Imaging with a 196 beam SEM

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Large area and volume imaging with a scanning electron microscope has gained a lot of traction in recent years. Brain tissue is being mapped and large datasets are used for ultrastructural studies. One of the challenges in this field is to reduce the time required to obtain a significant dataset. Imaging even a small area, for instance, a square millimeter, at high resolution, already requires several hours. When a large volume has to imaged, the acquisition time easily becomes months or years[1].

To tackle this problem, we have developed a multi-beam scanning electron microscope (MBSEM) with 196 electron beams which scan the sample in parallel[2]. For signal detection, standard SEM detectors cannot be used, as the signals of the beams will be mixed on the detector. Here we will describe our imaging system, analyze the contrast generation and show an imaging example.

The MBSEM developed at Delft University of Technology is a standard FEI Nova NanoSEM 200 where the source module has been replaced by a custom multi-beam generator. Using the original magnetic lenses, a grid of 14x14 electron beams are focused to spots as small as 5nm on the sample. The sample, a tissue section with a thickness between 50 and 200 nm is placed directly on top of a scintillator. The electrons passing through the sample generate light in the scintillator. This light is collected by a high NA objective and an image is formed by analyzing the light intensity for every scan step. Figure 1 shows the result of an analytical analysis comparing this imaging method to backscatter imaging in a regular SEM. It shows that transmission imaging has a contrast-to-noise ratio (CNR) comparable to or even better than backscatter imaging at certain energies.

To analyze the transmission signal of each beam in the MBSEM, an optical image of the scintillator is taken with a CMOS camera for every scan step, at a maximum framerate of 10 kfps. The intensity of the light spots is analyzed by means of an FPGA and an image is formed. This allows for live focusing and stigmation of all 196 beams simultaneously. A transmission MBSEM image of mouse brain tissue formed with 196 beams at 10kfps is shown in figure 2. It shows that we can obtain similar image quality with the MBSEM as with a single beam SEM. We are presently increasing the image acquisition speed to 10 Mfps.

[1] Zheng, Zhihao, et al. *bioRxiv* (2017): 140905.
[2]Ren, Yan, and Pieter Kruit. *Journal of Vacuum Science & Technology B*, 34.6 (2016): 06KF02.

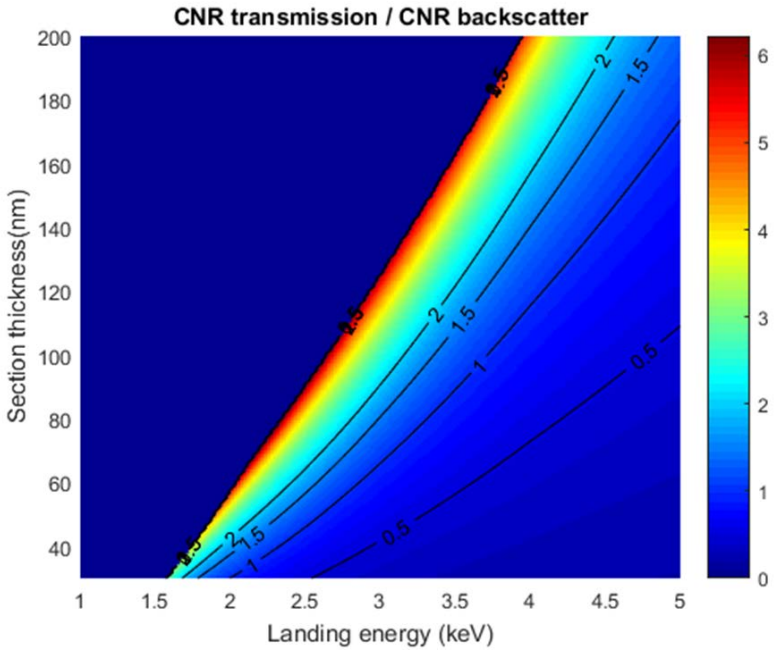

Figure 1: CNR comparison: Contrast-to-noise ratio as a function of landing energy and section thickness of transmission imaging in the MBSEM compared to backscatter imaging. Values above 1 indicate superior transmission imaging.

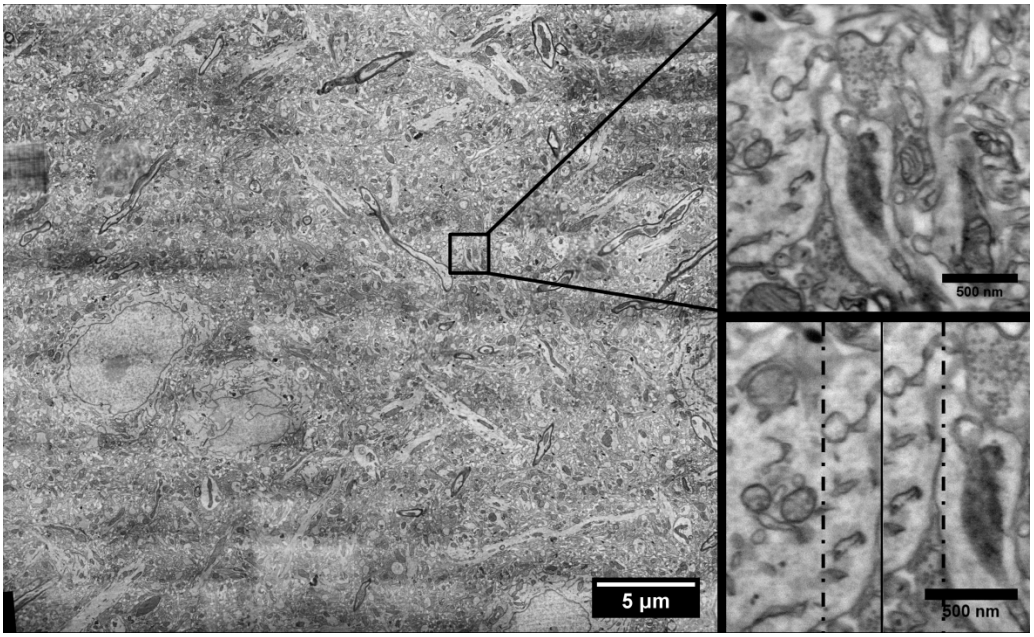

Figure 2: MBSEM imaging result: ***(left)*** 14000x14000 pixel stitched transmission image of mouse brain tissue. Imaged by the 196 beams of the MBSEM. (top-right) Highlighted part of the image showing details (bottom-right) Showing the overlap between areas scanned by neighboring beams. *Sample courtesy of Briggman (NIDS, US)*