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Optical depth sectioning in the aberration-corrected scanning transmission and scanning confocal electron microscope

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Abstract. The use of spherical aberration correctors in the scanning transmission electron microscope (STEM) has the effect of reducing the depth of field of the microscope, making three-dimensional imaging of a specimen possible by optical sectioning. Depth resolution can be improved further by placing aberration correctors and lenses pre and post specimen to achieve an imaging mode known as scanning confocal electron microscopy (SCEM). We present the calculated incoherent point spread functions (PSF) and optical transfer functions (OTF) of a STEM and SCEM. The OTF for a STEM is shown to have a missing cone region which results in severe blurring along the optic axis, which can be especially severe for extended objects. We also present strategies for reconstruction of experimental data, such as three-dimensional deconvolution of the point spread function.

1. Introduction
Transmission electron microscopes (TEMs) have proven to be invaluable for determining the local structure of materials. However, for many years the resolution of the TEM was limited by spherical aberration in the electron optics. The use of a small objective aperture was the only effective way to block out the strongly aberrated beams, but reduced the numerical aperture of the objective lens. Recently, with the development of spherical aberration correctors for the transmission and scanning transmission electron microscopes (STEMs) resolution has been greatly improved. In the case of STEM resolution is now below 0.1 nm.

While the lateral resolution is proportional to the aperture size, we find that the depth of field of the microscope decreases as a function of the square of the aperture size [1]. Three-dimensional (3D) imaging of a sample is now possible by recording a focal series in a similar way to scanning confocal optical microscopy using a technique known as optical depth sectioning [2].

In uncorrected electron microscopes the depth of field extends over a much larger distance than the thickness of the sample. The images that are acquired can then be treated as two-dimensional projections of a three-dimensional object and any information about depth is lost. To reconstruct the structure of a three-dimensional object a series of projections at many different tilt angles is required, an approach known as tomography. A volume resolution of approximately 1.0 nm³ can be achieved using this method [3].

While optical depth sectioning is not yet at the stage where it can compete with tomography on resolution in the depth direction, its promise lies in its speed. As the probe can be focused at the specific depths of interest, less images are required for a reconstruction. Image acquisition takes
minutes as opposed to hours. Future developments in the correction of higher order aberrations would also allow the depth resolution of optical sectioning to approach 1.0 nm.

If a second lens is situated post specimen in the column then a new imaging mode known as scanning confocal electron microscopy (SCEM) can be used [4]. In confocal mode electrons scattered in the vicinity of the confocal plane are focussed on an aperture placed before the detector. The contribution from other planes to the recorded image is greatly reduced as these electrons are not focussed on the aperture and are rejected. SCEM should in theory offer better depth resolution than STEM especially when both the lenses have been corrected for spherical aberration [5].

Confocal microscopy is now ubiquitous in light optics and 3D imaging of specimens by optical depth sectioning is common. However there is significant activity in light optics involving 3D imaging with a single lens and then deconvolving the blurring caused by the point spread function [6]. At present we are working on implementing SCEM experimentally, and we have attempted optical depth sectioning of extended objects with a STEM and the use of deconvolution to reduce the blurring from the point spread function of the electron microscope in a similar way to light optics.

2. STEM and SCEM comparison
For annular dark-field STEM imaging, and images formed with inelastically scattered electrons using both STEM and SCEM, the imaging mechanism can be described as being predominantly incoherent. Point spread functions for both incoherent STEM and SCEM and their corresponding optical transfer functions were calculated (see reference [5] for details on calculation). The parameters for each calculation were for the double aberration-corrected JEOL 2200MCO at Oxford University with an accelerating voltage of 200 kV and a semi-angle of convergence of 30 mrad. For the confocal mode a point pinhole aperture was used. Also presented here are the optical transfer functions of both imaging modes for the same conditions. The optical transfer function is the 3D Fourier transform of the relevant point spread function and Fig. 1 shows a plane within the transfer function of each imaging mode with axes along the reciprocal radial and reciprocal depth directions.

With SCEM mode the depth resolution is improved over SCEM. More importantly though, the transfer function for STEM has a missing cone region where there is no transfer of spatial frequencies, whereas in SCEM there are no such missing regions (see Fig 1). As the Fourier transform of a laterally extended object will have a lot of components at low radial spatial frequencies, the missing cone becomes important in this case. A simple explanation of this is to note that we are determining depth by observing how objects go out focus in the images, which is not easily observed for extended objects. Following the lead of light optics we have attempted to use deconvolution methods to reduce the blurring caused by the 3D point spread function.

3. Deconvolution algorithm
To collect the focal series the probe is focussed at different depths in the sample and raster scanned to collect the HAADF image. As the depth of field of the microscope is reduced with a corrector only a small volume of the sample will be in focus in each image. HAADF images are an example of incoherent imaging with the recorded intensity being a convolution between the intensity of the complex probe function \( P \) and the object function \( o \).

\[
I = |P(r)|^2 \otimes o(r) = p(r) \otimes o(r)
\]  

where \( r \) is the three-dimensional position, \( p \) is the point spread function and \( \otimes \) denotes a convolution in three dimensions. The intensity of the probe function can either be calculated
for each image plane or measured experimentally by imaging a small particle over a range of focus values.

It should now be possible to perform a three-dimensional deconvolution on equation 1 to recover the object function. We used the Richardson-Lucy (RL) algorithm (see equation 2) [7; 8] which is a popular algorithm that estimates the maximum likelihood solution of the object function. This particular algorithm has the benefit of being convergent and also enforcing the non-negativity constraint when provided with an initial estimate and point spread function that are themselves all positive [9]; therefore clipping of the data is not required after each iteration.

\[
O_{k+1}(r) = O_k(r) \left( \frac{i(r)}{p(r) \otimes O_k(r)} \right) \otimes p(-r) \tag{2}
\]

For each iteration, equation 2 was evaluated to generate an estimate for the object. Here \( r \) is the three-dimensional position, \( o_k \) is the \( k \)th estimate of the object function, \( p \) is the point spread function and \( i \) is the original dataset.

We present here results from a three-dimensional deconvolution of a synthetic dataset that consisted of a sphere approximately 3.0 nm in diameter blurred by a STEM probe using the same parameters for the JEOL 2200MCO to calculate the point spread function. There was no noise or background intensity. The deconvolution was performed in IDL on a Dell PC with a 2.8 GHz Intel Pentium Processor with 0.99 GB of RAM. The whole process took approximately 1.5 hours and ran for 20 iterations.

The important result of the RL algorithm is that the deconvolved volume still has a much greater amount of blurring in the \( z \) direction than you would expect from the PSF. This is due to the OTF of the STEM having a missing cone region. Maximum-likelihood methods cannot
reconstruct information in the missing cone region and no improvement in depth resolution is possible using these techniques. This problem does not exist when using confocal mode and deconvolution would be successful there.

4. Conclusion
For incoherent imaging it has been shown that the depth resolution for a SCEM is an improvement on a STEM which should allow for much better optical depth sectioning performance with the SCEM. When in confocal mode the pinhole aperture blocks electrons that have been scattered from outside the confocal plane greatly reducing the out of focus contribution to the recorded image.

As can be seen from Fig. 2 the Richardson-Lucy algorithm is not able to deconvolve the blurring along the optic axis for extended objects due to the missing cone region in the optical transfer function. This is a key difference between STEM and SCEM and indicates the importance of supplying prior information when using deconvolution for a single lens system.

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References