doi:10.1017/S143192762200592X
Microsc. Microanal. 28 (Suppl 1), 2022
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# Characterization and Optimization of OSTEM; A Novel Detection Method for Single- and Multi-Beam Scanning Electron Microscopy

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Recent developments in large scale and volume electron microscopy (EM) have revolutionized the understanding of biological systems across different length scales. However, the inherent low throughput of electron microscopes remains a major bottleneck for further progress. This is a result of a trade-off between acquisition speed and sufficient image quality for reconstruction and biological interpretation.

To increase throughput of electron microscopes, it is vital that as much signal can be collected in the least amount of time. Optimization of current detection conditions in scanning electron microscopes (SEM) can yield up to 20-fold faster imaging [1]. New techniques such as multibeam scanning electron microscopy (MB-SEM) [2, 3], where the sample is scanned in parallel by an array of beams, can even lead to orders of magnitude increase in image acquisition speeds compared to single-beam microscopes. Existing detectors, however, are incompatible with MB-SEM as they would integrate the signal from all beams. Zuidema & Kruit (2020) demonstrated transmission imaging with a scintillator in an SEM which offers a way to separate the individual signals. It may also be preferred for imaging thin biological sections as it can yield better signal to noise and improved dynamic range [4].

We have further characterized and optimized this novel detection method for SEM, optical scanning transmission electron microscopy (OSTEM), which is currently employed in a new commercial MB-SEM system. In the setup, the ultrathin tissue sections are directly mounted on a YAG:Ce scintillator evaporation-coated with a 30nm layer of molybdenum. The scintillation photons are collected using a SECOM light microscope (Delmic B.V.) retrofitted into a single beam Verios SEM (FEI) and detected with a multipixel photon counter (Hamamatsu).

We first performed a landing energy optimization by recording high magnification images for a series of landing energies and calculated the corresponding signal-to-noise ratios of the images. With the optimal settings, we show that OSTEM yields images with similar contrast and signal-to-noise ratio compared to regular backscatter electron detection (BSE) in SEM **(Figure 1**), though we notice that relation between dwell time and signal-to-noise ratio in OSTEM is different from BSE. We performed large-scale acquisitions of rat pancreas and zebrafish larval tissue (**Figure 2**) to demonstrate that OSTEM can be used for large scale or volumetric studies.

We conclude that OSTEM can as an alternative for regular BSE detection or for a seamless imaging alternative to regular STEM. Further research into a complete model of the detection system is necessary for understanding the signal output as a function of imaging parameters.

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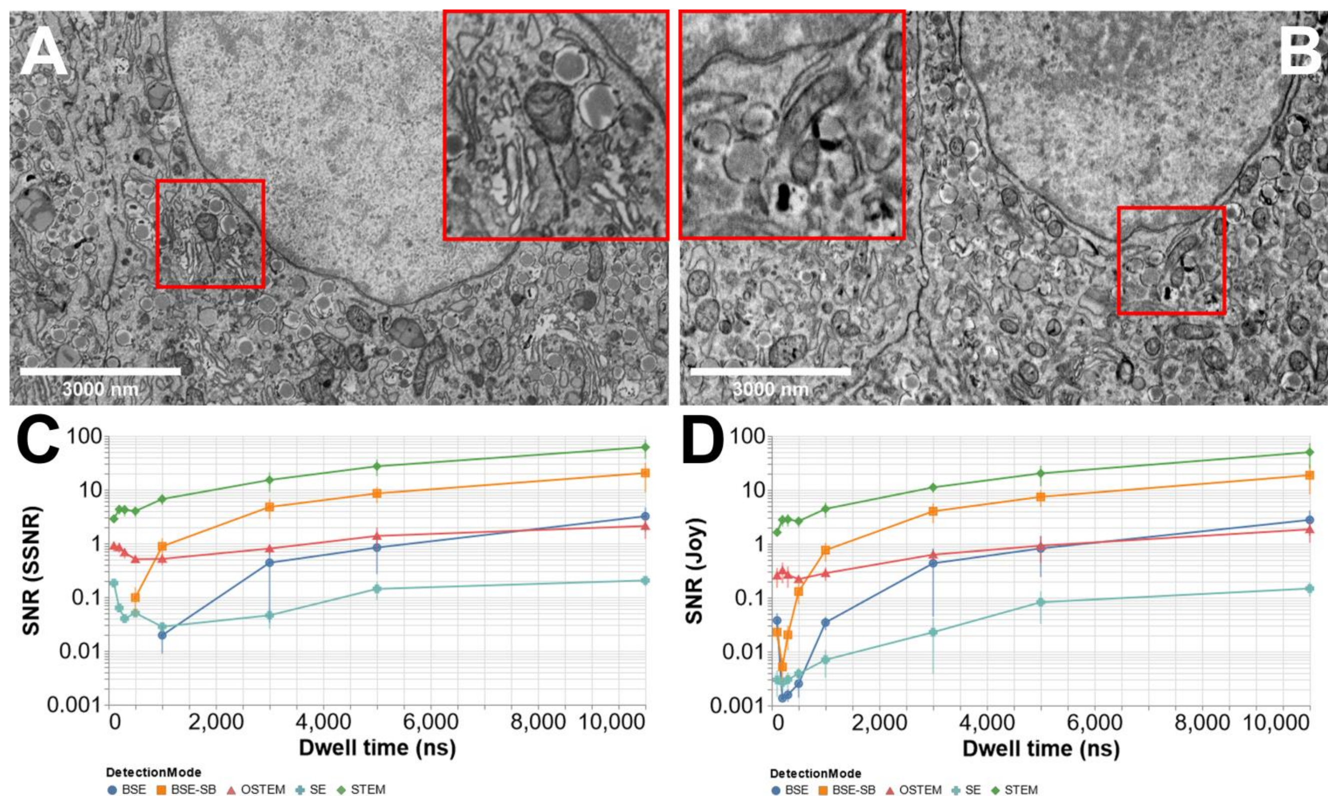


**Figure 1.** Comparison of OSTEM detection vs other detection methods. A: Backscatter electron image, 2keV landing energy. B: OSTEM image, 4keV landing energy. Current: 400pA, Pixel dwell time: 5 µs, Resolution: 4nm/pixel. C: Signal-to-noise ratio (SNR, method by Joy [5]). D: Spectral SNR (SSNR, method by Unser *et al*. [6]). BSE: Backscatter electron detection (CBS detector), BSE-SB: Backscatter electron detection with -1kV negative stage bias. SE: secondary electron detection (TLD detector), STEM: Scanning Transmission Electron Microscopy. LE: 1.5keV for BSE, BSE-SB, SE, 28keV for STEM, 4keV for OSTEM. Sample A-B: rat pancreas with *en bloc* OTO staining. Sample C-D: zebrafish with 4% NdAc *en bloc* staining.

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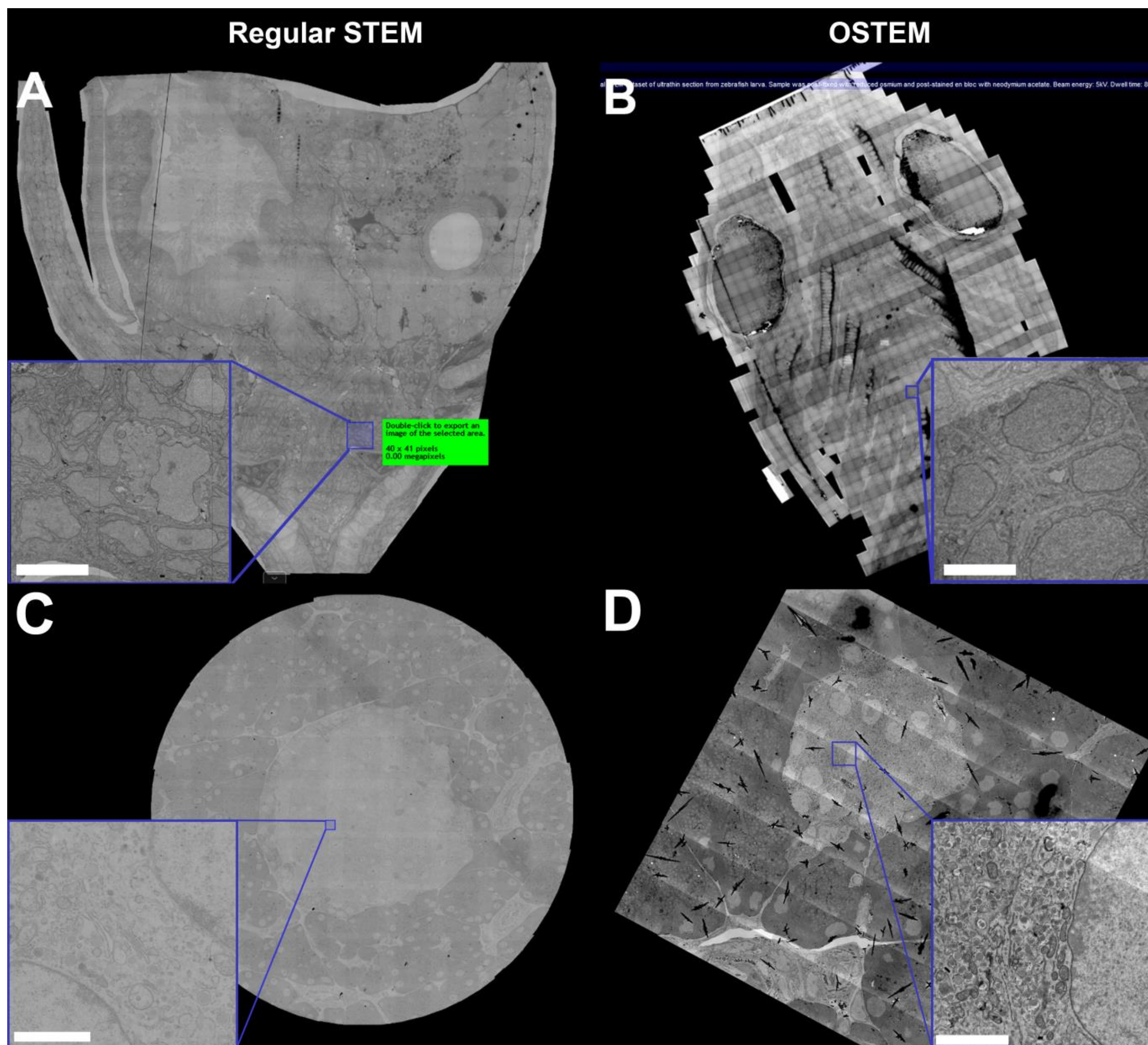


**Figure 2.** Large scale acquisitions of zebrafish larva (*en bloc* 4% NdAc) (**A-B**) and rat pancreas tissue (post-stain 4% NdAc in STEM / OTO protocol in OSTEM) (**C-D**). Settings for regular STEM: Landing energy: 28 keV. Dwell time: 3 µs. Pixel size: 4 nm. **B**: OSTEM. Landing energy: 5 keV (B), 4keV (D). Dwell time: 3 µs (A, C), 800 ns (B), 5 µs (D). Pixel size: 4 nm (A-D). Current: 400 pA (B, D). Scale bars: 5µm (A-B), 2µm (C-D).

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