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Optimizing optical STEM detection for faster acquisition speeds in scanning electron microscopy

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Background

Optimization of acquisition speed is important for large-scale and volume electron microscopy (EM) experiments of tissues and cells, as these are characterized by long acquisition times due to the low inherent throughput of electron microscopes. Developments in scanning electron microscopy (SEM) techniques have increased acquisition speed by orders of magnitude through the use of more sensitive detectors, electrostatic and magnetic immersion fields, and multibeam scanning electron microscopes (mSEM), in which the sample is scanned in parallel with an array of beams (1,2). Optical scanning transmission electron microscopy (OSTEM) offers a way to discriminate the signals from the individual beamlets in mSEM (3). In OSTEM, ultrathin biological sections are placed on a thin film-coated scintillator substrate, which converts transmitted electrons into photons. These photons are collected by a high NA optical objective and projected onto a multipixel photon counter (Figure 1A).

We have recently shown that OSTEM performs similar to backscatter electron detection (BSD) and SE detection on ultrathin biological samples (3), in terms of contrast, image resolution and signal-to-noise ratio (SNR). At short dwell times, OSTEM outperforms BSD and SE detection in SNR. However, BSD in combination with electrostatic immersion still outperforms OSTEM. Moreover, the SNR of OSTEM stagnates for moderate to high beam currents, suggesting a potential saturation point in the detection scheme. In general, signal generation and collection in OSTEM has not been thoroughly investigated and as a result, optimization of the OSTEM detector layout has not yet been performed.

To better understand signal generation and collection in OSTEM, a physics model is required that describes electron scattering in the substrate, its conversion to light, and the subsequent collection of this light signal. This model then must be verified with experimental results to be used to optimize the substrate design. We aim to develop this OSTEM signal generation model and use it to obtain a rational substrate design optimized for detection efficiency and acquisition speed.

Methods

In OSTEM, ultrathin biological sections are placed on a cerium-doped single-crystal yttrium aluminum garnet (ce:YAG) scintillator, coated with a thin (~30 nm) conductive Molybdenum coating. OSTEM is implemented in a Verios 460 (FEI Company) equipped with a SECOM integrated fluorescence microscope (Delmic) without the emission filters (Figure 1A). In an alternative setup, photons are directly detected by a multipixel photon counter array placed underneath the scintillator (Figure 1B). The photon output and SNR are evaluated by focusing the electron beam on the empty substrate surface or a biological sample respectively, and recording the photon intensity with the MPPC or a CCD camera, for a given beam current, dwell time and landing energy. The SNR is computed by averaging the spectral SNR over the full frequency space of every electron micrograph.

Electron scattering is modeled with Monte-Carlo simulations in CASINO (4) and NEBULA (5), by computing the electron energy loss per voxel. The material properties of ce:YAG and molybdenum are taken from literature. We assume that energy conversion to photons fully relies on low energy loss events (<50eV). Different possible saturation mechanisms are modeled by limiting the photon output as a function of the input energy per voxel. Additionally, the loss of photons in the detection is taken into account by a transmission coefficient that depends on the thin-film coating composition.

Results

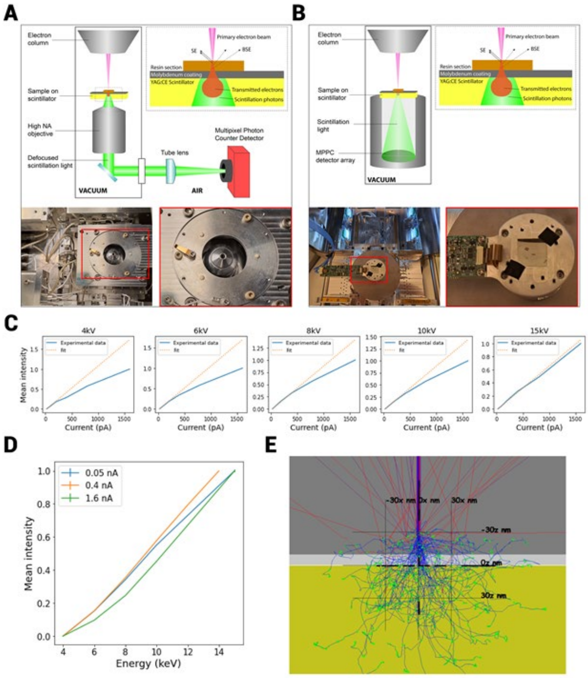
The photon output of the empty substrate demonstrated a sublinear relationship with the beam current (Figure 1C), suggesting a partial saturation effect. MPPC saturation was excluded as the main cause, since validation experiments with a neutral density filter and a CMOS camera showed similar sublinear relationships. The photon output does increase linearly at higher beam energies (Figure 1D), indicating the partial saturation is prevented in a larger electron interaction volume. Initial modelling with CASINO software showed proportionally more scattering events in the thin-film coating than in the scintillator (Figure 1E), although the majority of secondary electron generation takes place in the scintillator. However, CASINO does not accurately model low-energy secondary electrons. To better understand the signal generation and simulate the energy deposition at low energy (0-50eV), for which most of the energy transfer to the active scintillator dopant is expected, we intend to use NEBULA to calculate the energy loss per voxel as a function of the electron beam energy and current. NEBULA has been developed to provide fast and accurate simulations of low energy electron-matter interactions with first-principle physical models (5).

Conclusion

Optical scanning transmission electron microscopy (OSTEM) is an alternative SEM detection technique for imaging thin biological samples, and can be used for electron detection in multibeam scanning electron microscopy. Partial saturation of the scintillator and energy loss in the thin-film coating of the scintillator are hypothesized to limit the signal generation in OSTEM. In this work, a first attempt is made to model energy conversion from a focused electron beam to a photon signal. With a physically valid model, different substrate combinations can be tested. Subsequent optimization of the OSTEM substrate may increase acquisition speeds in single-beam and multi-beam scanning electron microscopes.

Caption
Figure 1: Optimization of optical STEM detection. A: Optical STEM detection with optical detection path. B: Optical STEM detection with direction detection of photons. C: Photon intensity on detector as a function of beam current, demonstrating a sublinear relationship. D: Photon intensity as a function of beam energy, demonstrating a linear relationship for higher energies. E: CASINO simulation of interaction volume of a 4keV beam in ce:YAG (>0z) with 30nm molybdenum (-30z to 0z). Blue: primary electrons, red: backscattered electrons, green: secondary electrons.

Graphi



Keywords:

SEM, OSTEM, Scintillation, Simulations

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