Development of a high throughput 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 millimetre, 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, a multi-beam scanning electron microscope (MBSEM) can be used. Recently, Carl Zeiss released the MultiSEM with secondary electron detection which has shown to give good imaging results [2]. Previously we have developed and built a MBSEM based on an FEI Nova NanoSEM which scans 196 beams in parallel and employs a novel transmission imaging method [3]. Here we will elaborate on the transmission imaging and show our plans for the next generation MBSEM.

The next generation MBSEM we are developing is based on a Thermo Scientific Apreo SEM. Using the original lenses, a grid of electron beams is focused 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 and an image is formed by analyzing the light intensity for every scan step. The sample stage with an objective lens is based on a Delmic Secom system, which adds an optical microscope to the electron microscope. The light is collected with a high NA objective and the grid is imaged onto an array of multi-pixel photon counters outside of the vacuum chamber. Figure 1 shows an example of imaging with a single beam using this scheme. It demonstrates that we can get a similar contrast as in backscatter imaging and we can obtain a high quality image at low dwell times.

Scanning the beam would result in the light spots moving off their detector. To avoid this problem, the stage moves in one direction and the beams scan 1/8 of the beam-pitch perpendicular to the stage scan, resulting in less motion of the grid on the detectors. A schematic overview of this acquisition method is shown in figure 2. An added benefit is that stage overhead is severely reduced, as the stage is constantly moving. With this system, we are aiming at an acquisition speed of 640 megapixels per second.

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[2] Crosby, Kyle, Anna Lena Eberle, and Dirk Zeidler. MRS Advances 1.26 (2016): 1915-1920.

[3] Ren, Yan, and Pieter Kruit. *Journal of Vacuum Science & Technology B,* 34.6 (2016): 06KF02.

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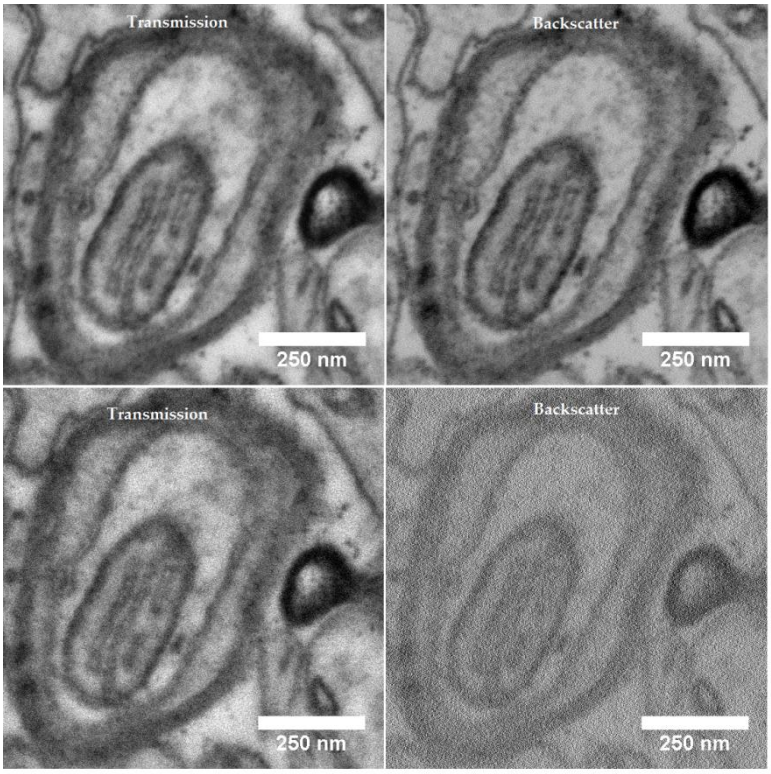

Figure 1: (left)Transmission and (right)Backscatter SEM images of a mouse brain tissue section acquired at 4 keV with a 0.8nA beam current. With a dwell time of (top) 1s and (bottom) 100ns. Samples courtesy of Briggman (NIDS, US).

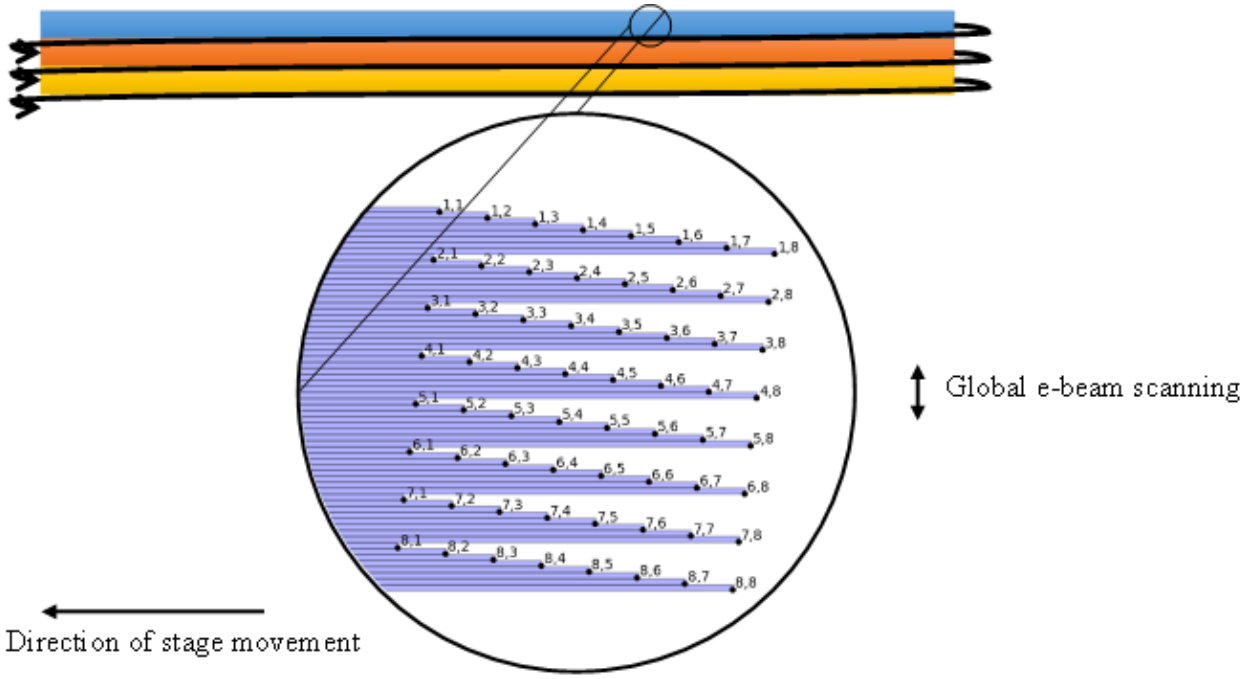

Figure 2: A schematic overview of the continuous acquisition scheme proposed for the MBSEM. (top) Ribbons of the image generated by scanning the stage in one direction and the beams perpendicular to this movement.(bottom) A close up overview of an array of 8x8 beams scanning the sample while the stage move perpendicular to this movement.