doi: 10.1017/S1431927609092435
Microsc Microanal 15(Suppl 2), 2009

Fast Throughput Low Voltage Scanning Transmission Electron Microscope Imaging of Nano-Resolution Three Dimensional Tissue

Mehdi Bolorizadeh*, Harald F. Hess*

*Howard Hughes Medical Institute, Janelia Farm Research Campus, Ashburn, VA 20147

Excellent methods exist to obtain structures of molecules at atomic, organelles at electron microscopic, and tissue at light-microscopic resolution. A gap exists, however, when 3D tissue structure needs to be reconstructed over hundreds of micrometers with a resolution sufficient to follow the thinnest cellular processes and to identify small organelles such as synaptic vesicles. Such 3D data are, however, essential to understand cellular networks that, particularly in the nervous system, need to be completely reconstructed throughout a substantial spatial volume [1]. The processes of obtaining such data are time consuming (orders of months) and labor intensive. Therefore there is always a need to increase the throughput of such a 3D imaging.

A good biological candidate for reconstructing hundreds of μm of tissue structure is the Drosophila brain, which has a dimension of ~ 200 × 200 × 300 μm^3. Our goal is to fully image the Drosophila brain in the fastest manner with a 4nm/pixel resolution, so the neuro-structures can be clearly traced through the tissue. The Drosophila brains were embedded in Epon 812 resin and fixed with Glutaraldehyde, and sliced into ~50 nm sections which were laid on a 50 nm Pioloform TEM slot grid. After sectioning it is Os stained, were the Os reacts with membranes increasing their electron density.

Using an Ultra 55 Zeiss Schottky FE-SEM system we have developed a low voltage (20 – 30 kV) high throughput STEM, LVSTEM. Since SEM has a high capability of modification it is an effective and low cost candidate for LVSTEM. With its relatively large chamber (compared to TEM and STEM) SEM allows a number of other instruments and detectors to be added to the system. Even with the additions, there is still plenty of space for a large array of biological sections. Furthermore, the column design of the SEM, having no cross over point and having a capability of upgrading to high current modes (tens of nA), made it an ideal candidate for our effort.

To push the high throughput imaging further we developed our own detector. The detector is based on a scintillator and photo-multiplier tube technology. A number of parameters were explored to optimize the STEM detector and increase the Signal to Noise Ratio (SNR). SNR is dependent on several factors, including contrast, beam current, sampling rate, and the transmitted electron coefficient of the sample for a specific beam energy. In order to study the contrast, double Monte Carlo (MC) simulation of the electron matter interactions [2] (10^6 traces) for different energies, 1-30 keV, were performed. By comparing the angular distribution of transmitted electrons in Os stained and stain-free brain slabs, the relation between the divergence angle of the transmitted electrons through the sample and image contrast were determined (Table 1). Fig. 1 shows a schematic of the detector and the contrast plot for a 30 keV electron beam, which gives an optimum bright field (BF) half angle of $\beta = 4.1$. This value falls in the

expected range as the aperture half angle is a few mrad. The geometry of the STEM detector was optimized to increase the image contrast with the type of sections and electron optics used.

Scanning and image acquisition are externally obtained through National Instrument's (NI) function generator, arbitrary wave generator, and digitizer. Images are obtained at 30 kV, 4nm/pixel resolution. Scanning fields were as large as 64 μm × 64 μm with a bandwidth range of 1-50 MHz. Experimentally different beam currents and sampling rates (with the appropriate low pass filters) were studied

The above STEM set up can be utilized to perform both tomography and normal serial section (no tilt) imaging. Also because of the large SEM chamber, batch of samples can be loaded at once, decreasing the sample handling and increasing the imaging throughput. Our effort demonstrates that a LVSTEM is a strong candidate for fast throughput imaging of long and thin cellular processes. Images can be taken at sampling speeds greater than 10 MHz with adequate SNR.

Reference:
[1] W. Denk, H. Horstmann, PLoS Biol 2 (2004) 1900.
[2] M. Bolorizadeh, D. C. Joy, J. Micro/Nanolith. MEMS MOEMS 6 (2007) 023004.

E (kV)	Max BF Contrast (%)	BF Half Angle (Degrees)
7	33.2	28.5
15	33.5	9.1
30	25.1	4.1

Table 1. MC simulation results of maximum bright field (BF) contrast with BF half angles for 7, 15, and 30 kV primary electron beams. It was assumed that the weight fraction of the brain cut contains 10% Os.

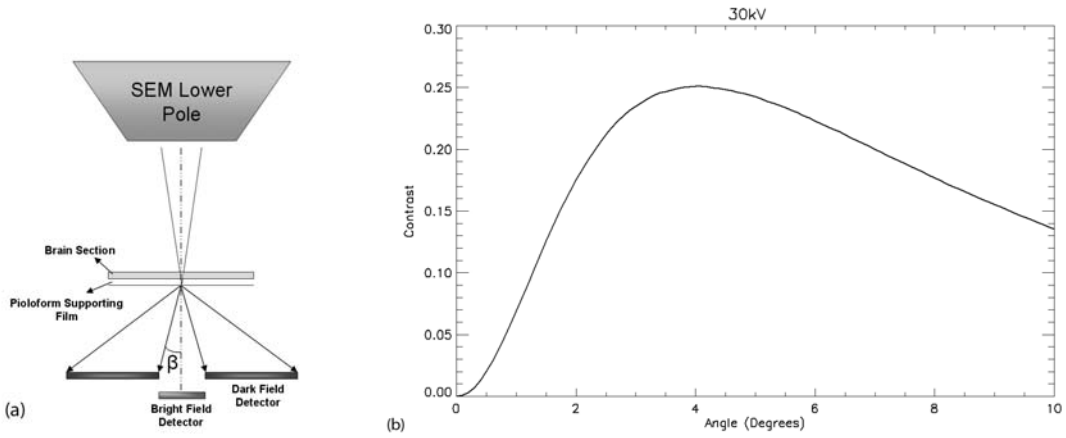

Fig. 1. (a) A schematic of the experiment and the STEM detector. (b) The relation between the divergence angle of the transmitted electrons through the brain section and contrast, determined by the MC simulation for 30 kV electron beam.

https://doi.org/10.1017/S1431927609092435 Published online by Cambridge University Press