564
doi:10.1017/S1431927617003506
Microsc. Microanal. 23 (Suppl 1), 2017

Improved Image Quality in SEM Imaging of Thin Tissue Sections

W. Zuidema[1], J.P. Hoogenboom[1] and P. Kruit[1]

[1.] Delft University of Technology, Department of Imaging Physics, Delft, the Netherlands.

To achieve faster imaging in scanning electron microscopy, novel imaging methods are needed, such as multi-beam scanning electron microscopes[1, 2]. Our scanning electron microscope has 196 beams that scan the sample simultaneously at the same rate as a regular SEM[1]. The secondary electron signals of these multiple beams can be separately detected, but, e.g., for biological imaging, back-scatter detection might be preferred. Here, we present an alternative way of obtaining signals from a multi-beam SEM and show it can deliver similar or even better quality images compared to back-scatter detection.

The imaging method presented here is used to image thin tissue sections, which are regularly imaged in backscatter mode in an SEM. Backscatter detection, however, is very difficult in multi-beam mode. In our method, the tissue section (thickness <200 nm) is placed directly upon a scintillator screen with a conductive coating. Cathodoluminescence of the scintillator, created by the electron beam, is then collected by a high NA objective lens beneath the scintillator. This light is then focused on a detector outside of the vacuum chamber. Here we investigate image quality for this way of imaging both by means of an analytical model and by experiment.

In the analytical model we check the ability to see a slight change in osmium concentration of both our transmission imaging and backscatter imaging. As a figure of merit, we use the contrast-to-noise ratio (CNR), which shows whether the contrast generated in the imaging method is large enough to overcome the noise on the signal, which is only shot noise for a high DQE detection system.

Using the analytical model, we calculated the CNR of our imaging method. As tissue section an EPON-osmium mixture was assumed with a step difference in the osmium concentration from 2 wt% to 6 wt%. The result of the model for transmission imaging is plotted in figure 1(left). The ratio between the transmission imaging CNR and the backscatter imaging CNR is shown in figure 1(right). As one goes to lower landing energies, the CNR increases markedly for all section thicknesses. This increase is mostly due to increased backscatter coefficients at these low energies and the added contrast from the energy loss in the section. Added to this, the relative noise at these energies stays fairly low, due to fact that the total amount of electrons transmitted is much bigger than the amount of backscattered electrons. These effects immediately show up in the comparison between the two imaging methods, shown in figure 1. Clearly the transmission imaging we devised can be similar to or even superior to backscatter imaging.

A qualitative comparison between the contrast in backscatter mode and transmission mode imaging is shown in figure 2. These images are taken in a single beam SEM with a standard commercial backscatter detector. It is clear that the image contrast in both imaging modes is very similar, qualitatively confirming our analytical result.

We have shown that the image quality of the transmission imaging method presented here can compete with the image quality of backscatter imaging. This leaves us with a very good imaging strategy for imaging thin tissue sections in our multi-beam SEM.

References:

[1] Ren, Y., & Kruit, P. Journal of Vacuum Science and Technology B, **34**, (2016)
[2] Eberle, A. L. *et al*, Journal of Microscopy, **259(2)**, (2015), p.114.
[3] Timischl, F. (2015). SCANNING, **37**(2014), p.54.

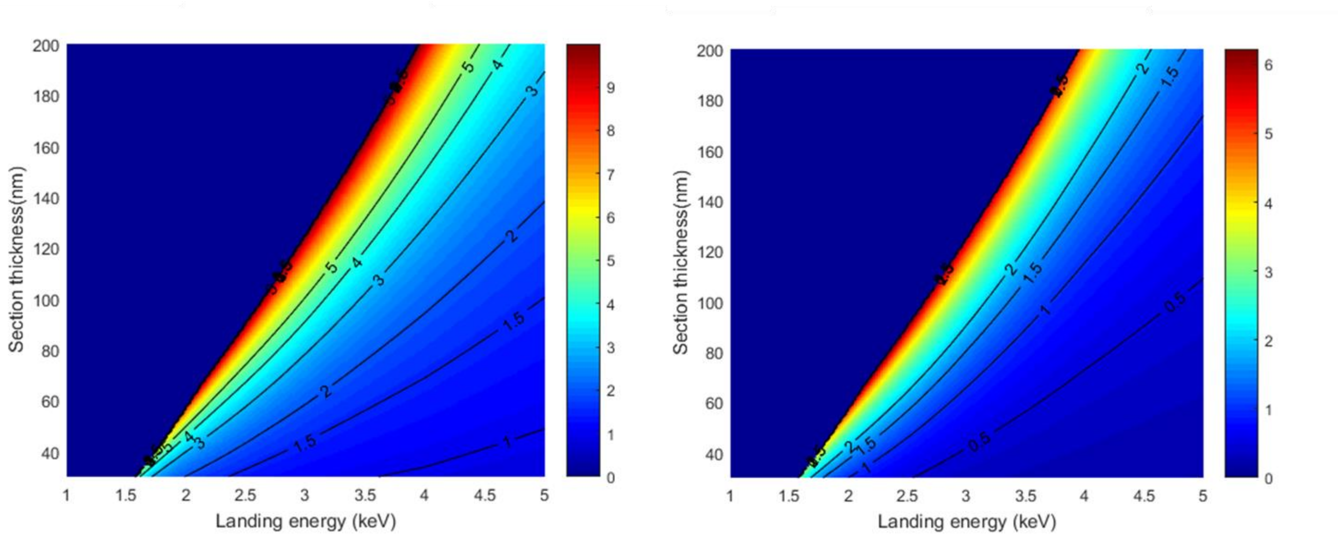

Figure 1. (left) CNR of transmission imaging as a function of landing energy and section thickness (right) Ratio between the CNR of the transmission imaging and backscatter imaging.

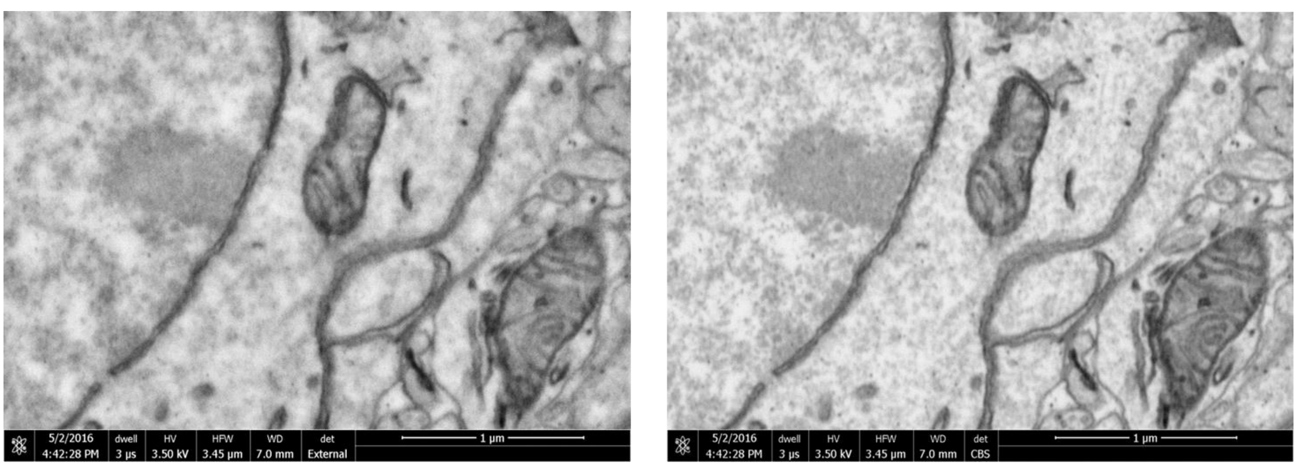

Figure 2. Image of Unaryl acetate stained and Osmium tetroxide fixated rat-brain tissue in both (left) transmission mode imaging and (right) backscatter imaging. Samples courtesy of Briggman (NIDS, US)

https://doi.org/10.1017/S1431927617003506 Published online by Cambridge University Press