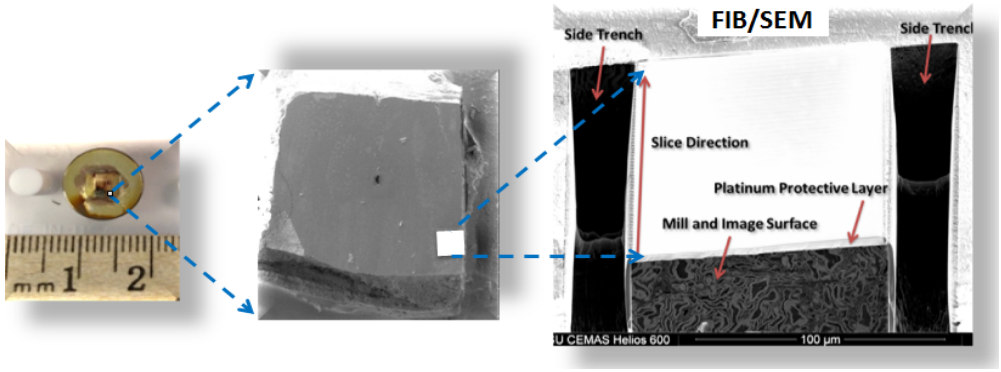

Figure 1. Field of view in FIB/SEM

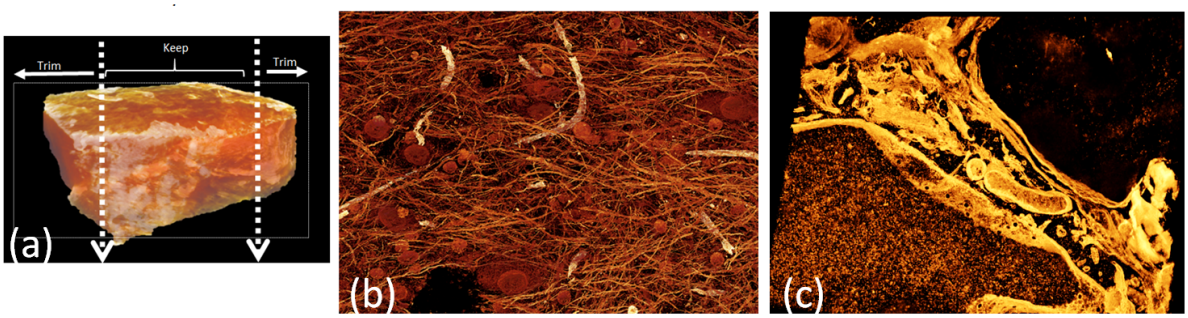

Figure 2. 3D view of mice spinal cord obtained using X-ray CT (a), SEM tomography (b) and STEM tomography (c)

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