Multibeam scanning electron microscopy with transmission detection

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Electron microscopy (EM) is increasingly being used in large-scale biological projects, either for volume imaging or large area mapping. In both use cases, a need for increased throughput, reliability and automation of electron microscopy highlights the importance for new approach to large-scale imaging. Here, we demonstrate the working principles and image collection in a streamlined multibeam STEM system based on the prototypes developed in collaboration between Delmic, TU Delft, Thermo Fisher Scientific and Technolution.
We developed multibeam electron optics that produce an 8x8 square array of electron beamlets with a current of 200 pA per beamlet, which are focused on the sample in one plane (figure 1). The 64 beamlets are scanned over the sample simultaneously, so that every beamlet scans an area of 3.2x3.2 μm. Thus, one scan covers a field of 25.6x25.6 μm. Electron detection is achieved through an optical detection pathway, where electrons transmitted through a sample produce light in a scintillator substrate[1] (figure 1). Since light intensity depends on the number and energy of electrons hitting the scintillator plate, fluctuations in the light intensity are a metric for the electron density of the sample. As a detector, a rapid multi-pixel photon counter array is used, enabling fast scan rates.

Using the transmission detector high quality field images can be acquired of biological material (figure 2). With stage movement routines, controlled by an interferometer, these fields can be rapidly tiled to form 'megafields', comprising of many multibeam fields (figure 3). This 4.1 gigapixel megafield was captured in 12 minutes. Such a dataset would take a minimum of 2 hours in a single beam SEM at similar dwell time settings. While this result is not yet a 64-fold increase in throughput, further optimizations in the acquisition chain will help realize that throughput. Development is underway to reduce stage settling time, and reduce overlap required between individual fields, which adds significant overhead in a routine workflow[3]. Together with optimizations in the image acquisition pipeline, these developments will help realize a throughput increase of 64 fold.

[1] Ren, Y. & Kruit, P. Transmission electron imaging in the Delft multibeam scanning electron microscope 1. *JVST B,* **34**, 06KF02 (2016).
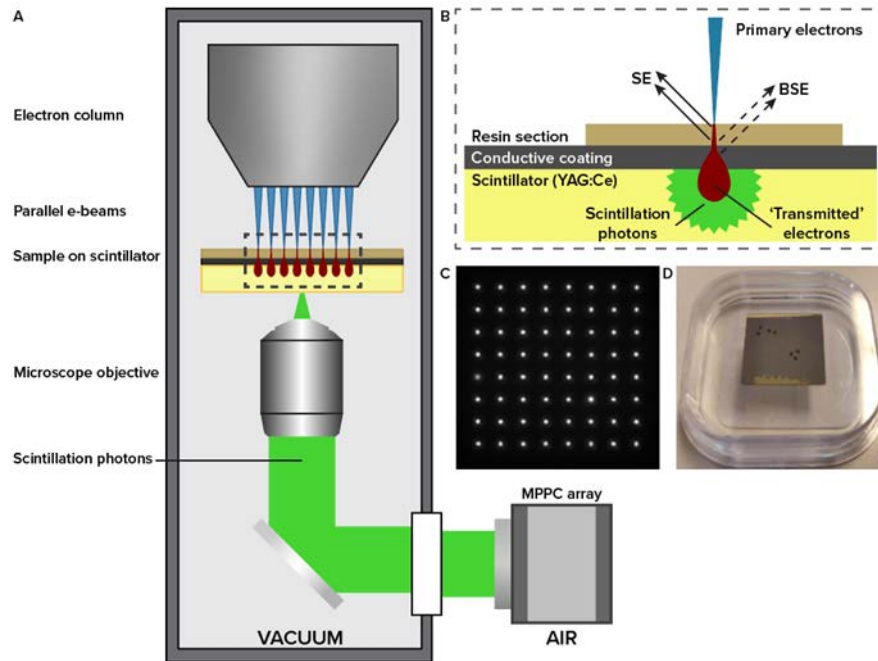
Figure 1 Working principles of the multibeam EM. (A) Schematic (B) Sample geometry for the multibeam. Electrons transmitted through the sample produce photons in the scintillator, which are detected on the MPPC array. (C) (D) Photograph of 80 nm epon sections deposited on scintillator substrate.
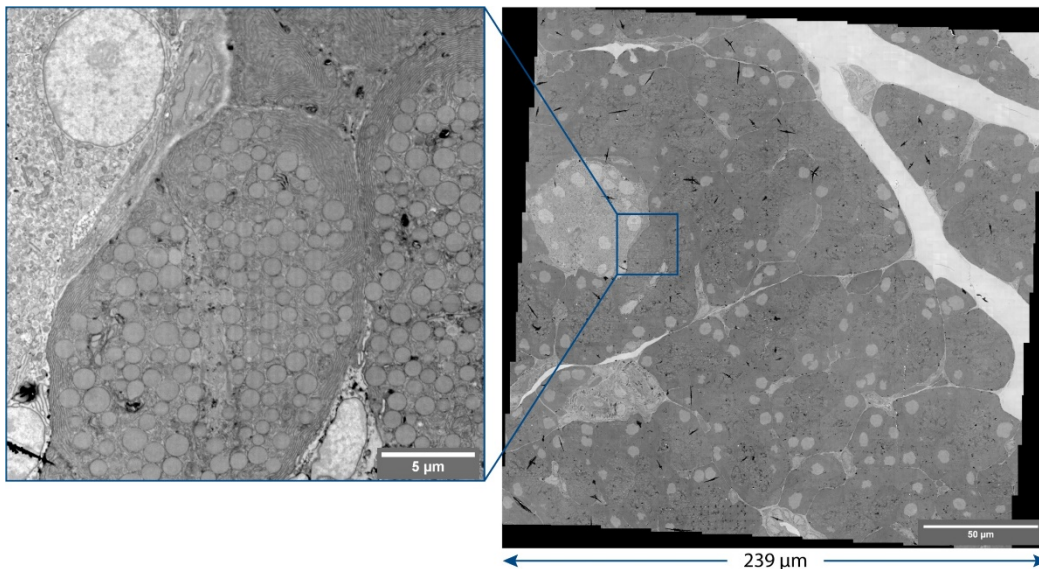

Figure 2 Single field (A) and megafield (B) of rat pancreas acquired in 64 beam multibeam STEM. (A) Single image showing the 8x8 pattern. Images were acquired at 5kV landing energy, 4 nm pixel size, 3200 ns dwell time. (B) 10x10 megafield. Raw pixel count is 64000x64000pixels (4.1 gigapixels), acquired in 12 minutes. Samples courtesy of Ben Giepmans.