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# STEM Axial Resolution Calculated by Monte Carlo Simulations in Micrometers-Thick Substrates

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The study of a whole cell can be done with a scanning transmission electron microscope (STEM) using micrometers-thick specimens with resolution sufficient to image individually tagged proteins [1]. Noise or beam broadening limits the spatial resolution of thick samples, because of the electron beam interaction with the specimen [2]. The relation between lateral resolution and beam broadening is well studied [3]. Knowledge of the axial beam broadening can potentially be used to enhance the resolution of 3D datasets by using deconvolution procedures.

The axial resolution of thin sample is well understood and analytical formulas are available to estimate the axial resolution value from experimental conditions. The axial resolution is either diffraction limited or limited by the particle size, if the particle is larger than the point spread function [4,5]. For a thick sample, where the beam broadening due to elastic scattering in the substrate is important, the axial resolution is not well known.

In this work, the effect of the sample thickness on the 3D shape of the electron beam was studied, and a method was developed to calculate the axial resolution in micrometers-thick substrates from the point spread function (PSF) of the convergent electron beam. The method includes the effect of the geometrical broadening, and beam blurring in the sample. The PSF was calculated using the Monte Carlo program CASINO [6]. The axial resolution was calculated from intensity line scans of the number of electrons passing through a small area (located at a depth z) inside the sample for different vertical focal positions. This small area represents either the pixel size or the feature of interest, i.e., a gold nanoparticle marker. In this calculation, the effect of beam spreading by the semi-angle is also included.

Examples of vertical line scan in 5 µm-thick carbon film are shown in Fig. 1. The pixel size of 30x30 nm$^2$ was chosen to have sufficient number of electrons to obtain a peak in the axial line scan at the bottom of the sample of 5 um. For a feature at the top of the sample, the axial beam profile is defined by the geometry of the convergent beam interacting with a sample area, leading to a full-width at half-maximum (FWHM) of 1.4 µm. In that case, the resolution is limited by the pixel size. The FWHM increases at deeper position because of the broadening by the beam-sample interactions. A FWHM of 5 µm was obtained at the center (2.5 µm) of the sample. As shown in Fig. 1, the number of electrons passing through the small area decreases for thicker film. The number of electrons passing through the small area represents the maximum signal possible at this position. The signal is only reach this maximum value if all electrons interact with the feature of interest and are detected. A 33 times decrease of the number of electrons was observed between a feature at the top and at 2.5 µm inside a carbon substrate for a pixel size of 30 nm. The Monte Carlo simulation was used to calculate the axial resolution in thick substrate, and study the dependence of the axial resolution and contrast with the sample thickness and pixel size. [7]

## References

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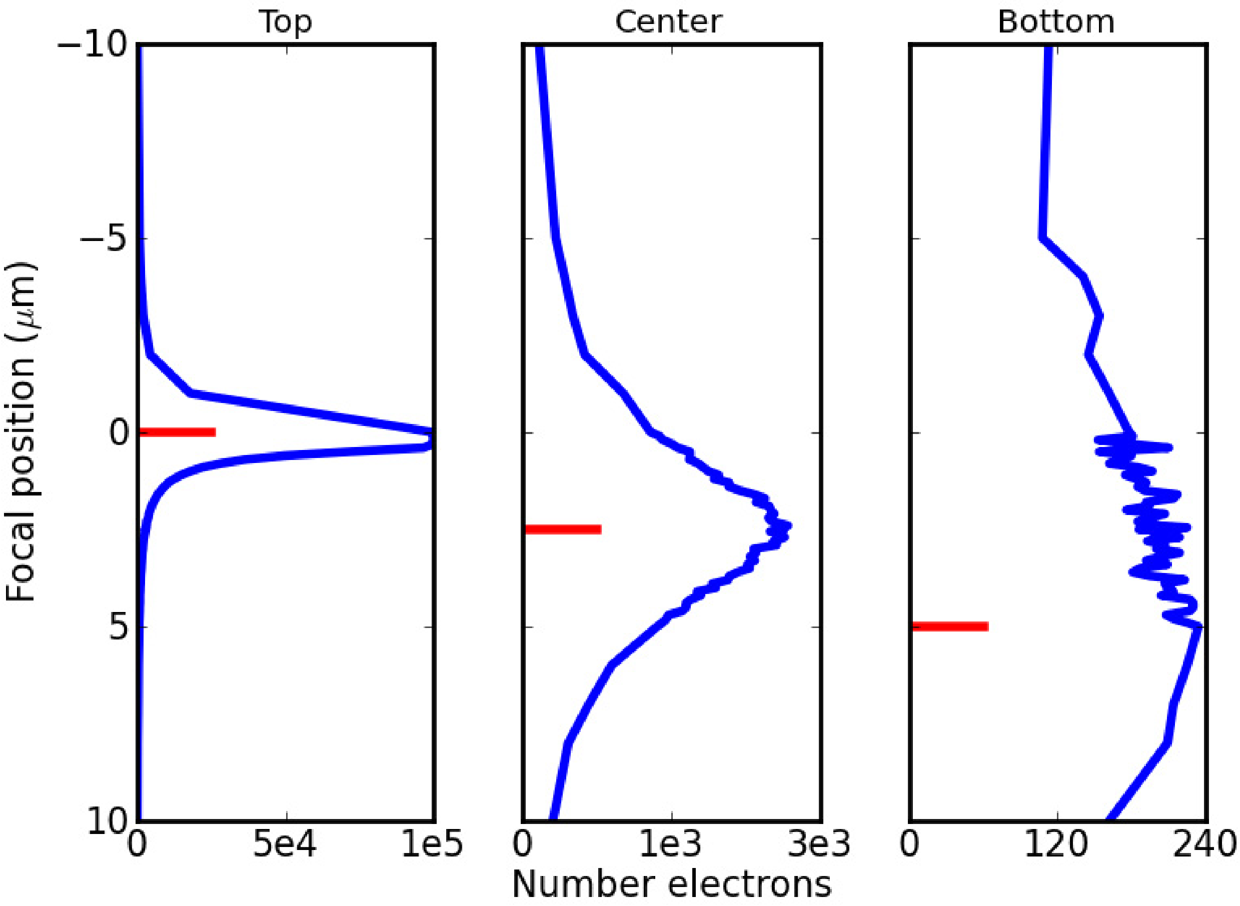

FIG. 1. Monte Carlo simulation of vertical focus line scans in a 5 μm-thick carbon sample. The number of electrons passing through a small area and located at top (0 μm), center (2.5 μm), and bottom (5 μm) of the sample was calculated for each vertical focus position. The red bar indicates the vertical location of the small area. For all line scans, the same area size was used: 900 nm$^2$ (30 nm x 30 nm). 100,000 electrons were simulated. The electron beam semi-angle was 41 mrad and the incident energy was 200 keV.