New Approaches to Tilt-Corrected Bright-Field Scanning Transmission Electron Miscroscopy

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(Dated: August 12, 2024)

Abstract

In structural biology, achieving high-resolution imaging of thick specimens poses a significant challenge due to inelastic scattering, which causes a substantial loss of energy in transmitted electrons and prevents proper focusing. Recent advances in pixelated detector technology have led to the development of a more dose-efficient and unfiltered imaging technique known as tilt-corrected bright-field scanning transmission electron microscopy (tcBF-STEM). In this study, we explore the capabilities of tcBF-STEM through the collection of a 4D-STEM dataset. Despite its advantages, tcBF-STEM does not correct for the aberrations inherent in every image obtained from the tilt series. We hypothesize that addressing these aberrations will lead to improved resolution and the ability to acquire meaningful depth information at higher resolutions. Our results demonstrate that our aberration-corrected tcBF-STEM (actcBF-STEM) method offers superior resolving capability and enhanced depth resolution. This study highlights the potential of actcBF-STEM as a powerful tool for high-resolution imaging of thick biological specimens and establishes precident for further research into its experimental depth resolution capabilities.

Introduction

Within the field of structural biology, a common problem is the ability to obtain high resolution images of thick specimens. Due to inelastic scattering, a large portion of transmitted electrons lose energy, making it difficult to achieve proper focus. The current method for solving this issue is to filter out any inelastic scattering, as done in energy-filtered transmission electron microscopy (EFTEM). However, this method reduces the collected signal and dose-efficiency compared to an ideal microscope. Recent advancements in pixelated detector technology have allowed researchers to explore a new dose-efficient and unfiltered imaging method known as tilt-corrected bright-field scanning transmission electron microscopy (tcBF-STEM). This method has shown enhanced contrast and a 3-5x improvement in collection efficiency for 2D images of bacterial cells compared to EFTEM for thicknesses greater than 500 nm[1].

tcBF-STEM starts with the collection of a 4D-STEM dataset, in which an electron beam gets focused to a small point on the sample plane and a 2D diffraction pattern is collected at each probe position across a 2D grid across the material (Fig. 1).

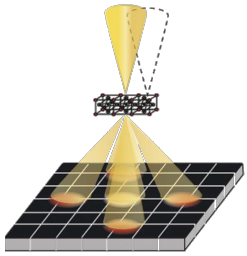

Figure 1: In 4D-STEM, and electron beam gets focused to a small probe by a magnetic lens and then rastered across the sample along a 2D grid. At each probe position, a 2D diffraction pattern is obtained.

4D-STEM produces a wealth of information, which can be extracted post-processing through the use of a virtual detector. tcBF-STEM makes use of a virtual bright-field detector, which creates a virtual image using both the unscattered electrons, as well as the electrons scattered from the lighter elements in the sample. By the theory of reciprocity, the image obtained from the central pixel in the bright-field disk is equivalent to a conventional transmission electron microscopy (TEM) image and the images obtained from any off-axis detector pixels are equivalent to TEM images formed with tilted illumination[2]. As shown in Fig. 2, the virtual images obtained from these off-axis detector pixels are shifted relative to the image obtained from the optic axis pixel.

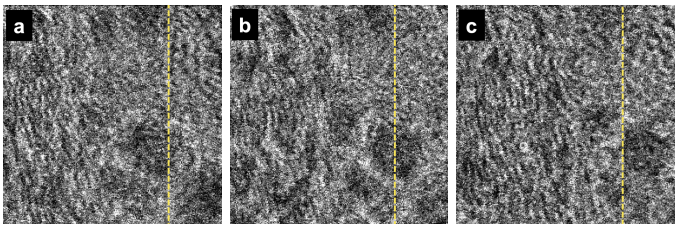

Figure 2: The virtual images shown in (a) and (c) are obtained from the pixels off the optic axis in the virtual bright-field disk, whereas the image shown in (b) is obtained from the detector pixel on the optic axis.

tcBF-STEM operates by correcting these shifted images through cross-correlation with the virtual image from the central pixel, and then combining each shift-corrected image

to obtain the final tcBF-STEM image.

In its current adaptation, tcBF-STEM does not correct for the aberrations present in every image obtained from the tilt series. We believe that correcting for these aberrations will result in greater overall resolution, as well as the ability to obtain useful depth information at higher resolutions. We refer to this method as aberration-corrected tcBF-STEM (actcBF-STEM), and test our hypotheses in both simulation and experiment.

Methods

At the heart of any contrast transfer function (CTF) correction is the aberration function. Aberrations occur when electron (or light) rays spread out over some region of space rather than focused to a point, which can cause images formed by a lens to appear blurred or distorted.

We used Eq. (1) to characterize many different aberrations:[3]

$$\chi(\omega, E) = \mathrm{Re}\left\{\frac{1}{2}\bar{\omega}^2 A_1 + \frac{1}{2}\omega\bar{\omega}C_1 + \frac{1}{3}\bar{\omega}^3 A_2 + ...\right\}. \quad (1)$$

Here, $A_1, C_1, A_2, ...$ represent different aberrations found in reference [3]. Plugging this into Eq. (2), we obtained the phase contrast transfer function (PCTF)[4], which we then used to compensate for any distortions introduced by aberrations.

$$\mathrm{PCTF}(\omega) = \frac{i}{\Omega_0}A(\Theta)\Big\{A(\omega-\Theta)e^{-i[\chi(\omega-\Theta)-\chi(\Theta)]} - A(\omega+\Theta)e^{i[\chi(\omega+\Theta)-\chi(\Theta)]}\Big\}. \quad (2)$$

Here, $A(\Theta)$ is the aperture function, ω and Θ are momentum vectors projected onto the detector in the diffraction plane and normalized as scattering angles, and $\Omega_0 \approx \pi\alpha^2$ is the solid angle subtended by the objective aperture[1].

We collected 4D-STEM data on platinum nanoparticles grown on carbon flakes using the Thermo Fisher Titan 300 S/TEM (60-300 kV) equipped with an electron microscopy pixel array detector (EMPAD).

To compare depth resolution, we conducted a qualitative comparison of tcBF-STEM and actcBF-STEM by examining the cross section of a simulation of a single atom floating in space after performing a depth section. Depth sectioning involves capturing a series of images, each with a different plane in focus, allowing one to obtain detailed information from the many different layers of a sample.

Results

Experiment

Figure 3 is a comparison of the final tcBF-STEM (left) and actcBF-STEM (right) images. These images are obtained from the same 4D-STEM dataset. A detailed description of how this 4D-STEM dataset was acquired can be found in the introduction section.

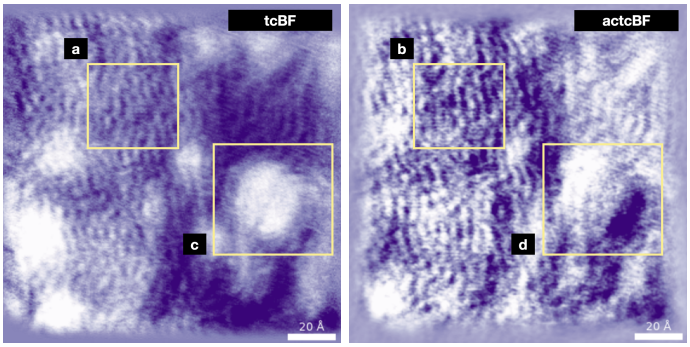

Figure 3: (a) and (b) directly compare the resolution of tcBF-STEM and actcBF-STEM on the carbon flakes that the platinum nanoparticles were grown on, which can be seen in (c) and (d).

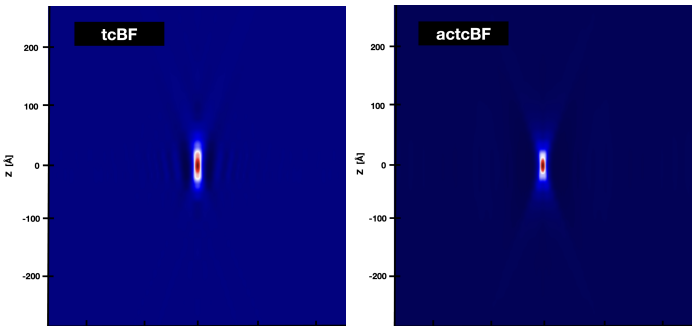

Figure 4: A cross-sectional slice through the center of the x-axis from a stack of 33 images obtained from a depth section of a simulation of a single atom floating in space.

Simulation

Figure 4 displays the tcBF-STEM and actcBF-STEM depth resolution comparison via depth sectioning of a simulation of a single atom floating in space. The figure shows a cross-sectional slice through the center of the x-axis from a stack of 33 images obtained via depth sectioning.

Discussion

Experiment

In Fig. 3, actcBF-STEM displayed greater resolution, allowing one to view the flakes of carbon—which one can see as the vertical lines throughout the image—and the platinum nanoparticles—the bigger structure highlighted in (c) and (d)—more clearly. actcBF-STEM also allows for a better qualitative analysis of the surface texture of the sample. In the actcBF-STEM image, one can see more clearly the roughness of the material, which appears smoother in the tcBF-STEM image.

However, there is what appears to be missing information in the final actcBF-STEM image. For example, some of the platinum nanoparticles—the bigger white regions—that appear in the final tcBF-STEM do not appear in the final actcBF-STEM image. There are some possible reasons for this: the CTF that we use for actcBF-STEM is not as accurate as it could be, the actcBF-STEM CTF correction is

not as effective, there could be a slight tilt (or shift) in the final actcBF-STEM image causing these particles to be out of frame.

There are possible solutions for these problems in the final actcBF-STEM image. For example, taking better 4D-STEM data where the predominant aberration is only defocus will help tremendously. Additionally, a more robust CTF correction, such as a Richardson-Lucy deconvolution, would obtain a better image.

Simulation

In Fig. 4, the tcBF-STEM cross section shows strange oscillations which do not represent what one would expect to see from a single atom floating in space. In the actcBF-STEM image, we managed to greatly diminish the presence of these oscillations, showing greater depth resolution than tcBF-STEM. We were not yet able to qualitatively compare the two methods in experiment due to time constraints.

Conclusion

In summary, researchers interested in high resolution imaging of thick samples ($\sim 500 - 800$nm) are presented with challenges obtaining these images due to inelastic scattering. The current imaging method they are exploring, tcBF-STEM, has been shown to enhance contrast and possess a higher collection efficiency than other imaging methods used. However, there is room for improvement.

We set out to improve the overall resolution and obtain better depth information by correcting for the aberrations present in every virtual image obtained from the tilt series, something that the current tcBF-STEM method does not do.

In doing so, our method actcBF-STEM showed greater resolving capability in both the ability to resolve the carbon flakes used to grow platinum nanoparticles, as well as the platinum nanoparticles themselves (Fig. 3).

Additionally, to compare the depth resolution of the two methods, we performed a depth section on a simulation of a single atom floating in space. When viewing the cross section of a stack of images obtained from depth sectioning, actcBF-STEM was able to show greater depth resolution than tcBF-STEM, diminishing the presence of strange oscillations present in the tcBF-STEM image.

Further work needs to be done to compare the depth resolution of the two methods experimentally.

Acknowledgements

I would like to thank my mentor, Steve Zeltmann, and my PI, David Muller, for their guidance and support. Thank you to the entire Muller group for welcoming me with open arms and extending your generosity. I always felt welcomed. Thank you Jim Overhiser and Brenda Fisher for all that you do. Thank you to Schuyler Shi for growing the platinum nanoparticles. Lastly, thank you to the National Science Foundation for funding this project under NSF Cooperative Agreement No. DMR-2039380.

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