

10kfps Transmission Imaging in a 196 Beam SEM

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Increasing imaging speed has been one of the key challenges in scanning electron microscopy for a long time. In order to scan a surface area of a square millimetre using a regular scanning electron microscope at a satisfactory resolution, multiple hours of measurement are needed. When one moves to volume imaging, a cubic millimetre for instance, this number gets outside of what would be considered a reasonable amount of time. This limitation is especially a problem when imaging biological tissue, where large areas or volumes can be of great importance to get better understanding of biological systems.

In order to overcome the throughput limitation, various parties have suggested using multiple beams instead of a single beam. One of these systems, with up to 91 beams in parallel, is now made commercially available by ZEISS [1]. We have developed a multi-beam scanning electron microscope with 196 beams in parallel where the signal can be detected in transmission mode as well as secondary electron detection mode [2]. Here we will show our first results of tissue imaging using our first-generation detector, which is a high-speed camera with frame-rates up to 10,000 frames per second (fps).

The multi-beam SEM was built using a standard column of a FEI Nova NanoSEM 200. The 196 electron beams are generated from a single high-brightness Schottky electron source, making use of a square aperture lens array grid of 14 by 14 lenses. Modified source optics allows focusing of all beams in the sample plane, with the same probe current and probe size as in a single-beam SEM. Both secondary and transmission electron signals can be detected in the system, but this work will focus on the transmission imaging only. For the detection of the transmitted electrons, the sample of interest is placed on a scintillating screen and the light generated by each beam is collected through an optical objective lens. This light is focused on a CMOS camera placed outside the SEM chamber and the image is produced through online processing of the intensity of each beam.

As the electron beams scan over the sample, their position on the CMOS camera changes which requires that different pixels on the camera have to be integrated in order to obtain the correct signal for each beam. To know which pixels have to be integrated, first a calibration step is performed after which the pixels to be integrated are calculated for every scan-step. Both the calculation of the pixels to be integrated and the integration are performed online by means of a FPGA. We show that using this method we can do online processing of each beam position at camera frame rates of up to 10,000 fps.

To obtain optimal contrast in the image, the electron beam was retarded from 15 keV to 4 keV using a high voltage sample bias. We show results of imaging of Pancreatic tissue, stained with the OTO protocol, at 10,000 fps, a dwell time of 100 μ s. The image created in this fashion is shown in figure 1, which shows both the megafame and a zoom-in of part of the image. The sub-images were stitched

using the calibration data obtained in the calibration step, which is also shown in figure 1. Problems occur due to beam damage, namely small tears in the tissue section, and due to an inhomogeneity in the contrast of the sub-images. We expect to solve these problems in due time.

The results obtained in this work show good perspective for a high speed high resolution multi-beam scanning electron microscope. With this microscope, we will be capable of speeding up scanning electron microscopy with a factor of almost 200 with good image quality and resolution [3].

References:

- [1] A, Eberle *et al*, *Journal of Microscopy*, **259(2)**, (2015), p.114.
- [2] Ren, Y., & Kruit, P. *Journal of Vacuum Science and Technology B*, **34**, (2016).
- [3] This research was funded by an NWO-IPP project.

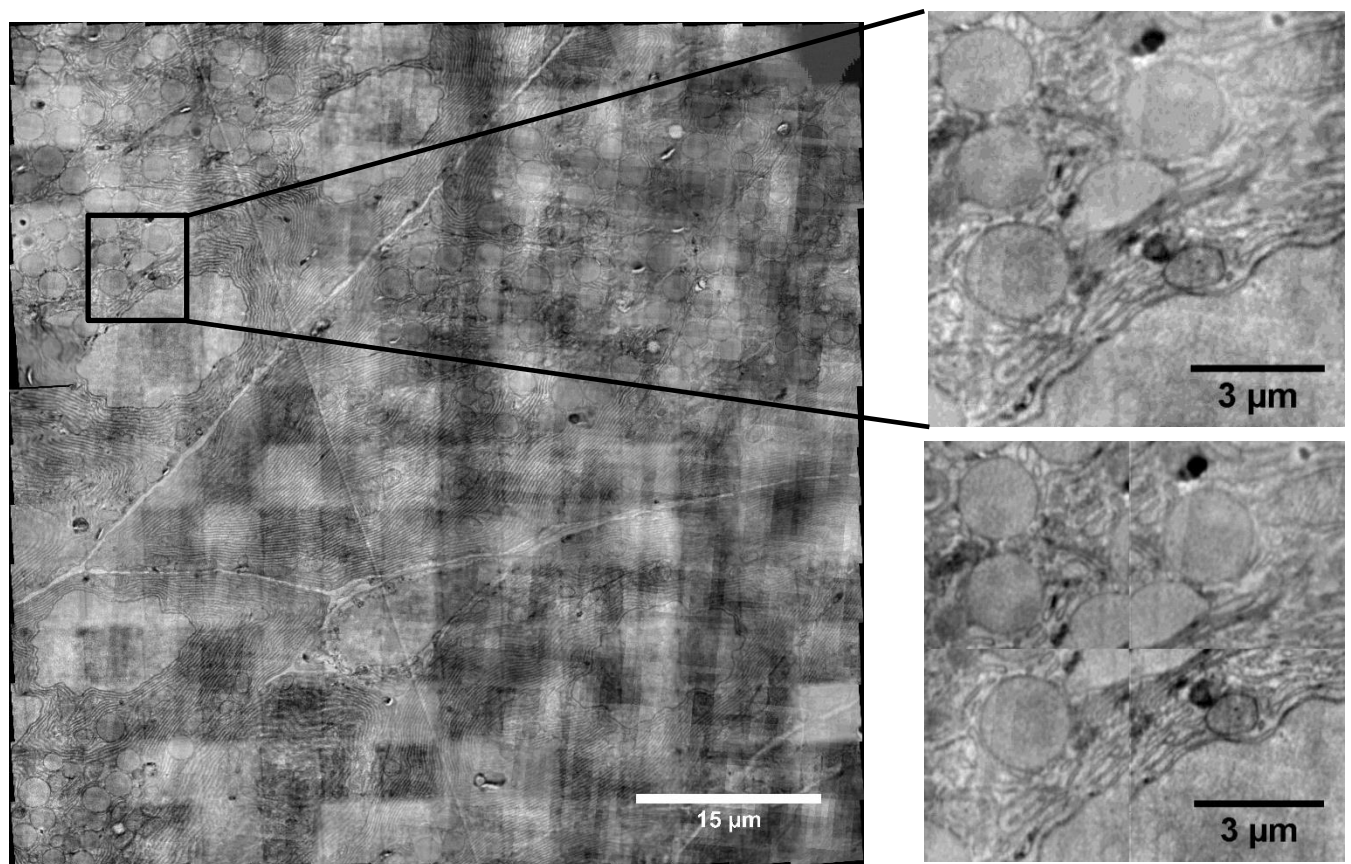


Figure 1. (left) Full stitched image of rat pancreas imaged by the 196 beam multi-beam (top-right) Stitched part of the image SEM (bottom-right) Unstitched part of the image