2019

Point Spread Function Determination in the Scanning Electron Microscope and its Application in Restoring Images Acquired at Low Voltage

Mandy C. Nevins
mcn6405@rit.edu

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Point Spread Function Determination in the Scanning Electron Microscope and its Application in Restoring Images Acquired at Low Voltage

by

Mandy C. Nevins

B.S. University of Wisconsin-Eau Claire, 2014

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Chester F. Carlson Center for Imaging Science

College of Science

Rochester Institute of Technology

2019

Signature of the Author ________________________________________________

Accepted by ________________________________________________________

Coordinator, Ph.D. Degree Program                                        Date
The Ph.D. Degree Dissertation of Mandy C. Nevins has been examined and approved by the dissertation committee as satisfactory for the dissertation required for the Ph.D. degree in Imaging Science.

Prof. Richard Hailstone, Dissertation Advisor

Dr. Lea Michel, External Chair

Dr. Eric Lifshin

Dr. Nathan Cahill

Date
To my darling husband
and to my loving parents
Point Spread Function Determination in the Scanning Electron Microscope and its Application in Restoring Images Acquired at Low Voltage

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Submitted to the
Chester F. Carlson Center for Imaging Science
in partial fulfillment of the requirements for the Doctor of Philosophy Degree at the Rochester Institute of Technology

Abstract

Electron microscopes have the capability to examine specimens at much finer detail than a traditional light microscope. Higher electron beam voltages correspond to higher resolution, but some specimens are sensitive to beam damage and charging at high voltages. In the scanning electron microscope (SEM), low voltage imaging is beneficial for viewing biological, electronic, and other beam-sensitive specimens. However, image quality suffers at low voltage from reduced resolution, lower signal-to-noise, and increased visibility of beam-induced contamination. Most solutions for improving low voltage SEM imaging require specialty hardware, which can be costly or system-specific. Point spread function (PSF) deconvolution for image restoration could provide a software solution that is cost-effective and microscope-independent with the ability to produce image quality improvements comparable to specialty hardware systems. Measuring the PSF (i.e., electron probe) of the SEM has been a notoriously difficult task until now. The goals of this work are to characterize the capabilities and limitations of a novel SEM PSF determination method that uses nanoparticle dispersions to obtain a two-dimensional measurement of the PSF, and to evaluate the utility of the measured PSF for restoration of low voltage SEM images. The presented results are meant to inform prospective and existing users of this technique about its fundamental theory, best operating practices, the expected behavior of output PSFs and image restorations, and factors to be aware of during interpretation of results.
Acknowledgements

This has really been quite the journey! I am so thankful to have been graced with the support, wisdom, and love of so many people along this challenging and meaningful path.

Firstly, I’d like to thank Prof. Richard Hailstone for introducing me to the tiny worlds within electron microscopy and advising me in my doctoral research. His quiet enthusiasm for science on the nanoscale inspired me, and his guidance has helped me develop into a scientist capable of performing independent research, which was the whole idea! I am thankful for the example he set as an approachable lab manager who is always open to assist students and microscope-users in solving their problems. Thank you for helping me stay determined, even when the microscopes and software decided to be less than user-friendly.

I would like to extend my gratitude to Prof. Eric Lifshin for his mentorship and collaboration in my thesis work. Thank you for bringing all your know-how and questions about the SEM point spread function to the Rochester Institute of Technology (RIT). Additional thanks to Matthew Zotta for his work in developing the Aura Workstation on which much of my research was performed, Kathy Quoi for her insight regarding SEM simulations, and Jeffrey Moskin for providing the funding necessary to launch the Aura Workstation.

While in the world of nano, I would also like to thank my friend and lab mate, Najat Alharbi. Our lab may have been small, but it is just like the microscopic subjects we studied…. small but scientifically mighty.

I am appreciative of my entire thesis committee, including Dr. Nathan Cahill, and Dr. Lea Michel. Thank you for supervising and enriching my work! I am grateful to Dr. Cahill for sharing his mathematical expertise and bringing attention to important aspects of this work which may not have been addressed otherwise. Thank you to Dr. Michel for contributing to this discussion and for being an excellent role model of a woman in science.

There are many people within the Center for Imaging Science that have helped me grow in knowledge and competence. I would like to thank the entire CIS faculty and staff for teaching me the fundamentals of imaging science and then assisting me as I entered into my own research. This is an academically vibrant department, and I’m happy I could learn from such an assembly of experiences and viewpoints. I also enjoyed the comradery of my fellow graduate students and
friends at RIT, including Grant Anderson, Emily Berkson, Lucy Chu, Ryan Ford, Sanghui Han, Kushal Kafle, Michal Kucer, Elisabeth McClure, Elizabeth Moore, Emily Myers, Lauren Taylor, Anton Travinsky, Jared Van Cor, Oesa Weaver, and Jacob Wirth. Thank you for sharing your encouragement, problem-solving, and love of science.

Rochester would not have felt like home with the friendship and care of Bridget Connolly, Matthew Rasmussen, Vera Delfavero, Anne Namocatcat, John McDermott, and the late Betsy McDermott. Thank you all. So much.

Before Rochester, I had the opportunity to study with the amazing physics folks at the University of Wisconsin-Eau Claire (UWEC). A big thank you to Dr. Erik Hendrickson for helping me through my foundational physics courses with countless office hours and for encouraging me to apply for a Research Experience for Undergraduates (my first experience with true scientific research, which inspired me to pursue my PhD)! Another great deal of gratitude is owed to Dr. Lauren Likkel for fueling my excitement for science and for improving my confidence as a speaker of science through many fun, cheesy planetarium presentations. Additional thanks to Dr. Nathan Miller, Dr. Kim Pierson, Dr. Matt Evans, Dr. Scott Whitfield, and Dr. George Stecher for sharing their knowledge and creating a welcoming environment for every student. A special note of thanks to Dr. Tom Lockhart for his delightful insight that a PhD is truly a test of stubbornness, as you have to try and try again when all your experiments break and break again.

Sincere thanks to all those friends who helped me learn about the brilliant, challenging subject that is physics, including the late Roxanne Accola, Shalaine Buehl, Ryan McCarty, Jake Pederson, Blake Smith, Sean O’Connell, James Truchinski, and Michael Yohn. Thank you also to my friends in the languages, businesses, and other sciences, for sharing your diverse knowledge and support, including Becca Anderson, Josh Bauer, Colleen Carhuff, Jon Ecker, John Fisher, Katie Fisher, Robert Fisher, Oscar Lopez-Martinez, Steve Searing, and Mitch Wood.

Before Eau Claire, my education began in the small town of Spencer, WI, where, “Rockets start their engines.” Thank you to all who helped get my engine going! Much appreciation to Randy and Diane Veale, for encouraging and enriching the creativity that continues to drive me in my problem solving. A great amount of thanks to Jackie Pickett for
teaching me how outline, organize, and write about research, setting me on the path to tackle a thesis! Sincere thanks to Doug Benton for sharing his scientific know-how, his encouragement, and for preparing me to succeed. Thank you to all of those who shared their friendship throughout that time, especially Ben Burnett for his exuberant confidence and sense of self which inspired me to embrace my inner scientist.

I give immense gratitude to my oldest friend, Tanika Reckner. She has seen me and supported me through it all, always there to lend a hand or an ear (and always willing to give more of herself than what is asked of her). Thank you for always being you, no matter what.

I have so much appreciation and admiration for my parents, Ron and Lori Neumann. They helped me grow into the person I was meant to be, and have been infinitely supportive on this journey. Thank you for showing me the value of hard work, and also the significance of bringing kindness and compassion into whatever I do. I am so, so lucky to have such a wonderful family supporting me. Thank you to my brothers and their wives, Michael and Christina Mroz, and Christian and Courtney Neumann, for living and working with love and for modeling joyous strength in family and faith. Thank you to my nieces and nephew, Hannah, Kaleb, Elsa, and Elianna Mroz for reminding me about all the exciting wonders and possibilities in this world. Thank you to all of my family, and please know that your support has meant so much to me. I would also like to give a shout-out to the crazy smart and generally crazy Nevins bunch who welcomed me into their family and have always been willing to share their stories and “gifts”. A special note of thanks to Dr. Amanda Nevins for providing much-appreciated wisdom about navigating graduate school and PhD research.

Finally, I give my deepest love and thanks to my husband, Thomas Nevins. I truly would not have made it through to the finish without his encouragement and constant support. More importantly, without him I would have never even realized I had enough ability and worth to finish, nonetheless go on to achieve it. Thank you, Thomas, for your strength in mind and character, your unflappable sense of responsibility, your aspiration for greatness, and your profound insightfulness. You transformed this journey into an epic. Thank you for helping me build the confidence and strength of will I needed to shine.
Author Publications

* indicates the authors contributed equally on this publication.
† indicates that a modified version of this publication is included in this dissertation.

Refereed Publications


Submitted/In-Review

† Nevins MC, Quoi K, Hailstone RK and Lifshin E (In Review) Exploring the parameter space of point spread function determination for the scanning electron microscope – Part I: Effect on the point spread function. In review at *Microscopy and Microanalysis*.

† Nevins MC, Hailstone RK and Lifshin E (In Review) Exploring the parameter space of point spread function determination for the scanning electron microscope – Part II: Effect on image restoration quality. In review at *Microscopy and Microanalysis*.

Conference Papers


• Nevins MC, Hailstone RK, Zotta MD and Lifshin E (2017) Viability of point spread function deconvolution for SEM. *Microscopy and Microanalysis* 23(S1), 126-127.

Magazine Articles

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1 Introduction

1.1 Context

A point spread function (PSF) is the impulse response of an imaging system. An image can be described as the convolution of the PSF and the scene being imaged. Knowledge of the PSF can be utilized for system characterization and to restore image quality. Addressing the resolution limits in the scanning electron microscope (SEM) requires the ability to characterize the microscope’s focused electron beam \( i.e \), PSF. PSF deconvolution could provide a versatile, low-cost solution for improving SEM image quality, particularly at low voltages. Measuring the PSF of an SEM is difficult because an image is gathered pixel-by-pixel, instead of all at once, by rastering a focused beam of electrons across a sample-of-interest, and collecting the signal generated by electron-matter interactions at each pixel.

1.2 Objectives

A method for measuring the PSF of an SEM using nanoparticle dispersions has been introduced in recent years. Further study was necessary to understand the viability of this method and its capabilities for image restoration purposes. The research presented here aims to characterize the effectiveness and limitations of the PSF measurement method and the utility of the measured PSF for image restoration of low voltage SEM images. This includes examining the robustness of determining the PSF using a dispersion of nanoparticles, investigating how various parameters within this method affect the determined PSF, and evaluating the image restoration capabilities of PSF deconvolution in low voltage SEM images.
1.3 Approach

Images of calibration samples (e.g., nanoparticle dispersions) and samples-of-interest are captured on a TESCAN MIRA3 field emission scanning electron microscope (FESEM). The PSF can be determined by comparing the average observed particle with a simulated reference particle. The PSF can be studied on its own or used to perform PSF deconvolution on any image taken with the same beam shape as the calibration image. The PSF determination method is evaluated under a range of SEM operating conditions to establish the method’s robustness and practicality. Applications of this PSF determination method are presented. The parameter space of the PSF determination method is explored in terms of the effects on the resulting PSF and image restorations (PSF deconvolution). The Aura Workstation was utilized for PSF determination and deconvolution.

1.4 Contributions

The PSF determination method presented here is the first to provide a full visualization and measurement of the SEM electron probe. This enables new research into SEM probe shape and behavior which was previously inaccessible, with applications in microscope characterization, maintenance, and image restoration.

Low voltage SEM imaging is beneficial for viewing biological, electronic, and other beam-sensitive specimens. Compared to high voltage imaging, beam damage and charging are reduced, and surface topography is greatly accentuated. However, image quality suffers at low voltage from reduced resolution, lower signal-to-noise, and increased visibility of beam-induced contamination. Most solutions for improving low voltage SEM imaging require specialty hardware, which can be costly and may not be available for certain systems. PSF deconvolution for image restoration provides a software solution which is comparable to specialty hardware systems and more versatile in application.
1.5 Thesis Layout

Chapter 1 – Introduction provides an overview of the research presented in this thesis. Chapter 2 – Background describes fundamental concepts to assist in the understanding of this work, including SEM imaging and challenges, the PSF, and a brief introduction to PSF deconvolution for image restoration. Chapter 3 – The Determination and Application of the Point Spread Function in the Scanning Electron Microscope lays the foundation of the SEM PSF determination method and gives a few examples of its application. Chapter 4 – Parameter Space Exploration: Effect on the Point Spread Function delves deeper into how data collection and PSF calculation parameters affect the PSF shape and accuracy. Chapter 5 – Parameter Space Exploration: Effect on Image Restoration Quality extends the work of Chapter 4 by investigating the effect of PSF calculation and image restoration parameters on the quality of images restored through PSF deconvolution. Appendix A provides the results of preliminary investigations of this PSF determination and deconvolution method. Appendices B, C, and D provide supplementary information for Chapters 3, 4, and 5, respectively. Appendix E contains additional examples of image restoration from the PSF deconvolution method.
2 Background

2.1 Image Formation in the SEM

Electron-optical systems generate images using electron wavelengths which are orders-of-magnitude smaller than wavelengths of visible light. This means the electron microscope has the capability to examine materials at a level of detail much finer than is capable with a traditional light microscope. Resolution on the order of nanometers is achievable by high-end modern SEM technologies, allowing scientists to examine specimens with nanoscale detail. But, achieving this resolution is dependent on many factors, importantly the electron probe size and current, energy of beam electrons, and material composition.

In an SEM (Figure 2-1), the image is generated pixel-by-pixel by focusing an electron beam onto a bulk sample and then rastering the beam over an area (Figure 2-2). To perform imaging with electrons, the SEM gun, column, and chamber must be under high vacuum. This ensures that the electrons are not deflected by air molecules during focusing, imaging, and signal collection. A voltage, usually on the order of kilovolts, is applied to a metal filament or crystal (the electron gun) which emits electrons. The emitted electrons travel through magnetic lenses which focus the electrons into a small electron probe.

The probe interacts with one point on the sample-of-interest, and this generates signal electrons to be collected by a detector. The collected signal from the “sample pixel” makes up one “image pixel” on the computer or television monitor. The intensity of an image pixel is determined by the amount of the measured signal at the sample pixel. Scanning coils control the electron probe location on the sample. When one image pixel has been collected, the beam is moved by a certain step size to the next pixel and so on down a single row. When that row is complete, the beam returns to the beginning of the next adjacent row and scans in the same manner. This continues
until all pixels have been collected, meaning the entire area to be imaged has been scanned and we have one complete image frame on our monitor.

2.1.1 The Electron Probe

The electron beam can be defined by four main parameters where it impinges the sample-of-interest (see Figure 2-3 for visualization). The accelerating voltage, $V_o$, is the voltage applied to the electron gun. (The energy of the electrons, $E_o$, in the beam is then equal to $eV_o$.) The electron probe diameter, $d_p$, is simply the diameter of the electron beam where it makes contact with the sample. The electron probe current, $i_p$, is the number of electrons impinging the sample per second. The electron probe convergence angle, $\alpha$, is the half-angle of the cone of electrons which make up
the probe at the sample. These factors play an important role in the quality and resolution of the eventual image.

The magnification of an SEM is determined by the ratio of the distance scanned on the display to the distance scanned on the sample (e.g., scanning a smaller area results in a larger magnification, because the display size is constant). But, magnification is not infinite! It is limited by the minimum electron probe size. As magnification is increased, the step size between pixels is

![Diagram of raster scan in SEM for pixel-by-pixel image collection](image)

Figure 2-2. *Raster Scan in SEM for Pixel-by-Pixel Image Collection.* The electron (e⁻) beam is scanned across a sample, collecting signal at each sample pixel which becomes an image pixel on the display (e.g., computer monitor). After completing one row, the beam is scanned across the next row and so on, until the entire area has been scanned.
decreased, and the probe size remains constant (Figure 2-4). Eventually, the step size between pixels becomes smaller than the diameter of the electron probe. This leads to “empty magnification”, where the oversampling of sample pixels results in a blurry image. Because of this, electron probe diameter is one of the limiting factors of resolution in the SEM.

An approximation of probe diameter can be found in Equation 2.1 (Bell and Erdman, 2013).

\[
d_{eff}^2 = d_d^2 + d_s^2 + d_c^2 + d_g^2
\]  

(2.1)

where \(d_{eff}\) is the effective probe diameter, \(d_d\) is the beam width of the Airy disc corresponding to the diffraction limit, \(d_s\) is the size of disk of least confusion caused by spherical aberration, \(d_c\) is the size of the disk of least confusion due to chromatic aberration, and \(d_g\) is the minimum Gaussian focus of the beam. These values are given by Equations 2.2-2.5.

\[
d_d = \frac{0.61\lambda}{\alpha} \quad (2.2) \quad d_c = C_c\alpha \frac{\Delta E}{E_o} \quad (2.3)
\]

\[
d_s = \frac{1}{2} C_s\alpha^3 \quad (2.4) \quad d_g = \frac{4i_p}{\sqrt{\beta\pi^2\alpha^2}} \quad (2.5)
\]
In the given equations, \( \lambda \) is the wavelength of the electrons, \( C_s \) and \( C_c \) are coefficients of aberration, \( \Delta E \) is the energy spread of electrons, and \( \beta \) is the brightness of the beam. The electron wavelength is dependent on the energy of the incoming electrons. Higher energies mean smaller wavelengths, and therefore a smaller beam width due to diffraction. The electron energy is also important when determining the contribution of lens aberrations to the beam size. As beam energy decreases, the ratio of the spread of electron energies to the beam energy (\( \Delta E/E_0 \)) increases. This makes chromatic aberration the dominating term for effective probe diameter at low voltage. The minimum probe size becomes larger than at high voltages, resulting in degraded resolution.

### 2.1.2 Electron-Matter Interaction

The electron beam interacts with the sample, causing the sample to emit various signals, including backscattered electrons (BSE), secondary electrons (SE), X-rays, and other types of radiation. The incoming beam electrons penetrate a certain depth into the sample, dependent on the energy of the electrons and the elemental composition of the sample (Figure 2-5). The region from which generated signal is collected is called the interaction volume. If the interaction volume is larger than the sample pixel size, signal could leak into adjacent pixels, reducing resolution (Figure 2-6).

Each signal reveals different information about the sample due to the mechanism by which the signal is generated. BSE are the original beam electrons which have scattered and then reemerged from the sample. In materials with high atomic number (Z), more high-angle scattering occurs which generates increased BSE signal. Imaging with BSE provides great contrast between
materials of different elemental composition. While the beam electron is in the sample, it may ionize atoms, creating SEs. SEs generated within a few nanometers of the surface (within the escape depth) emerge from the sample and can be collected for imaging by the SE detector. This makes SE useful for imaging surface topography. Looking at Figure 2-6, SE1 are the SE that are good for imaging, as they emerge from near where the beam impinges the sample and therefore represent the pixel currently being imaged. While exiting or once exited from the sample, BSE can instigate extraneous SE far from the beam impingement site on the sample (SE2) or from interactions with the microscope chamber (SE3). This decreases SE resolution because SE2 and SE3 add information to the overall SE signal that is not from the pixel currently being imaged.

Figure 2-5. *The Effect of Beam Energy on Interaction Volume*. The beam electrons enter from the top of the specimen and scatter as they interact with specimen atoms. This forms the interaction volume. From left to right, beam energies are 2keV, 5keV, and 20keV. Higher energy electrons are able to penetrate deeper into the sample, creating a larger interaction volume. Low energy electrons stay closer to the surface, making them excellent for studying a sample’s surface topography. If the beam energy were held constant and the atomic number (Z) of the specimen were varied, the same behavior would be seen as above, where interaction volume would increase as Z decreased. Illustration inspired by Monte Carlo simulations of electron trajectories from Joy in Schatten and Pawley (2008).
An example of the role signal type and voltage play in image generation is given in Figure 2-7. It is important to select the appropriate microscope conditions to obtain the desired information about the material being studied.

### 2.2 Low Voltage Imaging

Modern SEMs typically operate at beam voltages between 2-30kV, and some have the ability to image at voltages down to tens of eV. Compared to higher voltages, lower beam voltages (<5kV) have a larger spot size (“beam footprint”) due to factors including longer electron wavelength and increased chromatic aberration. This results in reduced resolution at low voltages. But, low voltage imaging has many benefits and a variety of applications (Bell and Erdman, 2013).

At low voltages, beam-sensitive samples are at reduced risk of beam damage, which can change the physical make-up of the sample. This results in images which are poor quality and difficult to interpret. Some examples of beam-sensitive samples include semiconductors (Müllerová and Frank, 2003) which are integral to modern life in objects like smartphones and other computer-based technologies, photovoltaics which harvest solar energy (Masters et al., 2015), and medical
and biological samples (Schatten and Pawley, 2008). Lower energy electrons also cause less charging in non-conductive samples, which can eliminate the need to coat samples with a conductive film prior to imaging. Because low energy electrons do not penetrate as deeply into a sample as with high energies, the interaction volume is shallower and enables studies of sample surface topography and thin films. Lower beam energies also open up contrast mechanisms not available at high voltage (Bell and Erdman, 2013), which can be useful depending on the information a user wishes to obtain about their sample.

Unfortunately, the advantages of high voltage imaging are lost when imaging at lower voltages. Low voltages images tend to be noisy and blurry due to increased probe size and electron wavelength (i.e., diffraction limit) and a decline in signal-to-noise due to lower probe currents, a consequence of lower brightness which decreases with $E_0$. While higher probe currents could be used to increase signal-to-noise, it would also increase the size of the electron probe, producing further decline in resolution. The effects of chromatic aberration is greater at low voltages which hinders resolution even more. In addition, surface contamination becomes more visible in low voltage images because the small interaction volume now contains a larger number of the contamination layers. As one can imagine, finding the optimal focus under these conditions is difficult and time-consuming, with no promise of obtaining the desired image quality.

Many advancements have been made in low voltage imaging in an attempt to counter the challenging conditions. During specimen preparation, plasma cleaning can reduce and remove carbon build-up on samples, assisting in the mitigation of contamination effects during imaging.
CHAPTER 2. BACKGROUND

Higher resolutions are achievable with the development of field emission electron guns, which deliver higher brightness and smaller electron energy spread compared to earlier gun models (Egerton, 2010). Low Voltage SEMs (LVSEM) and Scanning Low Energy Electron Microscopes (SLEEM) were created specifically for the task of imaging at low voltages (Müllerová, 2001). LVSEMs are designed to emit very low energy electrons (<1keV), but can encounter limitations from lens aberrations. Aberration-corrected SEMs would lead to a major improvement in SEM performance, but systems that could account for multiple aberrations would drive the system’s complexity and cost quite high (Joy in Schatten and Pawley, 2008). Progress in aberration correction has primarily occurred in TEM and STEM, and only first steps have been made for the SEM (Hawkes, 2015).

A review of the developments in SLEEM technology is given by Frank et al., 2011. The technique introduced by SLEEM is also known as beam deceleration mode (BDM) or gentle beam mode. SLEEM instruments use a special retarding-field element to control electron energy around the specimen. A cathode lens encompasses the specimen, and excited electrons from sample emission are extracted and accelerated to an anode for collection at enhanced resolutions. Disadvantages of SLEEM include the strong electrostatic field applied at the specimen and the requirement that the specimen must be relatively smooth. Detectors are also an important variable in low voltage imaging. State-of-the-art SE and BSE detectors employ energy filtering techniques to improve the collection of low energy signals, where electron yields are low and noise becomes a greater concern (Bell and Erdman, 2013; Lewis et al., 2015).

Thus far, the prime innovations in low voltage imaging have relied on improvements to hardware design and functionality. The most advanced systems may not be reachable due to reasons such as cost, location, and instrument time availability. Upgrading and replacing on-site hardware can be quite expensive and unavailable for some setups. An accessible solution to the problems faced in low voltage imaging could be enabled by a software-based approach, which would reduce the expenses brought about by hardware and could be designed for use with any microscope.
2.3 The Point Spread Function

Addressing the issue of resolution in the SEM requires the ability to characterize the microscope, most importantly the electron probe. Understanding the shape and size of the probe opens the door to understanding how different operating conditions and technologies effect the probe, facilitating strategies for improvement. Along with microscope characterization, measuring the electron probe provides information about the system that is vital for image restoration, and also has prospective applications in microscope maintenance. In the case of the SEM, the electron probe can be described by the point spread function (PSF) of the microscope.

The PSF of any imaging system relates how a single point of light is transformed by the system during imaging. An imaging system with an ideal PSF (i.e., delta function) would produce an image of a perfect point, matching the input exactly. In reality, the PSF is not ideal due to factors like imperfect focusing elements and system noise, which causes the output image to be somewhat distorted compared to the scene being imaged. By measuring the PSF, the imaging system and its imperfections can be characterized, which creates potential for resolution improvement efforts.

Mathematically, an image can be represented as the convolution of the PSF and the scene being imaged, as seen in Equation 2.6.

\[ I_o = PSF \otimes I_T + \eta \]  

(2.6)

The observed image, \( I_o \), can be represented as the convolution (\( \otimes \)) of the scene being imaged (true image), \( I_T \), and the point spread function, \( PSF \), with addition of noise, \( \eta \), from the image generation process. A visualization of the blurring process can be seen in Figure 2-8.

Normally, \( I_T \) is inaccessible, as it has been changed by the PSF and has been affected by system noise, resulting with \( I_o \). If the PSF and \( I_o \) are known, careful arrangement of Equation 2.6 along with methods for handling noise allow us to use deconvolution to calculate \( I_T \). In essence, PSF deconvolution restores image information (i.e., resolution) which has been lost due to blur or distortion by the PSF.

PSF deconvolution has been used in many fields, both scientific and artistic, for the purpose of image quality improvement and modification. This includes applications in photography, optics, astronomy, microscopy, and any project which utilizes an imaging system. A well-known example
of the successful application of PSF deconvolution is in the early Hubble Space Telescope (Lindler, 1990; Lallo, 2012), where the technique was used to correct for spherical aberration caused by a flawed mirror that was not detected before launch (Figure 2-9). In microscopy, PSF deconvolution is regularly utilized in confocal microscopy (Pawley, 2006), a form of light microscopy which images samples by exciting fluorescent labels with lasers. Scientists in soft X-ray microscopy have applied PSF deconvolution for the purposes of image deblurring and improved experimental setup (Otón et al., 2016; Ekman et al., 2017). The application of PSF deconvolution in electron microscopy, specifically SEM, is the topic of recent work (Lifshin et al., 2014; Zotta, 2016) and is addressed in this thesis.

Thus far, image processing solutions for SEM have focused on image enhancement. Through enhancement, an image undergoes a mathematical transformation in an attempt to improve image quality, but the improvement is not based on information from the imaged scene.
or the system which captured the image. In electron microscopy, this includes techniques like denoising (Sim et al., 2007; Mazhari and Hasanzadeh, 2016) and edge enhancement (Bai and Zhang, 2014). This even includes “blind” deconvolution techniques (Yano and Nomura, 1993; Nakamae et al., 1994; Vanderlinde and Caron, 2007; Koshev et al., 2011; Lupini and de Jonge, 2011; Ramachandra and de Jonge, 2012; Bajic et al., 2016), which rely on estimations of the PSF because the PSF is not known. A thorough review and analysis of state-of-the-art image processing techniques utilized in electron microscopy is given by Roels et al. (2018).

PSF deconvolution presents a cost-efficient and widely applicable image restoration technique for resolution and image quality improvement in the SEM. This could introduce improvement for low voltage imaging, as well as possibly improve thermionic electron source imaging to the level of FESEM-type imaging. While the results of blind deconvolution have shown some improvement in image quality, they lack the unique knowledge of their system which is characterized by the PSF. Therefore, only an approximation of the true image can be attained by

Figure 2-9. Example of Deconvolution from Hubble Space Telescope. Hubble Space Telescope original image of Saturn (with WFPC1 F718 filter) is shown on top, and below is the image restored with PSF deconvolution. Notice the improved visibility of the rings and the bands in the planet’s atmosphere. Images from Lindler (1990).
the blind technique. The advantage of PSF deconvolution is the ability to restore scene information to an image based on knowledge of the imaging system. Roels et al. (2018) showed that denoising methods produced higher image quality when combined with PSF deconvolution than when using denoising on its own.

2.4 PSF Determination and Image Restoration in the SEM

Knowledge of the SEM PSF would be beneficial for microscope characterization, image restoration (especially at low voltages), and has potential for use in microscope maintenance. Unfortunately, the PSF is notoriously difficult to measure in the SEM. This is due to the lack of obvious point source, possible interference during the electron-matter interaction, and pixel-by-pixel image collection in the case of scanning systems. A review of methods for electron probe measurement and visualization is presented in Chapter 3.

The PSF determination method investigated for this body of work is the first to provide a full visualization of the SEM electron probe. The method for PSF determination is explained in detail in Chapter 3, along with example applications including PSF deconvolution. An exploration of the method’s parameter space is required to improve the interpretation of its resulting PSFs (Chapter 4) and image restorations (Chapter 5). Additional examples of image restoration with this method are given in Appendix E, as well as a visual comparison to BDM and high voltage imaging in Appendix A.4.
3 The Determination and Application of the Point Spread Function in the Scanning Electron Microscope

This chapter presents a novel PSF measurement method for SEM, laying the groundwork for subsequent chapters. Knowledge of the electron probe shape (PSF) is valuable for SEM performance characterization and monitoring, as well as for modeling and simulation in computational scanning electron microscopy. For example, the PSF can characterize astigmatism and also open up the opportunity to study the relationship between beam energy, beam current, working distance, and beam shape and size in ways which were previously inaccessible. In addition, the PSF can be used for the purpose of deconvolution to improve the resolution and quality of images obtained using various electron sources (e.g., thermionic, FEG, or Schottky). The presented method represents an improvement over previous methods for measuring the SEM beam and several practical applications are described. Preliminary work to that presented in this chapter can be found in Appendix A.

3.1 Introduction

In an SEM, a focused beam of electrons is scanned over the surface of a sample in a raster pattern generating a detected signal at each pixel sampled. Signal electrons are generated from the volume where the focused beam interacts with the sample. The measured signal can be backscattered (BSE), secondary (SE), or transmitted electrons. The detectors convert electron intensity to a digitized voltage stored for each pixel. As the magnification is increased, the spacing between sample pixels (nm/pixel) decreases, and at some point the footprint of the beam will be
larger than the spacing, causing what is known as oversampling (Goldstein et al., 2018). When this happens, the information obtained from each pixel will no longer be unique to that pixel, and the image on the display will become blurry. Therefore, the resolution of an SEM depends significantly, although not solely, on the ability of an SEM to produce the smallest possible beam at the sample surface. Thus, probe size reduction has been a major long term goal of SEM manufacturers. Currently, SEMs are commercially available with probe sizes that are claimed to be at or well below 1.0nm in diameter even at very low beam energies. These advances have been the result of significant improvements in electron sources, electron optics, improved specimen stages, and a number of other factors.

From a user’s perspective, a principal question is, “What is the resolution of a particular SEM for a given sample and set of operating conditions?” This question is difficult to answer because although methods have been developed to evaluate SEM image sharpness (ISO 2011) and measure resolution (Sato, 2009), the exact definition of resolution is ambiguous, and there is not a single mutually agreed upon standard. An alternative question is, “What is the probe size and shape?” as these attributes can be characterized independently of the specimen and can be associated with high resolution if there is an adequate signal-to-noise ratio (SNR) and sufficient contrast. Following the definition suggested by Orloff (1997), the spatial distribution of electrons in a focused beam will be referred to here as the point spread function (PSF). Its measurement and use will be the primary emphasis of this chapter.

Additional factors that affect SEM resolution include the overall interaction (information) volume, sample drift, contamination, sample damage, and the SNR which depends on aspects including sample composition, integrated probe current per pixel, signal type (e.g., BSE, SE), and detector. Microscope users are generally aware of these factors and take many steps to mitigate any problems. In particular, decreasing the excitation volume involves either the use of thin film samples or decreasing the landing potential of the electron beam. The latter is done either by lowering the beam energy at the source or the use of electrostatic fields to lower electron landing energy at the sample. Although these approaches can lead to resolution improvement, each is still limited by the final size of the electron probe. One way of improving the SNR involves increasing the total pixel dose (probe current multiplied by dwell time). Higher probe current usually increases the probe size as a side effect, therefore decreasing resolution. The key point to be made is that decreasing the probe size is a necessary condition for improved resolution, but may not be
sufficient. Fortunately, in some cases, experimental conditions can be set such that the radius of the excitation volume is close to that of the probe size allowing PSF deconvolution methods to improve resolution as will be described later in this chapter.

3.1.1 Prior Efforts at PSF Determination

Characterizing the PSF is quite difficult since no detector currently exists with sufficient spatial resolution, so it is done indirectly. Liddle et al. (2004) utilized a transmission electron microscope (TEM) to obtain a high-resolution reference image of a test structure, and the same area was then imaged at a lower resolution in an electron beam lithography tool. The two images were then compared and the PSF calculated based on determining the mathematical transformation (convolution) needed to go from one image to the other. While this process lays the groundwork for the approach described here, it is limited by differences in the contrast mechanism associated with the different modes of image formation as well as some of the assumptions needed for the mathematical model. Babin et al. (2008) developed a method for electron beam lithography applications, where intensity profiles of a 22nm test pattern were imaged and measured to determine the beam size in two orthogonal directions. The resultant profiles could then be rotated and interpolated to create a two-dimensional beam distribution. This technique is limited by only being viable for two dimensions at a time and has a built-in assumption of a Gaussian-shaped beam profile.

Kandel et al. (2015) used a pair of images of a distribution of gold particles on a carbon substrate to estimate the distribution of electrons in the beam. One image was taken with a high-resolution SEM yielding a low noise reference image. The second image captured the identical region with the SEM of interest, where the beam size was larger than that of the high-resolution reference SEM. The PSF was then calculated from the pair of images. Unfortunately, this technique is limited by the requirement of having a high-resolution reference SEM image available as well as the considerable time needed to locate a specific field in both microscopes. Perhaps the most common way to estimate the PSF is by the “knife edge” method, where a signal is monitored while the beam is scanned over a sharp edge, such as a razor blade or cleaved crystal (Michael & Williams, 1987; Reimer, 1998; Kološová et al., 2015). This technique can be useful for beams down to tens of nanometers, but getting a sufficiently sharp edge and avoiding transmission or excitation volume effects limit its usefulness for very fine probes.
3.2 Materials and Methods

The new approach described here is in part built on the earlier referenced work of Liddle et al. The basic idea is to compare a high-resolution image (true image) of a known structure with an observed image of the same structure obtained with an instrument whose PSF is to be determined. Essentially, the observed image is the convolution of the true image with the PSF. In the method described here, the known structure is a well-separated dispersion of fine gold particles with a fixed known shape dispersed on a substrate with low background signal, which can be a carbon film on a TEM grid. The structure is imaged in the microscope whose PSF is to be determined for a fixed set of operating conditions, termed the “observation microscope” which gives an “observed image.” A theoretically calculated reference image, as would be obtained with an ultra-high resolution microscope, acts as the true image. These two images are then used to compute the PSF.

An overview of the PSF determination process is given below with details to follow:

1. A specially designed calibration standard is imaged in an SEM of interest at an operator-selected set of experimental conditions that include accelerating voltage, lens settings, and working distance (WD).

2. The calibration image is processed in software to detect particles in the field and combine the detected particle images to a single stacked particle image.

3. A “true” image is theoretically calculated for the chosen particle size, beam energy, and detector used. Such reference images can be calculated in advance and can be selected for PSF determination at the time of use.

4. The PSF is then determined by one of a variety of methods including by a Wiener filter. Once determined, the PSF can be displayed or stored in a variety of ways including surface profiles, contour maps, and as a two dimensional matrix (image).

3.2.1 The Calibration Standard

A calibration standard has been developed which consists of a dispersion of gold particles (e.g., 20nm in diameter) having a known and narrow size and shape distribution (coefficient of variation
CHAPTER 3. DETERMINATION OF THE PSF IN THE SEM

<10%) supported on a thin conducting substrate such as ultra-thin carbon (typically <3nm thick) on a copper support TEM grid. The particles are dispersed on the grid such that they are well-separated. This ensures that the electron beam can uniquely sample each particle without contributions from adjacent particles. The particles should have a nearest neighbor distance of at least twice the diameter of the beam to ensure unique sampling of each particle independent of any adjacent particles. Additionally, the thin nature of the carbon substrate film mitigates background signal and minimizes the contribution of the interaction volume (Watanabe, 2011). This is an important feature of the calibration standard, as limiting the excitation volume creates a situation where the primary cause of modification of the observation image is due to the convolution of the PSF and the gold particles. It has been found in practice that BSE images are preferable for imaging the calibration standard. SE images often have a larger background signal, and even small levels of carbon contamination can cause an apparent increase in the particle size that is less evident in a BSE image.

3.2.2 Image Collection and Processing

The calibration sample is imaged in the SEM of interest with sufficient pixel size to ensure a high-resolution rendering of the PSF. As an example, if 20nm calibration particles are used the magnification is set to 1nm/pixel. This ensures more than 10 steps across the particle diameter leading to a detailed rendering of each particle. Currently, this is a best practice recommendation and a topic of ongoing investigation. Different particle size standards have been used depending on the range of probe sizes to be determined. A small particle size is most desirable for characterizing small probes. But, they may not even be visible with large probes, where a larger particle size may be more suitable. The desirability of small particles is based on the fact that the image of a point source is in effect the PSF. Unfortunately, if the particles are too small they may not give rise to sufficient signal levels and may be lost in the noise.

Once the calibration image is collected (Figure 3-1a), individual particles in the image are cropped and stacked together to form a single composite particle (Figure 3-1b). The stacked particle image is considered to be an adequate representation of a single calibration particle (of known size) convoluted with the PSF. Although best efforts are made to ensure a calibration sample contains only well isolated particles, often some particles will agglomerate causing proximity issues for the goal of unique sampling of single particles. In this case, particles that are
too close to each other or touching are rejected and not used to form the composite stacked particle. This composite particle represents a noise and contamination mitigated rendering of the particle under the observation conditions.

There are several advantages to using multiple particles to form a composite image over simply imaging one particle at low noise conditions. High current conditions and long dwell times per pixel can be avoided. The stacked image can be nearly noise-free due to the stacking of hundreds of particles together to form a single particle, thereby increasing the SNR. Additionally, the stacked particle image is virtually contamination-free as each particle is subjected to a relatively minimal electron dose by scanning the beam rapidly. The fast scan also reduces the effect of drift. Finally, averaging many particles yields a final stacked particle image that minimizes issues relating to the size and shape distribution of synthesized particles. Depending on the quality

Figure 3-1. Example Calibration Image with Extracted Stacked Particle and Theoretically Calculated Particle. (a) The calibration image taken with a TESCAN VEGA® LaB$_6$ SEM, probe current = 6.45pA, HV = 20keV, (b) the stacked particle image created from selected particles in the calibration image, and (c) the theoretically generated high-resolution reference image for the given operating conditions. Figure brightness enhanced for publication.
of particle synthesis, the particles will have a varying range of sizes and shapes. By averaging particles together, the size and shape of the particles will converge to the nominal size and shape that is expected for the distribution and will correlate better with the calculated reference image.

Processing the calibration image to create a stacked particle image was carried out using an Aura computer system from Nanojehm, Inc. Selecting particles for stacking is a semi-automated procedure carried out on the basis of particle size and shape. The procedure is referred to as semi-automated in that, if desired, the user has various options to aid in selecting the most representative particles. These options include image smoothing, applying a circularity index, and selecting portions of particles for stacking using an interactive histogram of particle sizes. In the histogram, the peak of the distribution is often near the nominal particle size, but the peak can be shifted away from the nominal size depending on the chosen measurement criteria and the degree of blurring from the convolution. The user should select the region containing the peak because it is the mode of the sizes reported, which corresponds to the nominal size of the particles. Any deviation from the expected size mode is attributed to the PSF, and therefore, those particles representing the mode shift should be used to generate the stacked particle.

3.2.3 Reference Image Generation

Since the particle size and shape is known, a noise-free reference particle image (Figure 3-1c) can be generated by a Monte Carlo simulation program such as CASINO 3D which was used here. The reference particle simulation takes into account the particle size, shape, and composition; the substrate thickness, shape, and composition; and the image generated can be either BSE or SE. In order to obtain a high-resolution image, the probe size was set at 0.1nm for the simulation.

3.2.4 The PSF Calculation and Visualization

The relationship between the measured image (i.e., 2D matrix) of the standard \( I_O(i,j) \) and the true image \( I_T(i,j) \) is given by:

\[
I_O(i,j) = PSF(k,l) \otimes I_T(i,j) + \eta(i,j) \tag{3.1}
\]

where \( \otimes \) is the convolution operator, \((i,j)\) denotes the coordinates of a specific pixel, and \((k,l)\) refers to coordinates of the PSF relative to its center. Since image acquisition in the SEM is
characterized by noise associated with electron emission statistics and scattering, an additional term $\eta(i, j)$ is added which denotes the noise contributions to the signal at location $(i, j)$.

Wiener deconvolution is one method of PSF determination, which uses the stacked particle image along with the theoretically determined reference image. The Wiener method involves taking the Fourier transform of Equation 3.1 to simplify the problem by replacing the convolution operation (spatial domain) with multiplication (frequency domain). This process and the following mathematics are described in detail by Gonzalez & Woods (2009). Prior to the Fourier transform, the PSF is padded with zeros to the same dimension as $I_O(i, j)$, $I_T(i, j)$, and $\eta(i, j)$. The Fourier Transform of the PSF is the optical transfer function (OTF), which describes the spatial frequencies that pass through the imaging system. Therefore, Equation 3.1 can be reformulated as the following:

$$O(u, v) = OTF(u, v) \cdot T(u, v) + N(u, v) \quad (3.2)$$

where $O$ is the transform of the observed image, $OTF$ is the optical transfer function, $T$ is the transform of the true image, and $N$ is the Fourier representation of the noise. The coordinates $(u, v)$ are in spatial frequency units. Since spatial frequency is not always easy to understand, consider a series of parallel lines such as in a microelectronic device that are 10nm apart. The spatial frequency is 0.1 lines per nanometer. As the lines become closer their spatial frequency increases.

Considering the noiseless case where $N = 0$, Equation 3.2 can be rearranged to give an estimate of the PSF:

$$PSF(k, l) = F^{-1}[OTF(u, v)] = F^{-1}\left[\frac{O(u, v)}{T(u, v)}\right] \quad (3.3)$$

However, it is well known that in most situations there are values in $T(u, v)$ that are either zero or close to zero. In practice, this leads to the equivalent of division by zero which is an undefined behavior.

The Wiener method modifies Equation 3.3 by the insertion of an attenuation term to mitigate the contribution of small valued entries in $T(u, v)$. This leads to the following expression:
CHAPTER 3. DETERMINATION OF THE PSF IN THE SEM

\[
\hat{\text{PSF}}(k, l) = F^{-1} \left[ \frac{T^*(u, v)}{|T(u, v)|^2 + S_N(u, v)/S_{OTF}(u, v)} \right] O(u, v) \tag{3.4}
\]

where:

\[ T^*(u, v) = \text{complex conjugate of } T(u, v) \]
\[ |T(u, v)|^2 = T(u, v)T^*(u, v) \]
\[ S_N(u, v) = |N(u, v)|^2 = \text{power spectrum of the noise} \]
\[ S_{OTF}(u, v) = |OTF(u, v)|^2 = \text{power spectrum of the OTF} \]

Notice that in situations where there is no noise or where the SNR is sufficiently high \((S_N(u, v) \rightarrow 0 \text{ or } S_{OTF}(u, v) \rightarrow \infty)\), then the noise-to-OTF term drops out and Equation 3.4 reduces to the inverse filter given in Equation 3.3.

Since neither the power spectrum of the noise nor the power spectrum of the signal can be precisely known in practice, \(S_N(u, v)/S_{OTF}(u, v)\) is traditionally replaced and estimated by

\[
K = \frac{S_N(u, v)}{S_{OTF}(u, v)} \tag{3.5}
\]

reducing Equation 3.4 to the following:

\[
\hat{\text{PSF}}(k, l) = F^{-1} \left[ \frac{T^*(u, v)}{|T(u, v)|^2 + K} \right] O(u, v) \tag{3.6}
\]

where \(K\) is a constant added to each value in \(|T(u, v)|^2\) and is chosen such that sufficient compensation is made for the noise in the images. As the noise increases, \(K\) must increase to effectively regularize the noise in the result. If the noise in the stacked particle image is minimal, \(K\) can be decreased leading to a more accurate depiction of the PSF. Figure 3-2 illustrates the various modes to display the PSF calculated from the images presented in Figure 3-1b and Figure 3-1c. Figure 3-3 shows an example of how to select an optimum value of \(K\). \(K\) and its effects on the PSF are discussed in further detail in Chapter 4.
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3.3 Results and Discussion

3.3.1 Calibration Robustness and the Effect of Beam Energy

A variety of experiments were performed to test the robustness and validity of the method for PSF determination described here. One approach to robustness is to see if different fields of nanoparticles on a calibration sample produce the same PSF, which is expected if the beam is unchanged between images. It should be noted that even when the beam is unchanged, the PSF is changing at each pixel due to the stochastic nature of electron emission. This method determines an average PSF over the field, which may be good enough for probe size measurement and other applications.

To test calibration robustness, six different regions of nanoparticles were imaged on a calibration sample (Figure 3-4). BSE images were collected at 2kV, 3kV, 4kV, 5kV, 10kV, and 20kV on a TESCAN MIRA3. The samples were situated at a WD of ~7.5-8.8mm using stage...
height control. The minimum probe size was determined by the microscope software which resulted in probe currents ranging from about 75pA to 205pA depending on the beam voltage.

At each voltage, six regions were imaged within an area equal to one 42μm x 42μm TEM grid square. The six regions were imaged using the same beam shape to test calibration robustness and repeatability, meaning no changes were made to the focus using beam parameters once the best focus image was collected. The best focus was found for the first region by making typical adjustments to the beam (using focus, stigmators, and wobbler to center the beam). The same magnification was used for the remaining five regions, with occasional changes to stage height to account for variations in sample surface height. This ensured a constant WD. PSFs were then calculated for each region using a Nanojehm Aura workstation which has all the needed software for the abovementioned calculations. The results are presented in Figure 3-5.

As a null case, six images of the same region were captured at 2kV. Small compensations were made to brightness and contrast between images to account for changes in the sample surface as it received more beam exposure. These images were also captured in BSE mode, which reduced the effects of contamination on the image. In SE images, small amounts of contamination can cause
the particles to appear larger, and there is a larger background signal from the support film. In BSE images, these concerns are mitigated.

Traditionally, a well-focused SEM electron probe is approximated as a Gaussian (Reimer, 1998). The probe size can then be conveniently characterized using the full width at half maximum (FWHM) which is the dimension that contains 50% of the probe current, and the full width at tenth maximum (FWTM) that contains 90% percent of the probe current.

A Gaussian fit for the determined PSF was tested against Lorentzian, Airy, and sinc function fits, with a single PSF at each 2kV, 5kV, 10kV, and 20kV. The goodness of these fits were compared using sum of squared error, root-mean-square error, and $R^2$. Overall, the Gaussian

Figure 3-5. Regional PSF Contours. The 10kV through 2kV plots show overlaid PSF contours of six different nanoparticle fields on the same sample. The 2kV* (null case) plot shows overlaid PSF contours taken of the same nanoparticle field imaged six times. The 2kV and 2kV* data were collected on different dates with different beam shapes, which is why their PSF shapes differ somewhat when compared to each other. In each plot, the inner ring corresponds to $0.5I_{max}$ and the outer ring to $0.1I_{max}$, where $I_{max}$ is the maximum intensity for a PSF. The first region (best focus) PSF is shown in black and the PSFs of the other five regions (same beam shape as first region) are shown in color.
produced the best fit for the tested beam voltages, with the Lorentzian matching performance at 2kV. For consistency and simplicity, we used the Gaussian fit to compare PSFs based on their FWHMs as shown in Table 3-1. See Appendix A2 for more detail on use of Gaussians.

As the beam voltage decreased, the minimum probe size increased, which is reflected in the behavior of the PSFs. Decreasing beam voltage also introduced more noise into the PSFs. More pixels are required to define the size and shape of the PSF as the beam size increases. This effect coupled with lower beam current at lower voltages results in reduced signal at each pixel, making the PSF more susceptible to noise.

The 2kV data and 2kV* (null case) data were taken at different times. Efforts were made to use the same operating conditions between data collections. An important difference between collections remained: the 2kV calibration fields were much sparser in terms of the nanoparticle dispersion compared to the 2kV* data. Less particles to stack means less average signal and poorer counting statistics. When compared to the 2kV* (null case) data, this could account for the increased amount of noise in the 2kV data.

While the 3kV beams’ best focus and astigmatism might have been improved to produce more circular beam footprints, the required changes would be subtle and do not affect the conclusions. Notably, even if the shape was not optimal, each region of nanoparticles produced the same PSF, respectively for each beam energy.

Table 3-1. Regional PSF FWHMs.

<table>
<thead>
<tr>
<th>Axis</th>
<th>Distribution of FWHMs [nm]a</th>
<th>20kV</th>
<th>10kV</th>
<th>5kV</th>
<th>4kV</th>
<th>3kV</th>
<th>2kV</th>
<th>2kV*gb</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>10.5 ± 0.2</td>
<td>11.1 ± 0.2</td>
<td>14.5 ± 0.5</td>
<td>15.0 ± 0.5</td>
<td>19.4 ± 0.5</td>
<td>24.2 ± 0.8</td>
<td>30.2 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>y</td>
<td>10.9 ± 0.4</td>
<td>11.2 ± 0.2</td>
<td>14.2 ± 0.2</td>
<td>15.2 ± 0.3</td>
<td>16.7 ± 0.8</td>
<td>28.7 ± 1.3</td>
<td>30.9 ± 0.9</td>
<td></td>
</tr>
</tbody>
</table>

a Each entry is the mean ± one standard deviation of the six FWHMs calculated from the PSFs for that voltage and axis. These FWHMs were calculated using PSFs determined with \( K = 1000 \).

b 2kV* is the null case (six images of the same field) for comparison with 2kV.
3.3.2 Comparison of the New Method with the Knife Edge Test

As mentioned previously, the knife edge test has become a fairly standard way of measuring the PSF. A scan across a knife edge produces a profile plot that has the form of an error function if the PSF is Gaussian. The differentiation of the profile will lead directly to a one dimensional PSF curve in the direction of the scan. If the curve is radially symmetric, then it can be described by a single scan. If not, multiple scans are required over a range of directions to determine a two-dimensional PSF, which can be rather time consuming. In practice, the FWHM is determined by first finding the distance between x-axis values corresponding to the locations of 25% and 75% of the full profile intensity \( (d_{25\%} - 75\%) \) obtained from the beam being completely on or off the knife edge depending on whether a signal is measured above the knife edge (SE or BSE) or below it (transmitted). The FWHM is then calculated from (Kološová et al., 2015):

\[
 d_{25\% - 75\%} = 0.57 d_{FWHM} \tag{3.7}
\]

A derivation confirming Equation 3.7 is shown in Appendix B.1.

The knife edge method and the previously described new method were compared using BSE data obtained from an EMS 79510-01 gold-on-carbon standard for the knife edge measurements and 18.2nm gold particles on a carbon film for the new method. At each tested beam energy, the particles and the gold-on-carbon standard were imaged with the same beam shape. Ten different profiles at various angles (perpendicular to a feature edge) were measured on gold-on-carbon features. The ImageJ plugin GaussFit (Schneider et al., 2012) was used to determine \( d_{25\% - 75\%} \) for each profile. The same ten angles were used to measure profiles of the corresponding Table 3-2. Comparison of FWHM Determined by Knife Edge Measurement and the New Method.

<table>
<thead>
<tr>
<th>Beam Energy [keV]^a</th>
<th>GaussFit FWHM [nm]^b</th>
<th>New Method FWHM [nm]^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>22.8 ± 1.5</td>
<td>20.8 ± 1.7</td>
</tr>
<tr>
<td>5</td>
<td>13.9 ± 1.7</td>
<td>13.5 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>10.8 ± 1.4</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>20</td>
<td>8.8 ± 0.8</td>
<td>10.2 ± 1.0</td>
</tr>
</tbody>
</table>

^aImages were captured using typical operating conditions for a Schottky source instrument.

^bResults are given as the mean ± one standard deviation of the ten profiles at each voltage.
PSF ($K = 1000$), determined from the particle image. Gaussians were fitted to the profiles in MATLAB, from which the FWHM could be calculated.

Table 3-2 summarizes the FWHM comparison of the two methods. The agreement is sufficiently close to indicate that the two methods give similar results with subtle differences, possibly due to factors such as the sample not being a perfect knife edge or the selection of the $K$ parameter.

### 3.3.3 Effect of Working Distance

WD is defined as the distance between the bottom of the final lens and the sample. Shorter WDs are related to higher resolution because smaller probe sizes are achievable as a result of reduced aberrations and greater source demagnification.

To study the effect of WD, calibration images were collected over a range of WDs at 2kV, 10kV, and 20kV using a Schottky field emission source on the MIRA3. For each WD, the sample’s z-axis position was altered and the beam refocused. The calibration standard consisted of 18.2nm gold spheres (measured by TEM) on a thin carbon film, similar to other measurements described above. The probe current was held constant for all WDs at a particular voltage.

The PSF size increases as WD increases at all voltages, which matches the expected behavior of the beam size. FWHM contours at 2kV and average values are shown in Table 3-3 and Figure 3-6, respectively. FWHM contours at 10kV and 20kV are included in Appendix B.2.

<table>
<thead>
<tr>
<th>WD [mm]</th>
<th>Average PSF FWHM [nm]$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2kV</td>
</tr>
<tr>
<td>8</td>
<td>22.0 ± 0.1</td>
</tr>
<tr>
<td>12</td>
<td>23.4 ± 0.1</td>
</tr>
<tr>
<td>16</td>
<td>27.4 ± 0.1</td>
</tr>
<tr>
<td>20</td>
<td>31.7 ± 0.1</td>
</tr>
<tr>
<td>24</td>
<td>34.6 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$The average FWHM was determined from the FWHM values in the x- and y-direction. The uncertainty shown is based on the 95% confidence bound from the Gaussian fit used to determine the FWHM. All values rounded to nearest 0.1.
The effect of WD was also used to evaluate the PSF above and below the best focus condition. This resulted in a cross-sectional look at the 3D shape of the electron beam, which is included in Appendix B.2.

3.3.4 Characterization of Astigmatism

Astigmatism is an aberration related to different focal lengths found on perpendicular intersecting planes parallel to and containing the optical axis of an electron optical system. Detailed descriptions can be found in a number of sources including Khursheed (2011) and Klemperer and Barnett (2011). When present, a circular object may appear as: 1) an ellipse in an image plane short of the desired WD, 2) a circle at the WD, and 3) a rotated ellipse in a plane beyond the WD. Consequently, even at the WD the circle is described as a disk of least confusion which will be larger than what would be obtained in the absence of astigmatism. In SEMs, astigmatism correction is normally done by electromagnets placed in the objective lens that can compensate for uneven focus in perpendicular planes along the optical axis. If left uncorrected, this aberration
distorts the size and shape of the PSF. Sources of astigmatism are poor column alignment, defects in the optics, and contamination.

From an operator’s perspective, astigmatism is one of the most difficult corrections to make since it often involves searching for the sharpest possible image as slight adjustments are made to the compensating field while observing a rapidly-scanned, noisy image. The objective of this phase of the study was to demonstrate that measurement of the PSF can provide information on the relationship between astigmatism settings and the size and shape of the probe at a desired WD and beam energy. This research could lead to a new method for automated astigmatism correction.

PSFs were, therefore, investigated at different beam voltages for various degrees of astigmatism using the method described in this article. At each voltage, a calibration sample of 18.2nm gold nanoparticles on graphene was imaged with each beam shape. Additionally at 10kV, a gold-on-carbon resolution standard (Electron Microscopy Sciences cat. #79510-01) was imaged after the calibration image was collected. The calibration sample images were used for PSF determination, and the gold-on-carbon images were used to visualize the effects of astigmatism on a sample-of-interest, as well as for testing image restoration using PSF deconvolution as will be described in a later section.

To collect an astigmatism series, best focus images and stigmated images of the samples were recorded. The best focus image was captured using standard focusing parameters (*i.e.*, WD, stigmation, and wobbler). Astigmatism was gradually added with one stigmator, while the other stigmator was held constant at the best focus value, and vice versa. Afterwards, a few images were collected where both stigmators were adding astigmatism to the image. Astigmatism series were captured at 2kV, 5kV, 10kV, and 20kV using a Schottky field emission source.

The resulting 10kV series is shown in Figure 3-7. The 2kV, 5kV, and 20kV series are included in Appendix B.3. The beam becomes more distorted as the degree of astigmatism increases. When stigmating along one axis, the stigmated shape is not reflected from one side of best focus to the other. That is, the effect of astigmatism is asymmetric. This is particularly noticeable along the *x* stigmator axis in this case. Similar results were found at the other voltages, along with the expected change in diameter of the PSF.
Figure 3-7. Astigmatism Series at 10kV. A box containing a contour plot is shown for each PSF. Each box has size 85nm x 85nm. The contours shown are $0.9I_{\text{max}}$ (inner contour), $0.5I_{\text{max}}$ (middle contour), and $0.1I_{\text{max}}$ (outer contour), where $I_{\text{max}}$ is the maximum intensity for the PSF within the box. Minor artifacts from the PSF determination process were minimized in the outer contours of a small number of PSFs to avoid distraction from the series as a whole.
3.3.5 Electron Source Type

To gain insight into how the PSF varies with source type, BSE images of a calibration standard were obtained with Schottky and thermionic source (LaB₆) instruments at nearly identical imaging conditions (i.e., WD and accelerating voltage), specifically at 20kV and WDs close to 9mm. The chosen operating conditions did not necessarily maximize resolution. The measured probe currents were 182.42pA and 6.45pA for the Schottky and LaB₆ instruments, respectively. Figure 3-8 shows the FWHM for both beams. Not surprisingly, the beam for the LaB₆ instrument shows a FWHM almost one-and-a-half times that of the Schottky instrument even though the measured specimen current in the Schottky is orders of magnitude above that of the LaB₆. Additionally, the 6.45pA of current for the LaB₆ is spread out over a much larger area than the Schottky. The PSF images show the current density per square nm. They were obtained by normalizing the PSFs and then multiplying the measured probe current by the normalized PSF matrix to give a map of the current density for the beam cross-sections. Knowledge of the current

![LaB6 vs Schottky](image_url)

Figure 3-8. Source Type Comparison. The FWHM is shown on the left and the spatial current density is shown on the right for both beams. This figure was created by Matthew Zotta.
density should prove useful for understanding the nature of exposure of resist materials in electron beam lithography.

3.3.6 Image Restoration

Once a PSF is determined (using a calibration image of nanoparticles), any images taken with the same beam shape can be restored. The restoration process described and reviewed previously by Lifshin (2014) is based on Equation 3.1. However, instead of having an observed and reference image pair to determine the PSF, the observed image and the PSF are known and the true image is determined. It is standard practice to recast Equation 3.1 in a stacked vector form where \( m \times n \) matrices (the observed image, the true image, and the noise) are described by \( mn \times 1 \) vectors and the PSF is described by an \( mn \times mn \) block circulant matrix derived from its coordinate dependent coefficients as follows:

\[
\hat{b} = A \hat{x} + \hat{\eta}
\]  

(3.8)

where \( \hat{b} \) is the vectorization of the observed image, \( A \) is the block circulant form of the PSF, \( \hat{x} \) is the vectorization of the true image, and \( \hat{\eta} \) is the vectorization of the noise. The determination of the true image requires the solution of this equation. In the absence of noise, Equation 3.3 could be readily solved by multiplying it by the inverse of the \( A \) matrix, \( A^{-1} \). However, \( A \) is highly sensitive to noise, and error can increase when values of \( A \) are near or equal to zero. The increased error produces an “ill-posed” problem. This means that in trying to solve the inverse problem (going from the observed image to the true image) multiple possible solutions may exist. Only through constraints or penalty terms can the most likely answer be found. A simple example of a constraint is that any solution where the true intensity is less than zero would be unacceptable. Finding a solution to Equation 3.8 involves converting it to a functional minimization problem:

\[
f(x) = \arg \min_x \{\|Ax - \hat{b}\|^2 + \lambda R(\hat{x})\} \quad s. t. \quad \hat{x} \geq 0
\]  

(3.9)

Varying the smoothing parameter \( \lambda \) allows the user to control the weighting of the regularization term, \( R(\hat{x}) \), with the least squares term, thus balancing the amount of sharpness and denoising.
while minimizing any artifacts introduced during deconvolution. These artifacts can include false background texture, shadows, and ringing.

In theory, PSF deconvolution is able to restore information lost to blur and distortion by the imaging system. This is because PSF deconvolution uses knowledge about how the image was formed. Restoration should be distinguished from image enhancement which does not use this knowledge, but rather modifies the image to accentuate specific features. Example restorations from the 10kV astigmatism series presented in Figure 3-7 can be seen in Figure 3-9. The observed images and corresponding restorations from the entire 10kV series are included in Appendix B.4. Restorations usually took a matter of seconds to perform for $n \times n$ images where $n = 1024$, 2048, or 4096 pixels.

PSF deconvolution was able to restore lost information to the astigmatic images and best focus cases. Qualitatively, the best focus images resulted in the cleanest restorations. As more astigmatism was added to the images, the restorations became less clear and more artifacts were introduced (e.g., ringing, shadowing).

![Figure 3-9. Restorations of Gold-on-Carbon from 10kV Astigmatism Series.](image)

Images (a)-(d) are of the scale given in (a). Image (a) is the observed image at best focus, and (b) is the corresponding restored image. Image (c) is the observed image at stigmatism $x$-0.4%, and (d) is the corresponding restored image. Intensity profiles of all images are shown in (e). The profiles were taken across two nearby gold features shown in (a). In (e), solid lines: observed images, dashed lines: restored images, black: best focus, pink: astigmatic focus. Profile intensities were normalized by the max possible intensity for a 16-bit image.
3.4 Conclusions

Details of a new method for determining the point spread function (PSF) of an SEM have been presented and shown to be consistent with commonly used knife edge methods. The advantage of this new method is not only speed, since it requires only a single measurement for the determination of the two-dimensional PSF, but also no assumptions need to be made about the shape of PSF which can be highly non-symmetrical about the beam axis. A variety of uses for the PSF were presented, including measuring astigmatism, quantifying the effect of experimental factors such as WD and beam energy, and improving SEM image resolution and quality through deconvolution and regularization.

While the presented results show clear benefits of using this method, additional research can lead to improvement and further understanding. The PSF is a mathematical bridge between a true image (the image of an object not subjected to distortion, noise, or any limitation to spatial resolution) and the image observed with an SEM. It is difficult, however, to fully understand how the standard used to measure the PSF may affect the result. For example, in the case of a knife edge experiment using SE, artifacts may result from lateral edge emission when the beam is near the edge of a particle or feature. This problem may also influence the results of the new method, but the degree of this effect has not yet been determined for either method. Lateral edge emission becomes more serious as beams become smaller and smaller. Edge emission (i.e., edge brightening) when imaging with SE is investigated as part of Chapter 4.

On one level, the PSF may be thought of as the spatial distribution of electrons in the plane of focus perpendicular to the electron beam axis without any sample present. However, when a sample is examined, the excitation volume may cause further convolution beyond the spreading due to the spatial distribution of the electron beam in free space. Understanding this excitation volume effect on the PSF and its applications is particularly challenging since the composition and structure of the material in three dimensions is generally not known, but may be needed. Different material thicknesses are tested as part of Chapter 4, in an attempt to learn about the effect of the interaction volume on the PSF.

Many other parameters are involved in the PSF determination process as shown in this chapter, including $K, \lambda$, the size of the reference particle. Each of these affects the output PSF in its own way, which may be critical to the application of the PSF. Therefore, deeper understanding
of their effects on the PSF in terms of size, shape, and accuracy is warranted, and is the focus of the remainder of this thesis (Chapters 4 and 5).

In addition, it is recognized that determining the PSF in STEM instruments by the method described here may have significant potential value and should be the subject of future research, but this is beyond the scope of this thesis.

Acknowledgements

This chapter is a reprint of the material in *Microscopy and Microanalysis* (Zotta et al., 2018) with modifications to improve readability and integration into this thesis. I was the co-author of this paper, along with Matthew Zotta. I contributed all results reported in this paper, except the results on electron source type (contributed by Matt). Matt contributed the mathematical discussion and the creation of the Aura Workstation used to produce the results. Additional contributions to the literature review and the fundamentals of this technique were made by Dr. Eric Lifshin and Prof. Richard Hailstone.

We wish to recognize Mr. Jeffrey Moskin, President of Nanojehm, Inc. for making company resources available for some of the measurements and software used in this study.
4 Parameter Space Exploration: Effect on the Point Spread Function

As shown in Chapter 3, the PSF of the SEM can be determined using the Wiener filter method. The PSF can then be used in applications such as microscope characterization and image restoration. But many parameters affect the final representation of the PSF, and practices for parameter estimation led to a previously unstudied amount of uncertainty in the PSF size and shape. This chapter investigates the uncertainty in the PSF due to various parameters involved in the PSF determination process.

The parameters under scrutiny are involved in the data collection process and the PSF calculation in Aura, including signal type, particle support material thickness, reference particle size, PSF smoothing ($K$), and background correction. Simulations of electron trajectories were performed in CASINO for validation of experimental results.

The main findings of this chapter are as follows:

- Differences in detector position between the observed particles and the method’s simulated reference particles caused shifting and possible minor shrinkage in secondary electron (SE) PSFs compared to backscattered electron (BSE) PSFs.

- Particle support material thickness did not have a practical effect on the PSF at the tested voltages.

- Uncertainty in reference particle size varied the PSF full width at half maximum (FWHM) within ±0.7nm at $2\sigma$, with virtually no uncertainty in some cases.

- The best estimates for $K$ and background correction within a reasonable range of values resulted in PSF FWHM differences within ±0.9nm, except at 2kV for $K$ with an upper
bound of ±1.9nm which may be due to increased noise. Customizing the selection of \( K \) and background correction case-by-case would result in smaller differences than those reported here. The noticeable interconnection of these parameters may help constrain the problem of calculating their best selection in future efforts.

These results are especially important for those wishing to characterize the performance of their microscope using the method presented in Chapter 3. This chapter (Chapter 4) also provides a basis for the extension of the parameter space exploration given in Chapter 5. Chapter 5 will provide further insight for those intending to use the presented method for the purpose of image restoration through PSF deconvolution.

### 4.1 Introduction

The point spread function (PSF) of an imaging system describes how a point of light is transformed by the system during imaging, for instance by the focusing elements. An ideal PSF (i.e., delta function) would output a perfect image representing the point of light. But a real-life imaging system has a non-ideal PSF, which causes the image to be distorted or blurred. If we understand how the imaging system distorts the image, not only will we understand our system better, we could remove distortions from the image in an informed manner.

The electron probe in the scanning electron microscope (SEM) is an image of the electron source (at crossover), but modified by the electron optics and their aberrations. This probe is focused and scanned across the surface of a sample for imaging. The PSF of this system can be thought of as a visualization of the beam shape (excluding the contribution of the electron-sample interaction). Having knowledge of the size and shape of the beam allows us to investigate aspects of SEM imaging previously inaccessible experimentally (e.g., astigmatism (Nevins et al., 2018; Zotta et al., 2018)), characterize the microscope and its focusing elements, restore information to images which was lost due to blur and distortion (Lifshin et al., 2017), and has potential use in microscope maintenance (e.g., filament replacement and comparison).

Chapter 3 presented a technique for PSF determination in the SEM, providing a novel, full visualization of the beam. The authors used the Aura Workstation, developed by Nanojehm, to perform PSF determination and image restoration, as does our approach. The method involves imaging a field of spherical nanoparticles of known size (“calibration image”), and then comparing
the average particle of the field against a simulated reference particle of the known size. The PSF is calculated using a Wiener filter, which in a basic sense compares the average observed particle to the reference particle. Details on the mathematics behind this technique were discussed in-depth in Chapter 3 and Lifshin et al. (2014). The authors presented various applications and measurements using this technique, including visualizations of PSF due to changes in working distance and astigmatism, comparison of electron source type, and an example of image restoration for resolution improvement.

In the Aura software, the user has the ability to optimize particle detection in the calibration image, as well as select values for certain parameters in the PSF determination process. The user can choose the calibration image signal type, reference particle size, PSF smoothing ($K$), and PSF background correction. These parameters affect the appearance and accuracy of the resulting PSF, but are not yet fully understood. Although this PSF determination method shows great potential, these parameters require further investigation to improve our understanding of the accuracy of the determined PSF (in terms of shape and size), the limitations of the technique, and the interpretation of its results.

Part 1 of this investigation is presented in this chapter (Chapter 4), and explores how the PSF is affected by various parameters involved during data collection and PSF calculation. The parameters studied here include signal type (i.e., backscattered electron (BSE), secondary electron (SE)), calibration sample support material (i.e., thin carbon film, thick carbon block), reference particle size, PSF denoising and smoothing ($K$), and PSF background correction. These parameters interact and intertwine to produce the output PSF, so care was taken to isolate parameters under scrutiny.

Part 2 of this investigation is presented in Chapter 5 and will extend to an important application of PSF determination: image restoration through PSF deconvolution, and studying how these parameters effect the quality of the restoration.

Some parameters involved in this PSF determination method have been touched on in previous work, including the brightness and contrast of the calibration image (Zotta 2016), number of particles in a calibration image (Appendix A.3), and variability of the PSF between calibration images (Chapter 3).
The results presented in this chapter are meant to act as a point of reference to base further in-depth investigations of individual parameters, especially for the purpose of devising educated procedures to select parameters like $K$ and background correction.

4.1.1 Selection of Parameters for Study

4.1.1.1 Signal Type

BSE mode has been favored for capturing calibration images (Zotta et al., 2018) because it has the capability to display image contrast based on a sample’s elemental composition. This is useful for the calibration samples, which is composed of high atomic number ($Z$) gold nanoparticles dispersed on low $Z$ carbon substrate. The disparity in $Z$ gives good contrast for particle detection by Aura. BSE mode minimizes the contribution of background signal, especially for thin film carbon support. BSE mode is also less sensitive to contamination effects, which could increase the size of particles in SE mode.

SE mode is widely used in the SEM community, so it is vital to understand if and how the PSF is affected by differences in signal generation between BSE and SE modes. Image generation differs between these signal types. BSE electrons are beam electrons which have scattered in the sample material and resurfaced. More high-angle scattering occurs in materials with higher $Z$, which creates more BSE signal. As explained by Goldstein et al. (2018), SE electrons were once bound to sample material atoms, and the atoms received enough energy from the scattering beam electrons to eject an electron. SEs can move and scatter through the material, but only those generated within a few nanometers of the surface emerge from the sample surface to be collected by a detector for imaging. Notably, SE electrons have lower energies than BSE electrons, traditionally defined <50 eV (Goldstein et al., 2018). This makes SE imaging desirable for imaging the sample surface, while the higher energy electrons used in BSE imaging can escape from deeper in the sample and better distinguish spatial variations in the elemental composition.

Another factor to be aware of when imaging with these two signals is that the BSE and SE detectors may be in different positions and orientations. With our particular setup, detector position and orientation causes signal to be collected more-or-less symmetrically from all sides of the sample by the overhead BSE detector, and asymmetrically by the side-mounted SE detector, where
more signal will be detected from the sample surfaces facing the detector (i.e., non-isotropic edge brightening) (Figure 4-1).

Differences in sample feature size between BSE and SE have been noted in prior literature, both experimentally (Osawa et al., 1999) and theoretically (Postek et al., 2016). We noticed this difference in our own calibration images (Figure 4-2). This could cause differences in calibration particle appearance and size. Particle size is important in the PSF determination method because the PSF is calculated by comparing the average observed calibration particle to a simulated reference particle. We examined the effect of these signal types on observed particle size through CASINO simulation and experimental data.

4.1.1.2 Particle Support Material

In the Chapter 3, the PSF determination method presented here primarily used calibration samples of spherical gold nanoparticles dispersed on thin carbon film (~3nm) on grids used in transmission electron microscopy (TEM). This was done to minimize any possible contributions to the PSF from the interaction volume, in an attempt to isolate the shape of the beam.

Many samples imaged with the SEM have non-negligible thickness (>3nm), so it is important to understand what role the interaction volume involving the substrate plays in the output.
PSF. For example, in image restoration applications, the same beam shape is used to image the calibration sample and sample-of-interest. The PSF is then used to restore images of the sample-of-interest through PSF deconvolution. How would the difference in thickness between the calibration sample and sample-of-interest affect the PSF and corresponding image restoration?

4.1.1.3 Reference Particle Size

The average observed particle from the calibration image is compared to a simulated reference particle to determine the PSF, which makes the size of the reference particle quite important. In Aura, the user has the ability to select the reference particle size. This reference particle is used as the “true” representation of the particle for comparison with the average observed particle from our SEM calibration image. The reference particle is the result of a CASINO (www.gel.usherbrooke.ca/casino/) simulation, and it is selected to match the calibration particle size and the signal type with which the calibration image was captured.

What size do we select for the reference particle? The calibration particles were measured in TEM images using ImageJ, and the average diameter was taken to be the reference particle size. But this measurement has a degree of uncertainty to it. How does this uncertainty in reference particle size affect the PSF size?

Figure 4-2. Visual Comparison of BSE and SE Modes. Magnified SEM images of 28.5nm gold nanoparticles on Kapton substrate were taken at 20kV in BSE (left) and SE (right) modes, captured simultaneously. The difference in particle size between the signal types is visually apparent, where the particles in SE appear larger than the same particles in BSE. The same scale bar applies to both images.
4.1.1.4 $K$

$K$ is used to balance noisiness and accuracy in the PSF (see Chapter 3 Materials and Methods subsection 3.2.4 The PSF Calculation and Visualization for more detail). As $K$ decreases, the PSF size decreases and the level of noise increases. Why don’t we use the lowest $K$ available, then? Eventually, the PSF can lose its integrity and become lost in the noise. Increasing $K$ helps with noise reduction, but it can cause overestimation of PSF size and loss of shape. Thus, finding the right balance is critical to accurately representing the PSF.

4.1.1.5 Background Correction

Background correction is used to reduce or remove artifacts from the PSF determination process, including background structures and ringing. Background correction levels are chosen to mitigate these artifacts while maintaining the PSF. This level is usually apparent to the user while sliding through correction levels in Aura, but sometimes the level can be ambiguous. Selecting too low a background correction can result in an overestimated PSF size, while selecting too high a background correction can result in an underestimated PSF size. Therefore, it is important to understand how the variation in background correction affects the PSF size.

4.2 Materials and Methods

4.2.1 Calibration Samples for SEM PSF Determination

The calibration samples from Nanojehm included spherical gold nanoparticles with 30nm nominal diameter dispersed on carbon material supports. The diameters of one hundred particles were measured in TEM images using the measurement tool in ImageJ. Particle edges were determined by eye. The measured size distribution came out to be 28.5±1.2nm ($\mu$±1$\sigma$). The particles were separately dispersed on both a TEM grid with thin carbon film support (~3nm) and a thick Kapton substrate (~25µm) which has a high carbon content and lesser amounts of oxygen and nitrogen.

The particles dispersed on Kapton were plasma cleaned prior to imaging. The particles were freshly dispersed on the TEM grid, having not seen any beam exposure prior to imaging for this study. These factors should have minimized the contributions of contamination.
SEM images of the calibration samples were collected at 1nm/px scale throughout this chapter.

### 4.2.2 TEM Magnification Calibration and Particle Sizing

Images for calibration particle size measurement were collected using a JEOL-2010 TEM with LaB₆ filament at 200kV with 340kX magnification. But how well do we know the measured particle size? To answer this, we calibrated the TEM magnification using the MAG*I*CAL standard from Technoorg-Linda Ltd. Forty measurements of the TEM standard were taken and compared to the calibrated value given by the manufacturer. Combining the uncertainty in the calibrated value (±2% at 2σ) with the uncertainty in our measured values in ImageJ (~±2% at 2σ) by adding the variances, we get a magnification uncertainty of approximately ±3% at 2σ. This results in an uncertainty on the mean particle size of ±0.9nm (at 2σ).

### 4.2.3 SEM

Images were collected using a TESCAN MIRA3 Schottky field-emission SEM with a retractable, overhead BSE detector and a side-mounted Everhart-Thornley SE detector. BSE and SE images were captured simultaneously of the same field. Due to the position of the BSE detector, the working distance (WD) was limited to ~7-8mm for much of the data collected. Smaller beams, and therefore PSFs, would be achievable with shorter WDs. Voltages were chosen to test PSF determination over a range for typical operation. Doing so, we could observe particle appearance and PSF behavior between low and high voltages. Based on the capabilities of our BSE and SE detectors and the calibration samples, the tested voltages include 20kV, 10kV, 5kV, and 2kV.

The smallest probe size for the given operating conditions was determined using the MIRA’s software by setting spot size to “0”, which activates the software’s calculation of an optimal minimum probe size. This affects the probe current, and therefore the signal-to-noise ratio of the collected image. Opting for smaller beams means lower probe currents and more noise, so images (and PSFs) presented here may have higher amounts of noise than if a larger probe current were employed. The spot size was determined using the grid sample for all voltages.

The SEM magnification calibration was also tested using the MAG*I*CAL calibration standard. The standard was imaged at maximum magnification (1MX) in BSE mode at 11mm WD
with a 20kV beam. BSE was chosen due to the nature of the MAG*I*CAL standard, which has lines of calibrated width made of contrasting elemental composition. The variance of the line width measurements in the SEM was larger than the variance given by the TEM measurements. Taking intensity profiles across these lines give us a clue as to why this may be (Figure 4-3).

The lines have a sharper cut off in the TEM than the SEM, which means measurements of the TEM line widths could be made more reproducibly in regards to selecting the location of the line “edge” (based on pixel intensity). To test this, we applied an approximated Gaussian PSF to the TEM calibration image as if we scanned an SEM beam over the TEM image. The approximated PSF matched the SEM probe size reported by the MIRA. The contrast and noise were also altered to mimic the SEM image. Interestingly, the newly modified TEM image gave line width

![Figure 4-3. Intensity Profile Comparison of MAG*I*CAL Line Widths in TEM and SEM. Top Row: Magnified region of the MAG*I*CAL sample is shown in SEM, TEM, and TEM with image processing to mimic conditions of SEM. Similar region used between SEM and TEM. TEM image intensities were inverted before measuring or profiling for a more-direct comparison. Each image in the top row is a 172nm x 172nm field. Bottom Plot: A plot comparing the average horizontal line profiles across the MAG*I*CAL lines is shown. The legend entries match the images in the top row. “B/C” means brightness and contrast.](image)
measurements with a variance similar to that of the original SEM image. A visual comparison can be seen in Figure 4-3.

4.2.4 Particle Sizing and Profiling in SEM

Initially, particles in SEM images were sized by hand with ImageJ (similar to the TEM particle measurements described under Materials and Methods subsection 4.2.1 Calibration Samples for SEM PSF Determination), with approximately 100 particles sized in a single image. We intended on using these sizing results to compare imaging conditions. While imaging conditions were controlled to have similar brightness and contrast, it became apparent that this was not sufficient when comparing signal types (i.e., BSE and SE) and different particle supports (i.e., thin carbon film and thick Kapton substrate). We were concerned that differences in brightness and contrast between images could affect particle sizing by human eye. Another method for particle sizing was pursued in the attempt to verify our results.

Next, particles were sized according to the procedure and practices laid out in ISO 13322-1 for particle size analysis in static images (ISO 2014). A MATLAB script was created to execute the outlined procedure and provide particle size measurements to verify the initial ImageJ findings. After trying a few thresholding techniques, MATLAB’s built-in graythresh function (Otsu, 1979) was chosen for its simplicity and generality. But even using this ISO standard, the issue remained where an intensity threshold needed to be adjusted by the user when the program could not determine an appropriate one. This left the human eye’s judgement of brightness and contrast to possibly bias the particle size measurements.

Finally, particle profiling was considered and performed as a simple check for comparing particles between images. This method eliminated the need for human-chosen intensity thresholds to determine particle edges. Particle profiling was performed in MATLAB. Isolated single particles within an image were detected using the methods described in ISO 13322-1. The centroid of each particle was calculated using MATLAB’s regionprops function, and then profiles were taken at various directions and angles through the centroid. The resulting profiles displayed pixel intensity as a function of position on the particle. Each detected particle was profiled, and the same-direction profiles (e.g., all the horizontal profiles) from all detected particles in the image were averaged. This provided an average particle profile for a particular direction and angle.
Profiling can show the relationship of particles between imaging conditions, as well as complement the ImageJ and ISO size measurements, which have been included in Appendices C.1 and C.2. To minimize the contribution of anisotropic edge brightening to the SE profiles, the profile was taken approximately perpendicular to the orientation of the SE detector. Line profiles were background-corrected and normalized, so that the maximum intensity in the profile became intensity “1” and the new background level became approximately intensity “0”. This normalization allowed for easier visual comparison of profiles from separate images.

4.2.5 PSF Determination

We used the Aura Workstation (v0.64) distributed by Nanojehm, Inc. to calculate our PSFs. PSF determination can be thought of as split into two parts, one being data collection with the microscope, and two being PSF determination and processing with the software. The parameters investigated in this chapter are listed where applicable.

1. Collect an SEM image of the calibration sample (which accompanied the Aura Workstation) for PSF determination purposes, choosing parameters:
   a. Signal type
   b. Particle support
   c. Beam voltage

2. Input the calibration image into Aura to perform PSF determination, where observed particles from the image in bullet (1) are stacked (averaged) and compared to a reference particle by Wiener filtering, choosing parameters:
   a. Reference particle size
   b. K
   c. Background correction

The results shown in this chapter were produced by the Wiener method of PSF determination. Please see Chapter 3 for the mathematics which fuel this method.

4.2.6 Parameter Space Exploration

Analysis involving the comparison of particle profiles and sizing of PSFs was performed in MATLAB. According to Reimer (1998), a well-focused electron probe in the SEM can be approximated as a Gaussian. The Gaussian fit was tested against Lorentzian, Airy, and sinc function fits at multiple voltages, and the Gaussian was shown to be the most applicable (Zotta et
al., 2018). The full width at half maximum (FWHM) of the Gaussian can be used to characterize the size of the probe. To estimate the PSF FWHM, a 2D Gaussian was fit to the PSF using MATLAB’s curve fitting tool, and the FWHM was calculated using the x- and y-axis standard deviations of the fit Gaussian.

4.2.6.1 Particle Support

The calibration sample supports have been described previously (see Materials and Methods subsection 4.2.1 Calibration Samples for SEM PSF Determination).

At a particular voltage, the beam was focused on the grid sample (using wobbler, WD, and astigmatism) and a calibration image captured. The Kapton sample was imaged immediately afterward with the same beam shape. The only change made was to stage height for focusing, to account for differences in sample height.

4.2.6.2 Signal Type

Images were collected of the same field simultaneously with BSE and SE detectors. This ensured the same imaging conditions in both BSE and SE images. Brightness and contrast were adjusted manually in an attempt to produce similar levels between the two signal types. This was not always achievable due to differences in signal generation, but the particle profiling analysis is robust to this effect. Calibration particles from the BSE and SE images were profiled for comparison. PSFs determined from the calibration images were also compared.

4.2.6.3 Reference Particle Size

For a single SEM calibration image of particles (i.e., observed image), we created multiple PSFs by using multiple reference particle sizes over a pre-determined range. We performed this for each voltage-signal-support case (e.g., 20kV BSE grid). The range of tested sizes was based on the TEM magnification calibration and its uncertainty (see Materials and Methods subsection 4.2.2 TEM Magnification Calibration and Particle Sizing). The mean size of the particle dispersion is known within ~±3% (at 2σ), which gave us a range of 28.5±0.9nm.

Note that the range does not account for the calibration particles having a distribution of sizes. The ~±3% range simply focuses on the mean size of the distribution. In theory, the particle stacking routine in the Aura software outputs the average observed particle in a calibration image.

For each calibration image voltage-signal-support case, background correction and K were held constant to isolate the reference particle size variable. This let us observe how the reference
particle size affected the PSF in each case, while providing best estimates for background correction and $K$ on a case-by-case basis; doing so enables better comparison between voltages, signal types, and particle support materials, which can require different background correction and $K$ to achieve similar PSF quality.

Except for this study on reference particle size, the mean particle size (i.e., 28.5nm) was used when determining other PSFs presented in this chapter.

4.2.6.4 $K$

In the Aura software, the user has the ability to select a $K$ value which best balances PSF noisiness and accuracy. To test the effect of $K$ on the PSF, PSFs with varying $K$ were generated for single calibration images. A best estimate for $K$ was chosen for an image based on the visual balance of noise and accuracy. For repeatability, $K$ was selected from the default values available using the Aura $K$ slider. After deciding on the best estimate, the default values on either side of this initial $K$ (i.e., one increment smaller and one larger) were also tested.

To isolate the $K$ variable, reference particle size and background correction were held constant for each PSF voltage-signal-support case. This allowed us to study how the selection of $K$ influenced the PSF in each case, while maintaining the ability to select the best estimate for background correction. Background correction was selected case-by-case to improve intuition when comparing different voltage-signal-support cases, which can require different background correction levels to output similar PSF behavior.

4.2.6.5 Background Correction

PSFs may contain background structure and ringing which are not part of the PSF itself, but rather an artifact from the mathematics and computational processing. For this study, background correction levels were estimated by eye to reduce or eliminate the aforementioned artifacts, while maintaining the PSF itself. Then correction values surrounding that level were tested, to see how the PSF was affected.

While Aura does perform background correction, it cannot currently save the PSF with the selected background correction level. So, we simulated the background correction process in MATLAB. Basically, when the new background level is chosen, any intensity values below that level are removed, and the PSF is then normalized so its new lowest intensity level is set to “0” while the max intensity level remains at “1”.
Reference particle size and $K$ were held constant for each PSF voltage-signal-support case to isolate the background correction variable. This allowed us to study how the background correction level affected the PSF in each case, while being able to choose the best estimate for $K$ for each case. Selecting $K$ for each case allows for simpler comparison between voltage-signal-support cases, which can require different $K$ values to generate similar PSF quality.

4.3 Results and Discussion

4.3.1 PSF Calculation Parameters

4.3.1.1 Reference Particle Size

The observed particle is compared to the simulated reference particle to determine a PSF for the imaging system. If the observed particle is held constant, and the reference particle size is changed, the output PSF size is expected to change accordingly. If the reference particle size is smaller than the observed particle size, then the PSF is expected to be larger. It would be as if the PSF caused the image of the particle to appear larger than the reference. With similar reasoning, the output PSF is expected to be smaller when the reference particle size is increased.

The results of this type of test are shown here. For a single observed image of particles, multiple reference particle sizes were used to produce PSFs. This allowed us to study how the uncertainty in our reference particle size measurement affects the PSF size. The PSFs from a single image were overlaid in contour plots to compare shape, and FWHMs were calculated to compare size. Results from the grid and Kapton images are shown in Figure 4-4 and Figure 4-5, respectively. In some cases, differences in PSF shape can be seen in the FWTM contour more than the FWHM contour. This is due to the background correction remaining constant over the range of reference particle sizes. Lower pixel intensity levels, such as those around the FWTM, are more sensitive to this effect.

In general, the PSFs follow the expectation that smaller reference particles result in larger PSFs. Interestingly, the expectation that larger reference particles results in smaller PSFs only holds true some of the time. In some cases, the trend in PSF FWHM is linear and decreasing, as we expect when increasing reference particle size (e.g., grid 20kV SE). In other cases, the change in FWHM size tapers off (e.g., Kapton 5kV BSE), or the FWHM increases as reference particle size increase (e.g., grid 10kV BSE). Because this behavior seems centered around the mean particle
size (28.5nm), it is attributed to the constant background correction value over the range of reference particle sizes. The background structure brightened as reference particle size increased. This appeared to “lift” the PSFs in intensity, as part of Aura’s normalization routine. Because the same background correction was used for each PSF, the correction did not overcome the brighter PSF background. This would account for the PSFs from larger reference particle sizes appearing larger, when the opposite is expected.

Figure 4-4. Reference Particle Size Range PSF Contours and FWHMs on Grid. Contour plots show FWHM and FWTM contours (inner and outer, respectively). The rainbow of colors from red to purple and then black represents reference particle sizes 27.6nm, 27.9nm, 28.2nm, 28.5nm, 28.8nm, 29.1nm, and 29.4nm. The axis labels on the top left contour plot applies to all contour plots, and similarly for the FWHM plot on the top mid-left. The 2kV SE calibration image did not have enough contrast between the particles and support material to detect particles.
The uncertainties in PSF size based on uncertainty in the reference particle size are shown in Table 4-1. The differences in upper and lower uncertainty are a result of the maximum differences in PSF size relative to the PSF FWHM for the mean reference particle size (i.e., 28.5nm), as shown in Figure 4-4 and Figure 4-5.

PSF FWHMs differed at most by 0.7nm (~2%, at 2σ bounds), seen in the expected linear behavior cases (e.g., grid 20kV SE). In other cases, virtually no change was experienced in PSF

Figure 4-5. Reference Particle Size Range PSF Contours and FWHMs on Kapton. Contour plots show FWHM and FWTM contours (inner and outer, respectively). The rainbow of colors from red to purple and then black represents reference particle sizes 27.6nm, 27.9nm, 28.2nm, 28.5nm, 28.8nm, 29.1nm, and 29.4nm. The axis labels on the top left contour plot applies to all contour plots, and similarly for the FWHM plot on the top mid-left. The 2kV BSE calibration image did not have enough contrast between the particles and support material to detect particles.
4.3.1.2 $K$ and Background Correction

To visualize the effects of $K$ and background correction on the PSF, PSFs were generated for the same voltage-signal-support case using a range of values for both parameters (Figure 4-6). The FWHMs for the PSFs in Figure 4-6 are plotted in Figure 4-7. Results for other voltage-signal-support cases than the one shown here are given in Appendix C.3.

At low $K$, the PSF is noisy and can become obscured, especially at low voltage. As $K$ increases, the PSF coalesces and becomes smoother. But, as $K$ becomes too large, it overcompensates for the noise with too much smoothing. This results in the exaggeration of PSF size and in the loss of PSF shape, as its features are smoothed over.

With no background correction, artifacts like background texture and ringing are apparent. PSFs with not enough correction retain residual artifacts, while PSFs with too much correction become undersized, leading to deceptively small FWHMs. A background level should be chosen to balance artifact mitigation and PSF preservation.

Mathematical differences between reasonable selections of $K$ and background correction were calculated and are given in Table 4-2 and Table 4-3, respectively. Values for differencing were based on prior experience with the method. With user experience, $K$ is usually distinguishable within one default value increment in Aura. Likewise, background correction is typically estimable within ±0.05 pixel intensity level (where normalized PSF intensity ranges from [0, 1]).
CHAPTER 4. PARAMETER SPACE: EFFECT ON THE PSF

Figure 4-6. Visualization of K Selection and Background Correction on the PSF. The 5kV BSE on Kapton case is shown here (see FWHMs in Figure 4-7), where the estimated best representation of the PSF appears around \( K = 100 \), background correction = 0.25. The horizontal axis represents the default values of \( K \) selectable in Aura. The vertical axis represents the background correction level, where the PSF intensity is scaled from [0, 1]. The top row contains the original PSFs with no background correction.

Figure 4-7. PSF FWHMs over a Range of \( K \) and Background Correction. The 5kV BSE on Kapton case is shown here (see PSF images in Figure 4-6), where the estimated best representation of the PSF appears around \( K = 100 \), background correction (“BC”) = 0.25. Note that the vertical axis is linear, while the horizontal axis is logarithmic. For Table 4-2, the FWHM values used for calculation include the best \( K \) estimate FWHM and the FWHMs found by moving right and left one “△” (\( K \) value) on the horizontal axis (along background correction = 0.25). For Table 4-3, the FWHM values used for calculation include the best background correction estimate FWHM and the FWHMs found by moving up and down one “■” (background correction level) on the vertical axis (along \( K = 100 \)).
For all cases presented in Table 4-2, the lesser $K$ FWHM is closer to the best $K$ estimate FWHM than the upper $K$ FWHM. It may be that the eye chooses to balance noise and smoothing when the PSF has finally converged out of the noise, but has not yet gained excess girth. Based on the shape of the curves presented in Figure 4-7, this may be the region of the curve where it transitions from a shallower slope to a steeper slope (going left to right).

Table 4-3 shows that the SE PSFs are more sensitive to changes in background correction than BSE PSFs. In general, the SE PSFs appear noisier than the BSE PSFs (see Results and Discussion subsection 4.3.2.1 Data Collection Parameters: Signal Type), especially the background texture. Extra noise and a larger signal contribution from the support material could explain the heightened sensitivity.

The best estimates for $K$ and background correction within a reasonable range of values resulted in PSF FWHM differences at or below 0.9nm, except at 2kV where $K$ has an upper bound of 1.9nm, which may be due to increased noise. Note that by intelligently adjusting the

**Table 4-2. PSF FWHM Differences based on Selection of $K$.** PSF FWHM differences [nm] are rounded to the nearest 0.1. The differences shown here are relative to the PSF FWHM for the best $K$ estimate and the two nearest default $K$ values. For example, if $K = 100$ is the best estimate, the differences shown are $K=31 – K=100$ FWHMs (left); and $K=316 – K=100$ FWHMs (right), respectively in the table column (see Figure 4-7).

<table>
<thead>
<tr>
<th>Beam Energy</th>
<th>Grid</th>
<th>Kapton</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSE</td>
<td>SE</td>
</tr>
<tr>
<td>20kV</td>
<td>-0.3, +0.4</td>
<td>-0.5, +0.6</td>
</tr>
<tr>
<td>10kV</td>
<td>-0.4, +0.4</td>
<td>-0.6, +0.7</td>
</tr>
<tr>
<td>5kV</td>
<td>-0.5, +0.6</td>
<td>-0.7, +0.8</td>
</tr>
<tr>
<td>2kV</td>
<td>-0.8, +0.9</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**Table 4-3. PSF FWHM Differences based on Selection of Background Correction.** PSF FWHM differences [nm] are rounded to the nearest 0.1. The differences shown here are relative to the PSF FWHM for the best background correction (BC) estimate and ±0.05 intensity level. For example, if BC = 0.20 is the best estimate, the differences shown are BC=0.25 – BC=0.20 FWHMs (left); and BC=0.15 – BC=0.20 FWHMs (right), respectively in the table column (see Figure 4-7).

<table>
<thead>
<tr>
<th>Beam Energy</th>
<th>Grid</th>
<th>Kapton</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSE</td>
<td>SE</td>
</tr>
<tr>
<td>20kV</td>
<td>-0.3, +0.4</td>
<td>-0.5, +0.6</td>
</tr>
<tr>
<td>10kV</td>
<td>-0.4, +0.4</td>
<td>-0.6, +0.7</td>
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<tr>
<td>5kV</td>
<td>-0.5, +0.6</td>
<td>-0.7, +0.8</td>
</tr>
<tr>
<td>2kV</td>
<td>-0.8, +0.9</td>
<td>n/a</td>
</tr>
</tbody>
</table>
combination of $K$ and background correction, similar PSF sizes and artifact mitigation are achievable. While this provides multiple estimated solutions of the PSF with variations in noise and shape, it also suggests that an interdependence exists between the $K$ and background correction parameters, which may help constrain the optimization of their selection.

### 4.3.2 Data Collection Parameters

#### 4.3.2.1 Signal Type

For each voltage-signal-support case, BSE and SE images were captured simultaneously of the same field of calibration particles (Figure 4-2). Results of particle profiling from the observed images can be seen in Figure 4-8. These are corroborated with the results of particle profiling from CASINO simulations in Figure 4-9. A direct comparison of particle images and profiles between simulation and observation is given in Figure 4-10. In these figures and those following them, there are no profiles or PSFs for the 2kV grid SE and Kapton BSE cases due to poor contrast between the particles and support material.

As voltage decreases, the probe size increases, which causes the particle to appear larger. This behavior is reflected in both observation and simulation. As beam voltage decreases, the electron probe size increases with increasing electron wavelength due to diffraction and chromatic aberration (Goldstein et al., 2018). At lower voltages, the simulated profiles appear somewhat smaller compared to their observed counterparts. The simulations also show larger dips at the edges of the particle, compared to the observed.

The SE profiles have a plateau-like shape, especially at high kV, while the BSE profiles do not show this behavior. The simulations predict similar profile shapes, although with accentuated edge brightening in SE. The enhanced edge brightening in the simulations is the result of using a high number of electrons to achieve high signal. Simulations using less electrons displayed less edge brightening. The observed SE images of the particles did show directional edge brightening, and the profile’s orientation was chosen to be approximately perpendicular to the SE detector’s optical axis to minimize this effect. The simulation assumes the SE detector is overhead. So if the real-world SE detector were stationed overhead, instead of side-mounted, the observed edge brightening would be symmetrical about the particle as shown in simulation.

To compare signal types, the observed BSE and SE particle profiles were overlaid (Figure 4-11). The SE particles are larger than the BSE particles at all testable voltages. No profiles were
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Figure 4-8. Average Observed Particle Profiles at Multiple Voltages. The legend in the top left plot (Grid BSE) applies to all plots. The direction of profiles shown here is approximately perpendicular to the orientation of the SE detector to reduce the appearance of directional edge brightening.
Figure 4-9. Simulated Particle Profiles at Multiple Voltages. The legend in the top left plot (Grid BSE) applies to all plots. To create the profile, 100k electrons trajectories were simulated at each pixel to improve signal (similar to averaging the observed particles).
Figure 4-10. Observed and Simulated Single Particle Images and Average Profiles on Kapton. Observed and simulated images for single particles are shown for each voltage-signal case to provide visual comparison of what a calibration particle would look like while imaging. The average profiles are shown to compare observation and simulation, while taking advantage of lower noise levels for simpler comparison. The solid line (−) represents the observed data, and the star line (*) represents the simulated data.
taken in the 2kV grid SE or Kapton BSE cases due to low contrast between the particle and background, so a comparison of BSE and SE could not be done at that voltage. The simulated profiles exhibit the same behavior as the observed profiles, as can be seen in Appendix C.4. The simulations also show SE particles appearing larger than BSE particles at 2kV.

The observed SE profiles appear shifted compared to the BSE profiles. As mentioned previously, the SE particle profiles exhibit directional edge brightening, in which the pixel intensity on one side of the particle is brighter and gradually decreases across the particle. When the centroids of the particles were calculated (for particle detection and profile averaging), the brightening of one side of the particle shifted the centroid towards the SE detector position. Because all the SE particles were affected this way, the average of the SE profiles is still valid, but may be shifted compared to the BSE profile.

For each profiled image, PSFs were generated over the reference particle range to incorporate that uncertainty into our visualization. Plots of FWHM and FWTM contours of BSE and SE PSFs were created for inspection (Figure 4-12).

SE PSFs appear shifted compared to BSE PSFs, which is the result of differences in detector position (similar to the shift in profiles in Figure 4-11). The PSF will be affected by the difference between the observed and reference particles, in this case manifesting in the form of a shift. Some of the SE PSFs have noisier FWTM contours than their BSE counterparts, although the FWHM noise levels appear similar and are a better point of comparison for PSF size. Even with the shift and noise, it is evident that the BSE and SE PSFs are of similar shape.

In general, these plots suggest that SE PSFs are larger than BSE PSFs for the current method, when they should be the same size. This could be due to various reasons, including the contribution of the interaction volume, the reference particle simulation, and the selection of other parameters (e.g., $K$, background correction). BSE and SE particle electrons emerge from different parts of the interaction volume. This implies that the electron-sample interaction should be included as a contributor to the PSF. This follows imaging intuition because the electron-sample interaction is part of the imaging chain, and therefore part of the output image. But, shouldn’t the selection of BSE or SE reference particle in Aura mitigate the effect of the interaction volume? This implies that the CASINO simulation used to generate the reference particle needs to be investigated more thoroughly when it comes to differences in signal type and detector orientation. The simulations for BSE particle profiles do appear to be more similar to observation, than the SE
Figure 4-11. *Signal Comparison using Average Observed Particle Profiles*. The legend in the top left plot (20kV Grid) applies to all plots. BSE and SE modes are compared at multiple voltages on both TEM carbon grid and Kapton substrate supports. The direction of profiles shown here is approximately perpendicular to the orientation of the SE detector to reduce the appearance of directional edge brightening.
Figure 4-12. *BSE and SE PSF Comparison over a Range of Reference Particle Sizes.* The contours for PSF FWHM and FWTM are shown, as inner and outer, respectively. The axis labels and legend in the top left plot (20kV Grid) applies to all plots.
shape, which includes taller peaks and a sharper cut-off at the particle edge (Figure 4-10). Otherwise, selection of $K$ or background correction could affect the relative sizes of the BSE and SE PSFs. For validation, this study should be repeated once a better way of selecting $K$ and background correction is determined.

4.3.2.2 Particle Support Material

Thin and thick carbon supports (TEM grid and Kapton, respectively) were compared with particle profiling (Figure 4-13) and PSF contours (Figure 4-14) as in the previous subsection on signal type comparison. Similarly, the CASINO simulations match the behavior shown in the observed data and have been included in Appendix C.4.

The average profiles between grid and Kapton are quite similar in both BSE and SE modes at the testable voltages. The SE profiles are noisier, especially for the grid, but even so the profiles are similar to the Kapton SE profiles. The simulated profiles display the same behavior, including at 2kV, as can be seen in Appendix C.4.

The PSFs between grid and Kapton are also similar in most cases, particularly when viewing the FWHM. An apparent difference in PSF shape can be seen in the 5kV case. This may be due to non-optimal focusing between the two samples, which can be seen in the observed particles in Figure 4-10. The Kapton particle is somewhat smudged on the right-hand side. Average particle images for the grid have been included in Appendix C.5 for comparison. The grid sample was imaged first at best focus, and then without altering the beam, the Kapton sample was focused by changes to stage height only.

Because the profiling shows little difference between the grid and Kapton particles, we would expect the PSFs to be similar, which they are in general. Unfortunately, profile and PSF comparison of observed data at 2kV was not possible due to poor contrast. CASINO simulations show that the grid and Kapton particle profiles remain quite similar in width at 2kV, suggesting that the 2kV PSFs should also be similar at that voltage. Even so, future efforts to explore the role of the interaction volume on the PSF at low voltage would be beneficial, as low voltage imaging is a prime application for image restoration with these PSFs.
Figure 4-13. *Support Material Comparison using Average Observed Particle Profiles.* The legend in the top left plot (20kV BSE) applies to all plots. TEM carbon grid and Kapton substrate supports are compared at multiple voltages for both BSE and SE modes. The direction of profiles shown here is approximately perpendicular to the orientation of the SE detector to reduce the appearance of directional edge brightening.
Figure 4-14. *Grid and Kapton PSF Comparison over a Range of Reference Particle Sizes.* The contours for PSF FWHM and FWTM are shown, as inner and outer, respectively. The axis labels and legend in the top left plot (20kV BSE) applies to all plots.
4.4 Conclusions

In this chapter, we investigated various parameters involved in the Wiener deconvolution method for determining PSFs in the SEM. These parameters are involved during data collection and PSF determination.

Our investigations of signal type comparison showed that SE particle profiles appear wider than BSE particle profiles, which agrees with previous reports (Postek et al., 2016). While the PSF determination method did incorporate the ability to select the signal type of the reference particle, some differences were seen between BSE and SE PSFs. The SE PSF appears shifted compared to the BSE PSF, which is mostly likely the result of detector position and orientation differences. This resulted in variations in edge brightening between the observed and reference particles (directional versus symmetrical, respectively). This study also suggests that the current state of the method may produce SE PSFs that are slightly larger than BSE PSFs. In the future, this PSF determination method should account for variations in edge brightening in SE calibration images, which should account for the PSF shift and may attend to the issue of size difference between signals. In practice, the shape of the PSF will be reflected in both signal types, and the user should attempt to minimize edge brightening while capturing the calibration image.

In regards to particle support material, the particle profiles and PSFs for thin and thick carbon supports (grid and Kapton, respectively) showed little difference. The contribution of the interaction volume may be smaller than our current precision with this PSF determination method, and should be revisited after optimization of software parameters is addressed. From a practical standpoint, the support material thickness does not have a major affect the PSF.

PSF determination parameters were examined individually to provide upper bounds on PSF size uncertainties and differences. The uncertainty in reference particle size varied the PSF FWHM under $\pm 0.7\text{nm}$ at $2\sigma$, in some cases causing virtually no change. In practice, the variation in PSF size caused by reference particle size uncertainty can be reduced by tailoring the background correction.

The best estimates for $K$ and background correction within a reasonable range of values ($i.e., \pm 1$ default $K$ increment in Aura, $\pm 0.05$ pixel intensity level for background correction) resulted in PSF FWHM differences within $\pm 0.9\text{nm}$, except at $2\text{kV}$ for $K$ with an upper bound of $\pm 1.9\text{nm}$ which may be due to increased noise. When selecting background correction, SE PSFs
were slightly more sensitive than BSE PSFs, which also may be due to increased background signal and a larger presence of noise. Low probe currents were employed to shrink the probe size, but higher probe currents could be used to increase signal-to-noise in practice, which may reduce these differences in FWHM. It is important to note that customizing the selection of $K$ and background correction to each PSF would result in smaller differences and uncertainties than those reported here. The interplay of these two parameters may help constrain the optimization problem of calculating the best selection.

Other parameters which deserve exploration in future efforts include scan speed, degree of focus or blur, and calibration particle size in relation to the level of magnification.

Part 2 of this investigation presented in Chapter 5 will explore the effect of the parameters explored here on this method’s application of image restoration.

Acknowledgements

This chapter has been submitted to *Microscopy and Microanalysis* for review. The version presented here has been modified to improve readability and integration into this thesis. I was the primary investigator of this work. Kathryn Quoi contributed the CASINO simulations of particle images and profiles.
5 Parameter Space Exploration: Effect on Image Restoration Quality

PSF deconvolution is an attractive software-based technique for resolution improvement in the SEM because it is able to restore information which has been blurred or distorted by challenging operating conditions (e.g., low voltage, noisy, beam-sensitive materials). In Chapter 4, the modern PSF determination method presented in Chapter 3 was explored regarding how various parameters affected the PSF shape, size, and uncertainty. In this chapter, the exploration is extended to encompass the application of PSF deconvolution for SEM image restoration.

The parameters under investigation in this chapter focus on the computation side of the process, these being reference particle size, PSF smoothing ($K$), background correction, and restoration denoising ($\lambda$). Image quality was assessed through visual qualitative inspection and Fourier analysis, with the inclusion of cross-correlations.

The main findings of this chapter are as follows:

- Low $\lambda$ corresponded to greater image sharpness at the cost of intense noise and reduced visual aesthetics. When $\lambda$ was too high, the image details were lost to over-smoothing. For the imaging conditions tested here, $\lambda = 37-57$ seemed to provide a good balance between feature preservation and denoising.

- Reference particle size within ±0.9nm had almost no effect on restoration quality.

- Differences in $K$ contributed to differences in noise level and artifacts between restorations. Fortunately, if $K$ was chosen within a reasonable range, it did not have much effect on useful image content.
• Restorations created using PSFs with background correction had superior quality compared to those using the PSF with no background correction. But, users should be cautious not to select too high a background correction, otherwise the PSF would be cut off and result in a blurrier restoration.

• Future efforts to automatically determine parameters like $\lambda$, $K$, and background correction would remove user guesswork, improve this method’s consistency, and maximize the amount of information from the PSF and restoration.

The results and practices outlined in this chapter are particularly important for those desiring to use this method for image restoration. This chapter should help users understand current best practices for this method and what to expect in image restorations, which should improve the method’s utility and the interpretability of its results.

5.1 Introduction

The electron microscopy community has endeavored to push the resolution limit of its microscopes ever since the dawn of the field. For the scanning electron microscope (SEM), technological advancements including field emission guns, stigmation, and beam deceleration techniques have allowed us to see what before went unseen. Much of the progress regarding resolution improvement has resulted from hardware-based technologies, which require microscope-specific integration. Software-based technologies could provide an image processing solution to increase resolution, and have the ability to reach a wider audience, as the methodologies are microscope independent. In addition, software-based approaches are more practical than in the past, now that computing times have become faster.

Roels et al. (2018) presented a valuable overview and comparison of state-of-the-art image restoration and enhancement techniques that have been applied to electron microscopy data. Some of the tested techniques are based on the Bayesian-inspired Richardson-Lucy method (Richardson, 1972; Lucy, 1974) for estimating a good image restoration using point spread function (PSF) deconvolution by balancing denoising with image feature preservation through iterative steps. Roels et al. showed that denoising methods, such as total variation regularization (Rudin et al., 1992), gave higher restoration quality when combined with PSF deconvolution than when employing denoising on its own.
As a brief review of PSF deconvolution, an image can be represented as the convolution of the scene being imaged and the PSF of the imaging system. The PSF describes how a single point of light travels through and is changed by an imaging system (e.g., lenses). An ideal PSF would be a two-dimensional delta function, meaning no spread to the point at all! Because real-world PSFs are non-ideal, the image we observe of our reference scene is blurred by the PSF. By measuring the PSF, this blur can be removed from the observed image in an informed manner using PSF deconvolution. This is done in the frequency domain to simplify the problem by converting the convolution operation (spatial domain) into multiplication (frequency domain).

In Chapter 4 (Part 1 of this 2-part series), we studied the PSF determination method for SEM presented by Zotta et al. (2018) and explored how various parameters in the method affected the shape and size of the PSF. SEM PSFs were determined using the Aura Workstation by Nanojehm, Inc., in which SEM imagery of nanoparticles (observed image) is compared to simulated nanoparticles from CASINO (reference image) (www.gel.usherbrooke.ca/casino/) with a Wiener filter (see Gonzalez and Woods, 2008). Tested parameters included signal type (backscattered and secondary electrons), nanoparticle support type (thin and thick carbon), reference particle size, PSF smoothing ($K$), and PSF background correction.

In this chapter (Part 2), we extend our exploration to encompass an important application of this method: PSF deconvolution for image restoration. For SEM, resolution at low voltages remains limited despite hardware advances, and finding optimal focus at low voltage can be a time-consuming challenge for even experienced users. Low voltage imaging is of particular importance for modern metrology tasks (e.g., semiconductors, nanotubes), imaging beam-sensitive samples, and imaging surface texture at high resolution. To accurately measure and interpret restored images, it is essential to understand how the parameters involved in the PSF deconvolution process affect the quality of the restored image. The parameters explored in this chapter focus on the computation side of the process: reference particle size, PSF smoothing ($K$), background correction, and restoration denoising ($\lambda$).

The quality of the restored images was evaluated using Fast-Fourier Transform (FFT) analysis and cross-correlations of FFTs to better distinguish signal from noise (Joy et al., 2000). Comments were also made regarding the visual aesthetics of the restorations. Metrics like peak signal-to-noise ratio (PSNR) and structural similarity index (SSIM) (Wang et al., 2004) were not applicable for this data, as the low voltage images did not have reference data of the same sample
region for comparison (tricky to do for tiny tin spheres!). A high voltage reference image was captured for qualitative comparison.

5.1.1 Selection of Parameters for Study

PSF determination and deconvolution are both functionalities of the Aura Workstation, and therefore it was used again for this chapter. The following software parameters are currently adjustable by the user.

5.1.1.1 Reference Particle Size

A reference particle is simulated with CASINO for the purpose of comparison with the average observed particle from the calibration image. The size of the reference particle is based on the mean particle size as given by the distributor of the particles or as measured in-house by TEM. In either case, there is some uncertainty to the mean particle size. In Chapter 4, we saw that the uncertainty in mean particle size of 28.5±0.9nm at 2σ caused variation in the PSF FWHM size under ±0.7nm at 2σ, and in some cases causing virtually no change. While in practice this variation could be reduced by tailoring the background correction, it remains beneficial to understand how the variation in the PSF due to reference particle size could alter the quality of the image restoration.

5.1.1.2 K

During PSF determination, the parameter K balances the accuracy and noisiness of the PSF. High K values result in a smoother but larger PSF (i.e., less high frequency content), and low K values in a noisier but smaller PSF (i.e., more high frequency content). The PSF can eventually lose its integrity if K is pushed too low, preventing users from always selecting the lowest K. Due to the variation in PSF size (as seen in Chapter 4) and frequency content between different K selections, the quality of the restoration could be affected.

5.1.1.3 Background Correction

During the PSF determination process, artifacts like ringing and texture are introduced into the background of the PSF. Background correction is used to mitigate those artifacts, which decreases PSF size (as seen in Chapter 4) and has been observed to improve restoration quality in practice. Introducing the threshold into the PSF changes its frequency content, which would therefore have an effect on the frequency content included in the image restoration. The difference in PSF size would also be expected to have an effect on the restoration quality.
5.1.1.4 $\lambda$

This parameter applies to denoising the deconvolution result specifically and was not involved in the PSF determination process. Deconvolution on its own can produce a noisy restoration which is difficult to interpret. Therefore, it is coupled with total variation regularization (Rudin et al., 1992) for denoising in Aura (see Equation 3.9). The denoising method aims to smooth the image while preserving edges. The user can choose the amount of smoothing, where lower amounts of smoothing preserve features but also noise, and higher smoothing does well at denoising but can blur edges. Understanding how this parameter affects the restoration quality may help in determining which value to use for various applications.

See Chapter 3 Results and Discussion subsection 3.3.6 Image Restoration for more detail on the origin of $\lambda$.

5.2 Materials and Methods

5.2.1 SEM and Samples

The calibration sample for PSF determination was a dispersion of 28.5nm gold nanoparticles on iridium-coated Kapton. Tin balls of size 75-100nm (from Electron Microscopy Sciences) were used as the sample-of-interest for restoration quality comparison. Images of example fields from these samples can be seen in Figure 5-1. We originally intended on using images of gold-on-carbon which were captured with the data from Chapter 4, but found that the tin balls had a smoother distribution of spatial frequencies. The tin balls data was also collected at a lower voltage than those tested in Chapter 4, which reflects a more real-world use of the technique and is a better test of the restorative capabilities of PSF deconvolution.

Images were collected using the TESCAN MIRA3 Schottky field-emission SEM at 1kV with side-mounted Everhart-Thornley secondary electron (SE) detector at WD $\approx$ 4mm, where WD is the working distance. Reference image of a similar areas were also collected at 20kV for visual comparison (Figure 5-1). Multiple nanoparticle fields were imaged and then stitched together to increase the number of particles used for stacking in Aura (to create the average observed particle). Both calibration and tin ball images were captured at 1nm/px scale, using the same beam shape.
The only change made when transitioning between samples was to the stage height for focusing, to account for differences in sample height.

### 5.2.2 Image Restoration through PSF Deconvolution

PSF deconvolution is attractive as a potential technique for resolution improvement because it is able to restore information which has been blurred or distorted by the imaging system. For the SEM, a PSF determined from a calibration image of nanoparticles can be used to restore any image taken with the same beam shape. To determine the PSF, we need knowledge of the average observed particle and a simulated reference particle. In the case of image restoration, the PSF is known, and when it is deconvolved from an observed image, the reference or “true” image can be restored. The mathematics involved in the process of restoration are described in Chapter 3 (Results and Discussion subsection 3.3.6 Image Restoration) and Lifshin et al. (2014).
5.2.3 Image Quality Evaluation

The observed image, restored images, and the PSFs were taken into account when evaluating image quality. First, the observed and restored images were compared by visual inspection (image brightness enhanced slightly for publication). This provided information on the perceptive image quality and presence of artifacts that the other metrics may not capture. Second, the FFT of each PSF was calculated, giving insight about the optical transfer function (OTF) and how the frequency content of the PSF affects the frequency content of the resulting image. Third, FFT and cross-correlation were used to analyze the frequency content of the observed and restored images. Details on this are provided in the following discussion.

Various metrics and methods have been developed for the purpose of image quality assessment, including those based on peak signal-to-noise (PSNR), structural similarity (SSIM) (Wang et al., 2004), and image sharpness (ISO, 2011; Joy et al., 2000). Both PSNR and SSIM (utilized in Roels et al., 2018) require an image-reference pair, and obtain their measurement by comparison. PSNR measures the pixel-wise error of an image relative to the maximum signal in the reference. SSIM evaluates the preservation of structures between the two images. Usually, these metrics are used in experiments where there is a reference image that has been modified with known distortions, sent through an algorithm which attempts to remove these distortions, and then the output image is compared to the original reference image. In our case, we only have the distorted image (i.e., low voltage image of tin balls), and thus do not have access to a high resolution reference image for comparison with our restoration. Evaluation of image sharpness through Fourier analysis (i.e., frequency content analysis) does not require a reference image. This metric is related to resolution and sheds light on how an image’s quality changes from observation to restoration and how the quality varies with changes in the restoration parameters.

Taking the Fourier transform of an image in the spatial domain (as we normally view an image) results in a representation of that image in the frequency domain. In the spatial domain, we use the units of “pixels” (px), and in the frequency domain this becomes inverse pixels or “cycles per pixel” (cyc/px). In our case 1px = 1nm, so we use cyc/nm. Imagine one sine wave period spanning multiple pixels; for example, if the single wave period spans 10 pixels, it would have a frequency of 1 cycle per 10 pixels = 0.1cyc/px = 0.1cyc/nm. The highest frequency we can measure is the Nyquist frequency (0.5cyc/px) to avoid aliasing. Low frequencies describe smooth areas with slow change in intensity, while high frequencies describe sharp edges and details.
The challenge with using the Fourier transform is distinguishing where signal ends and noise begins. In their proceedings on metrics for the resolution and performance of CD-SEMs, Joy et al. (2000) suggested using the cross-correlation of the Fourier transforms (power spectra, specifically) from two regions: one of the region-of-interest (ROI) and the other of the ROI shifted by 10-20nm. The cross-correlation measures the similarity of two functions as a function of shift between the two (Eq. 5.1 as given in Joy et al., 2000). The advantage of the cross-correlation is that it is simpler to distinguish signal (i.e., peak) from noise (i.e., flat region) compared to the Fourier transform (Figure 5-2). Note that the intensity profiles shown in Figure 5-2 and throughout this chapter are the horizontal and vertical profiles going through the center of the image.

\[
c(i, j) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} C(u,v) e^{2\pi i (ux + vy)} dudv
\]

(5.1)

In Equation 5.1, \(C(u,v) = F(u,v)^* G^*(u,v)\) where \(F(u,v)\) and \(G(u,v)\) are the two-dimensional power spectra of the ROI image, \(f(x,y)\), and the shifted-ROI image, \(g(x,y)\).

However, we note one point of confusion in these proceedings. Joy et al. claimed that the FWHM of the resulting cross-correlation corresponded to the size of the beam. But, if we take the example where the ROI and shifted-ROI FFTs capture more high frequency content (assuming that content reflects higher resolution and therefore smaller sample features), the FFT functions would look similar for larger shifts during the cross-correlation. This would make the cross-correlation peak appear larger, when a smaller beam would have been necessary to image that resolution in the first place.

Readers should note that while Fourier and cross-correlation analysis do tell us about the frequency content of an image, they may not provide a complete assessment of image quality. This is where the visual inspection provides complementary qualitative information.

The restoration FFTs and OTFs have been passed through a median filter to reduce noise for clearer comparison. For the cross-correlation of ROI and shifted-ROI FFTs, the ROI and shifted-ROI were each 1024px-by-1024px with a shift of 20px horizontally between them. For the resulting two-dimensional FFTs and cross-correlation, only the horizontal and vertical profiles are shown in the interest of simplicity and space. Because the FFTs, cross-correlations (in this case),
and OTFs are symmetric about the central axes, only half of the profile is shown for enhanced visualization. FFTs and cross-correlations are normalized so that the maximum intensity is “1”.

### 5.2.4 Parameter Space Exploration

Restorations of the 1kV SE tin ball images were created using PSFs determined from particle calibration images (imaged with the same beam shape). For each parameter under scrutiny, multiple restorations were made using a range of parameter values while holding the other parameters constant. This was done in the effort to isolate the effect of individual variables. In practice, a user can change the parameters so they work together to form an informative PSF and restoration.
The parameters involved during PSF calculation (reference particle size, $K$, and background correction) are described in detail in Chapter 3. In Aura, users can select $K$ on a continuous range from 1 to 100,000, but there are built-in default increments on the $K$ slider which increase along a logarithmic scale with the inclusion of intermediate values (default values approximately 1, 3, 10, 31, 100, 316, 1000, 3162, 10000, 31622, 100000). For background correction, the user can select a value on a continuous range from 0 to 1, which represent the minimum and maximum normalized PSF intensity. The restoration-specific parameter, $\lambda$, was described in the Materials and Methods subsection on Image Restoration through PSF Deconvolution. Users can select an integer between 0 and 100 for $\lambda$.

5.3 Results and Discussion

5.3.1 Restoration-specific Parameters

5.3.1.1 $\lambda$

The parameter $\lambda$ controls the balance of denoising and feature preservation in the restored image (see Equation 3.9). The same PSF was used for all restorations shown here (reference particle size = 28.5nm, $K = 3162$, background correction = 0.23). Aura allows the user to select $\lambda$ between 0 (i.e., no smoothing) and 100 (i.e., high smoothing). Restorations produced with a selection of $\lambda$ values are shown in Figure 5-3. The FFTs and cross-correlation are shown in Figure 5-4. Observed 1kV image and frequency content is shown for comparison.

Choice of $\lambda$ has an effect on the restoration which is visually apparent (Figure 5-3). Restorations with low $\lambda$ appear sharper, but can contain textural artifacts. Restorations with high $\lambda$ appear smoother, but may lose some image details.
appear sharper than the observed image, but are not as sharp as the low $\lambda$ cases. Also, high $\lambda$ restoration features display smoother texture than with low $\lambda$. As $\lambda$ increases beyond the values shown here, the features in the restoration become blob-like, and the information restored by PSF deconvolution is lost to over-regularization.

These qualitative observations are reflected in the frequency content analysis (Figure 5-4). Compared to the observed image, restorations contain increased frequency content in the 0.01-0.1 cyc/nm range. Lower $\lambda$ restorations have more of this content, which decreases as $\lambda$ increases. Looking at the cross-correlation, we can easily distinguish this frequency content as signal (i.e., the peak). Based on the cross-correlation, frequencies beyond this range (i.e., where the curve

![Figure 5-4. Frequency Content Analysis of Restorations with a Selection of $\lambda$ Values.](image)

The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. In the legend, “Observed” refers to the observed 1kV image of tin balls, and “Smooth” refers to restored images with given $\lambda$ (e.g., Smooth17 represents $\lambda = 17$). Note that the y-axes are plotted on log scale.
levels off) appear to be part of the noise. This would mean that the restorations have less noise content than the observed image for the $\lambda$ shown here. If lower $\lambda$ than those shown here are used, this noise level increases and can overtake the level of noise in the observed image due to the contribution of intense artifacts. Images and curves for a larger range of $\lambda$ are given in Appendix D.1.

5.3.2 PSF Calculation Parameters

5.3.2.1 Reference Particle Size

PSFs were calculated over a range of reference particle sizes (diameters of 28.5±0.9nm at 2$\sigma$), and then restorations were performed. All PSFs had the same $K = 3162$ and background correction = 0.23. Observed and restored ROIs are shown in Figure 5-5. Intensity profiles for the PSFs and OTFs are shown in Figure 5-6 (only half of OTF profile since it is symmetric about y-axis). FFTs and cross-correlations for the restorations ($\lambda = 37$) are shown in Figure 5-7.

Although the PSF shows some change due to reference particle size, it is not enough to have noticeable effect on the restoration quality (Figure 5-5). The OTF remains relatively similar for all reference particle sizes studied, with the greatest difference occurring at the highest frequencies (noise in our case) (Figure 5-6). The FFTs and cross-correlations show little difference in frequency content between restorations (Figure 5-7). Similar observations were made at $\lambda = 17$, 57, and 77 (included in Appendix D.2).

![Restorations with a Selection of Reference Particle Sizes](image)

Figure 5-5. Restorations with a Selection of Reference Particle Sizes. From left to right: 1kV SE observed ROI of tin balls and restored ROI with reference particle sizes = 27.6nm, 28.5nm, and 29.4nm to show the 2$\sigma$ range. Restorations shown here have $\lambda = 37$. Scale bar = 100nm.
Figure 5-6. **PSFs and OTFs for a Range of Reference Particle Sizes.** All PSFs have $K = 3162$ and background correction = 0.23. PSFs are shown in the top row, and OTFs are shown in the bottom row. Only half of the OTF is shown to improve visibility; the OTF is symmetric about the $y$-axis. The legend provides the reference particle sizes used to create each PSF. Note that the y-axes are plotted on log scale.
PSFs were generated using a range of $K$ values. $K$ is usually estimable within one increment of the $K$ slider in Aura (e.g., 1000, 3162, 10000), so a range encompassing two increments is shown here in the interest of caution (e.g., 316, 3162, 31622). Restorations were created with these PSFs and then analyzed. All PSFs had the same reference particle size = 28.5nm and background correction = 0.23. Observed and restored ROIs are shown in Figure 5-8, PSF and restoration comparison for lowest $K$ is shown in Figure 5-9, intensity profiles for the PSFs and OTFs are displayed in Figure 5-10, and FFTs and cross-correlations for the restorations ($\lambda = 37$) can be found in Figure 5-11.
Using different $K$ resulted in some subtle difference between restorations. When $K$ is too large, the “Cheerio effect” become noticeable. This artifact causes image features like the small tin balls to appear like they have holes in their centers (Figure 5-8). The PSF profiles show how $K$ balances PSF noisiness with accuracy (Figure 5-10), where low $K$ give smaller but noisier PSFs and high $K$ give smoother but larger PSFs. The user should use caution when selecting lower and
lower $K$ values in an attempt to get the “best accuracy” for the PSF because in some cases, especially at low voltage, the PSF can become lost in the noise and create uninterpretable restorations (Figure 5-9). The OTFs show that low $K$ PSFs contain additional content in the 0.01-0.1 cyc/nm range in general, which translates to more of that content in the restorations as well (Figure 5-10 and Figure 5-11). This is especially visible in the vertical profiles. But, the OTFs for low $K$ also contain increased content at higher frequencies, contributing to higher noise levels in the low $K$ restoration. That said, the low $K$ restoration has lower noise levels than the observed image. Similar observations were made at $\lambda = 17$, 57, and 77 (included in Appendix D.3), although differences between $K$ values are more noticeable at lower $\lambda$.

![Figure 5-10. PSFs and OTFs for a Range of K Values.](image)

All PSFs have reference particle size = 28.5nm and background correction = 0.23. PSFs are shown in the top row, and OTFs are shown in the bottom row. Only half of the OTF is shown to improve visibility; the OTF is symmetric about the y-axis. The legend provides the $K$ used to create each PSF. Note that the y-axes are plotted on log scale.
5.3.2.3 Background Correction

The purpose of background correction is to mitigate artifacts and computational effects in the PSF background while maintaining the PSF. This is done to improve understanding of the PSF and restoration quality. To test background correction, PSFs were determined over a range of suitable background correction estimations, along with using no background correction on the PSF. Based on experience, background correction is usually estimable within ±0.05 (where correction ranges from [0 1]), but to be careful, a range encompassing ±0.10 is shown here. Images were restored using these PSFs and then analyzed similarly to other studied parameters. All PSFs

Figure 5-11. Frequency Content Analysis of Restorations with a Selection of K Values. The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The data shown here were calculated from the $\lambda = 37$ restorations. Note that the y-axes are plotted on log scale.
had the same reference particle size = 28.5nm and \( K = 3162 \). ROIs from the observed and restored images are displayed in Figure 5-12, along with an enlarged comparison in Figure 5-13, intensity profiles for the PSFs and OTFs are plotted in Figure 5-14 (note that OTF x-axis plotted over shorter range than previous plots to improve visibility of 0.01-0.1cyc/nm region), and FFTs and cross-correlations for the restorations \((\lambda = 37)\) are given in Figure 5-15.

There is a visible difference between restorations from PSFs with background correction versus no background correction (Figure 5-12 and Figure 5-13). A striking difference in contrast is apparent between the two cases, where the no background correction produces a restorations with high contrast that can lead to saturation. Even though it appears that no background correction provides the most frequency content between 0.01-0.1cyc/nm (Figure 5-15), the OTF shows that it actually transmits the lowest amount of those frequencies (Figure 5-14). The zero background correction restoration only appears to have higher frequencies, but this is an artifact. When viewing the no background correction case versus the corrected case (Figure 5-13), notice the enhanced gapping or shadowing between objects and blob-like appearance of small features which are not present in the background corrected version. Because the PSF appears larger than it should in the no background correction case, it erroneously deconvolves image content from too large of an area, causing features to appear smaller than they should.

The OTF shows that as PSF background correction increases, high frequency content increases. This is a result of introducing a sharp edge into the PSF (i.e., where the PSF peak meets “0”). But, too high of a background correction cuts into the PSF. This means the deconvolution would draw from too small an area, and that incomplete picture of the PSF would limit the

![Figure 5-12. Restorations with a Selection of PSF Background Corrections. From left to right: 1kV SE observed ROI of tin balls and restored ROI with background correction (“bc”) = 0.00, 0.13, 0.23, and 0.33. Restorations shown here have \( \lambda = 37 \). Scale bar = 100nm.](image)
information content of the restoration. This can be seen in the FFTs and cross-correlations. When background correction becomes too high, we lose information and the image becomes blurry again.

Similar observations were made at $\lambda = 17, 57, \text{ and } 77$ (included in Appendix D.4), although differences between background correction are more noticeable at lower $\lambda$.

Figure 5-13. *Comparison of Restorations with PSF Background Correction versus None.* The top shows the ROI restored by a PSF with no background correction (“bc”). The bottom shows the same ROI restored by a PSF with a reasonable background correction. Restorations shown here have $\lambda = 37$. Scale bar = 100nm.
Figure 5.14. *PSFs and OTFs for a Range of PSF Background Corrections.* All PSFs have reference particle size = 28.5nm and $K = 3162$. PSFs are shown in the top row, and OTFs are shown in the bottom row. Only half of the OTF is shown to improve visibility; the OTF is symmetric about the y-axis. The legend provides the background correction (“bc”) used to create each PSF. Note that the y-axes are plotted on log scale.
5.4 Conclusions

In this chapter, we explored the parameter space of PSF deconvolution for application in SEM image restoration. Overall, the quality of the restored images was greater than the observed images for the tested conditions (i.e., low kV, noisy, high atomic number material), as shown by the inclusion of more high frequency signal content and the improved visual aesthetics.

The restoration-specific parameter $\lambda$, balances the amount of denoising (i.e., smoothing) and feature preservation in the restoration. Low $\lambda$ correspond to better feature preservation and

Figure 5-15. Frequency Content Analysis of Restorations with a Selection of PSF Background Corrections. The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The data shown here were calculated from the $\lambda = 37$ restorations. Note that the y-axes are plotted on log scale.
image sharpness at the cost of noise and reduced visual aesthetics. This may be useful in situations where feature measurement is vital and appearance does not matter as much. For the sample-of-interest and imaging conditions tested here, $\lambda = 37\text{-}57$ seemed to provide a good balance between feature preservation and denoising. When $\lambda$ is too high, the image details gained by performing PSF deconvolution are lost to the over-regularization. In general, higher $\lambda$ also caused the other parameters investigated here to make less of a difference in restoration quality.

Reference particle size had almost no effect on restoration quality within the approximate $\pm 3\%$ uncertainty in the mean particle size. So while minor differences in PSF size may be important to those studying the beam, this parameter should not be a concern for those wishing to use this method for image restoration purposes. That is, as long as the user knows the approximate mean size of the calibration particles.

Differences in $K$ contributed to differences in noise level and artifacts between restorations. Fortunately, if $K$ is chosen within a reasonable range, it does not have that much effect on useful image content. As $K$ increases and the PSF becomes smoother, featureless, and larger, the user can expect to see a rise in artifacts (e.g., Cheerio effect). As $K$ decreases, the restoration becomes noisier. In some cases like low voltage imaging, choice of very low $K$ allows too much noise to form a contiguous PSF. This causes restorations to be uninterpretable as they bear little semblance to the observed image features.

Restorations created using PSFs with background correction had superior quality compared to those using the PSF with no background correction. Background correction performed its role of removing false background texture and artifacts (e.g., ringing) from the PSF, which improved restoration appearance and information content. Users should be cautious when selecting a background correction to not pick one that is too high, which cuts off the PSF and results in a poorer, blurrier restoration. In most cases, estimating a reasonable background correction is not a problem.

Future efforts to automatically determine parameters like $\lambda$, $K$, and background correction would remove user guesswork, improve this method’s consistency, and maximize the amount of information from the PSF and restoration.
Acknowledgements

This chapter has been submitted to Microscopy and Microanalysis for review. The version presented here has been modified to improve readability and integration into this thesis. I was the primary investigator of this work.
6 Conclusion and Future Research

The goals of this work were to characterize the capabilities and limitations of a novel SEM PSF determination method, and to evaluate the utility of the measured PSF for restoration of low voltage SEM images. PSF deconvolution has the potential to provide a low-cost and widely-applicable solution for SEM image quality improvement, without the need for new or upgraded hardware. While methods do exist to profile or estimate the SEM PSF, this work outlined and examined the first method to provide a measurement of the complete PSF in the SEM. The results of this research are meant to inform prospective and existing users of this technique about its fundamental theory, best operating practices, the expected behavior of output PSFs and image restorations, and factors to be aware of during interpretation of results.

The PSF determination method presented in this work provides a two-dimensional measurement and visualization of the SEM PSF from a single measurement, making it quicker and more informative than past methods for PSF measurement. The method studied here makes no assumptions about the PSF shape in order to make its measurement, meaning that beams which are affected by high levels of astigmatism or lens aberration can still be measured. This is particularly useful for microscope characterization and the application of image restoration, which is intended to restore information to images affected by blur and distortion. It should be noted that while the presented method has been tested for its capabilities in low voltage imaging, the image restoration technique could also be beneficial for improving imaging with thermionic sources to achieve FESEM-type image quality. Additionally, no assumptions were made about the microscope’s operating conditions, and PSFs were determined for multiple beam energies, working distances, and signal types.

The parameter space of the PSF determination method was investigated regarding how choices made during data collection and PSF calculation affected the output PSF. The choice of signal type (i.e., BSE, SE) did affect the size of the observed calibration particles, but signal type
is a selectable option for the reference particle in Aura which accounts for the size difference. Variation in PSF size observed due to signal type is most likely due to differences in detector position and orientation between the observed particles and the simulated particles. For the time being, users should attempt to minimize edge brightening in SE mode to reduce discrepancies between observed and reference particle edge brightening symmetries. Further development of this method in terms of reference particle generation for variable detector orientation should be pursued.

The choice of particle support material thickness showed little effect on the PSF, and should not interfere with practical use of this method in its current state. Because the interaction volume is part of the imaging chain in the SEM, it is expected to have a contribution to the PSF, even if it’s only a minor one, and therefore, the role of particle support material and thickness should be reexamined after PSF calculation parameters are optimized.

Selection of $K$ and background correction can be tailored for each PSF, which shrinks the confidence interval in the PSF FWHM introduced by uncertainty in the simulated reference particle size. Currently, $K$ and background correction are chosen based on user judgement which introduces uncertainty on the order of ±1nm for the PSF FWHM. Automated methods to calculate $K$ and background correction should be considered in the future to make the parameter selection more repeatable and consistent across systems. Uncovering the mathematics required for automation may also improve the PSF determination method’s precision.

Investigation of the PSF determination parameter space was expanded to include PSF deconvolution and the effects on restored image quality. In the case of a noisy, low voltage image of tin spheres, the quality of the restored images was greater than the observed images. General improvement was seen in other restoration examples as well (see Appendices). Simulated reference particle size (within ~±3% uncertainty) had almost no effect on restoration quality, so if the calibration particles have been well-characterized, this is a non-issue for image restoration. Selection of $K$ affects the balance of noise and artifacts in the restorations, but fortunately, if $K$ is chosen within a reasonable range, it does not have much effect on restoration quality. Introducing a PSF background correction mitigated artifacts and improved restoration appearance and detail visibility. Users should be wary of selecting a background correction level that cuts into the PSF because this will result in a blurrier restoration as information provided by the PSF is cropped. Choice of $\lambda$ (for regularization which was paired with PSF deconvolution) affected the balance of
denoising and feature preservation in the restoration, so this parameter may be selected to suit the application. Users should avoid using an excessively high $\lambda$, which washes out the image details gained by performing PSF deconvolution as they are lost to over-regularization. Future research to automatically determine $\lambda$, $K$, and background correction would remove user judgement, improve restoration consistency, and maximize the amount of information available for scientific interpretation.

Additional investigations within the parameter space are important for understanding how this method operates under more extreme conditions. For example, in critical dimension SEM metrology, the microscope is pushed to its limits in terms of fast scan speeds and low electron doses. The effect of scan speed on the PSF and corresponding restorations has not yet been examined in detail, but is worthy of study to improve critical metrology tasks. As another example, biological samples are routinely imaged at low voltage to avoid beam damage. But, features important to biological study may be at a lower magnification scale than the 1nm/px scale used throughout this thesis. The particles used at 1nm/px may be barely visible at these low magnifications! Thus, it is important to determine the appropriate calibration particle size to use in different magnification regimes. While some examples of astigmatism and restoration were given here, it would be useful to examine restoration quality for PSFs suffering from severe distortion, such as if the PSF has been determined using very poor focus or has high amounts of astigmatism or aberration.

Further study to improve the practicality of this method would be useful for day-to-day users. While this method is far quicker than previous PSF measurement methods and provides more information by producing a two-dimensional PSF, the method can be time-consuming. After the calibration image is collected, the same beam shape must be used for any images taken afterward for PSF deconvolution to be valid. It would be useful to assess the PSF validity based on differences in sample height between the calibration sample and sample-of-interest (e.g., by knowing how out-of-focus the sample-of-interest can be, it may speed up image collection times). High volume imaging tasks where many images are taken one after another under the same conditions are already well-suited to this workflow.

The user runs into trouble if they need to capture images of multiple samples under different operating conditions (e.g., voltages, working distances). The calibration sample and a sample-of-interest will have some variation in surface height, leaving the user to refocus the
sample-of-interest by changing only the stage height to maintain the same beam shape. The stage may not have the mechanical precision required to achieve the exact focus that was used on the calibration sample. During this thesis work, the restoration process proved robust to minor differences in focus between calibration sample and sample-of-interest, but even so, switching between multiple samples with only stage height control for focusing can take some time.

If this method is being considered for use in a facility with many users, then all users who wish to use this method should be trained to collect calibration images. While preliminary results have shown that the beam shape remained the same before and after a sample exchange, users may switch voltages or working distances according to their imaging tasks. This would require a new calibration image be taken each time. The calibration image does not take long to collect, but if the user changes conditions often, then calibration imaging time builds up. Future study into workflow optimization or onboard microscope incorporation would improve the practicality of this method.
Bibliography


CASINO: [http://www.gel.usherbrooke.ca/casino/](http://www.gel.usherbrooke.ca/casino/)


Appendix A: Preliminary Work

This appendix contains excerpts from my thesis proposal that provide complementary information to that given in this thesis.

The following work was presented in a post-deadline poster at the 2016 Microscopy & Microanalysis (M&M) conference, and as a platform presentation at M&M 2017 (Nevins, 2017). The work presented at M&M 2017 was submitted for publication as equal contribution with the work of Zotta and Lifshin (2017) in the spring 2018, and is given in Chapter 3 of this thesis.

My preliminary work focused on examining the basic viability of PSF deconvolution when applied to SEM images. This included testing the repeatability of the measured PSF (given in Chapter 3) and comparing the deconvolution’s effectiveness to widely available image processing methods. Images were taken over a range of electron beam voltages to test the general applicability of the technique. The behavior of the SEM at different voltages is well known, so it was expected that measured PSFs would follow that behavior. These initial results also highlighted the struggles we may face at low voltages. The results confirmed that PSF deconvolution is generally applicable to SEM images and laid the foundation for the proposed work.

A.1 SEM Image Restoration using PSF Deconvolution

In the SEM, the PSF describes the shape and distribution of electrons in the beam which is scanning the sample. Nanojehm’s Aura Workstation uses the average nanoparticle of a calibration image (output image, $I_o$) and a simulated reference image (true image, $I_t$) to determine the PSF corresponding to that system (Figure A-1). The particle is stacked to get better statistics and average out the differences in shape between particles. Also, stacking the particles alleviates the need to park the beam on one particle for long, reducing the effects of contamination, beam
APPENDIX A: PRELIMINARY WORK

Figure A-1. PSF Determination using a Calibration Image. The calibration image of a nanoparticle dispersion at 2kV (left) is used to find the average particle, also known as the stacked particle (center). The stacked particle is compared to the simulated reference particle (center). The PSF is calculated through this comparison (right). The center images of the stacked and reference particles and the PSF have the same scale bar.

damage, and sample drift. So, the user must use the same beam shape to capture a calibration image of the particles for PSF determination, along with the images of their specimen-of-interest.

Calibration images have thus far been captured in BSE mode. The BSE mode is very sensitive to elemental composition. The high atomic number (Z) nanoparticle material and the low Z support material create a perfect opportunity for imaging with BSE, especially at low voltages where the signal-to-noise is lower. SE are sensitive to surface topography, which is not as useful because the features of interest (19-20nm gold nanoparticles) become difficult to distinguish from the features of the support structure. Plus, contamination builds up on the particles and increases their apparent size.

A single PSF should apply to multiple signals, like BSE and SE, since it refers to the electron distribution in space and should be independent of signal. The ability to use one calibration image for multiple signals would improve the applicability and practicality of this technique, since: 1) SE mode does not provide enough contrast at low voltages for an accurate calibration (Figure A-2), and 2) a user could avoid switching between samples multiple times. (This work is addressed in Chapter 4.)

All images in the preliminary work were collected with the TESCAN MIRA3 field emission scanning electron microscope (FESEM) using the minimum probe size. The probe size
was calculated by the microscope operation software. BSE images of a 19nm diameter gold (Au) nanoparticle dispersion on a TEM grid with carbon (C) film support were used for calibration. Restorations were performed on BSE and SE images of the calibration sample and a high resolution Au-on-C standard. Any changes made to the beam shape would result in a different PSF, so the beam shape was not changed after the calibration image was taken. Images were taken at 20kV, 10kV, and 3kV to examine the PSFs and deconvolution results over a range of voltages. After image collection, PSFs were calculated from the calibration images by Aura. The Aura Workstation also performed the deconvolutions. The results can be seen in Figure A-3.

The PSFs generated by Aura matched the expected trend in beam size (Eq. 2.1), where the beam has a sharp shape at higher voltages and becomes broader as the voltage decreases. This is due to factors like increased aberration effects and longer wavelengths at low voltages. The PSFs at lower beam energies were also noisier due to the decreased signal-to-noise. Upon visual inspection, PSF deconvolution improved the image clarity at all tested beam voltages for both BSE and SE images. The restorations were blurrier at lower voltages, but even so, the sample features were easier to distinguish compared to unrestored images. Restoration artifacts, such as halos and false textures, were minimized by optimizing the choice of regularization parameter in Aura. Since the time this analysis was performed, Aura has been updated, reducing these artifacts.

Figure A-2. Comparison of BSE and SE signals at 3kV. Similar regions of Au nanoparticles are shown in BSE (top) and SE (bottom) imaging modes. The scale bar applies to both images. SE imaging is more sensitive to surface topography than BSE, even more so at lower voltages. The greater contrast displayed in the BSE image makes it the preferable imaging mode for calibration.
Figure A-3. PSF and Image Restoration for BSE and SE Images. Top Row: 20kV, Bottom Row: 3kV. From left to right: PSFs generated using the calibration images (A) and (C) in BSE mode. Restorations of (A) and (C) are shown in (B) and (D), respectively. All BSE images have the same scale bar. The SE images of Au-on-C in (E) and (G) were taken with the same beam shape as (A) and (C), respectively. The restorations of (E) and (G) are shown in (F) and (H), respectively. All SE images have the same scale bar.

The measured PSFs followed the expected behavior of the SEM regarding beam voltage. Based on visual inspection, the PSFs could be used for restoring same-signal and different-signal images. Image restorations displayed sharpened feature boundaries and less noise, with particularly notable effectiveness at low voltage. These results matched our expectations and encouraged continued and more-detailed study of this technique.

Since this initial study, the FESEM has been outfitted with a BSE detector better-suited for low voltage imaging. Higher signals are now achievable with beam voltages under 3kV.

A.2 Fitted Gaussians as a PSF Comparison Metric

Being able to compare PSF measurements is essential to many of the proposed tasks. For example, in the case of determining PSF variability, it is important to know if repeated measurements of the PSF under the same experimental conditions yield the same results. Traditionally, the SEM electron probe is approximated as a Gaussian. The probe size can then be described with the full width at half maximum (FWHM) or occasionally the full width at tenth max (FWTM). The FWHM is also related to the spread, \( \sigma \), of the Gaussian. The PSF measurements include noise and other variations which make it difficult to consistently measure by hand. This is avoidable by fitting a normalized Gaussian (i.e., the integral under the curve is 1) to each PSF and then extracting the desired values.
Multiple imaging-related functions were tested for goodness of fit to ensure that the Gaussian was the best choice for our measurements. Gaussian, Lorentzian, Airy, and sinc functions were fitted to a single PSF at 20kV, 10kV, 5kV, and 2kV using MATLAB’s curvefit tool (Figure A-4). The fits were compared using sum of squared error, root-mean-square error, and $R^2$ (Table A-1). The sum of squared error is the sum of the squares of the residuals, which are the deviations of the data points from the corresponding fitted points. The root-mean-square error is another measure of residuals that compounds the individual differences into one value. The $R^2$ is the coefficient of determination, a measure of how well the fit matches the data based on the proportion of variation between the fit and the data. See Mathworks documentation on evaluating goodness of fit for equation definitions and more detail (Mathworks, 2017).

The Gaussian produced the best fit over the range of beam voltages, with the Lorentzian matching performance at 2kV. Only a single PSF was fit at each beam voltage, but it is clear that the Gaussian should be used for consistency between voltages. PSFs can now be compared using the spreads in $x$ and $y$, $\sigma_x$ and $\sigma_y$, respectively. This metric was used in some preliminary work and will also be beneficial in the proposed work. In the future, the beam spreads should be related to the FWHM to improve comprehension by matching well-used terminology.

Figure A-4. PSFs with Fitted Gaussians. PSFs (blue circles) and fitted Gaussians (color surface) are shown for different voltages. From left to right: 20kV, 10kV, and 5kV. The PSF becomes noisier as voltage decreases. The fitted Gaussians provide a good approximation of the beam shape which can cut through this noise and other variations. This fosters consistency in beam spread measurements. The rings in the PSF may be an artifact from the PSF determination process in Aura.
Table A-1. Goodness of Fit for PSF Functional Fits. The sum of squared error (SSE), root-mean-square (RMS) error, and $R^2$ are shown for each functional fit to each PSF (single PSF per beam voltage). The Gaussian fit performed the best overall for both the C film and Kapton substrate supports.

<table>
<thead>
<tr>
<th>Carbon Film</th>
<th>20kV</th>
<th>10kV</th>
<th>5kV</th>
<th>2kV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSE</td>
<td>RMS</td>
<td>$R^2$</td>
<td>SSE</td>
</tr>
<tr>
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<td>0.947</td>
<td>2.153</td>
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<tr>
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<td>0.965</td>
<td>1.593</td>
</tr>
<tr>
<td>Lorentzian</td>
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<td>0.046</td>
<td>0.917</td>
<td>4.690</td>
</tr>
<tr>
<td>Sinc</td>
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<td>0.070</td>
<td>0.810</td>
<td>8.571</td>
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</table>

<table>
<thead>
<tr>
<th>Kapton Substrate</th>
<th>20kV</th>
<th>10kV</th>
<th>5kV</th>
<th>2kV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSE</td>
<td>RMS</td>
<td>$R^2$</td>
<td>SSE</td>
</tr>
<tr>
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<td>0.039</td>
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<td>Sinc</td>
<td>9.505</td>
<td>0.069</td>
<td>0.790</td>
<td>10.03</td>
</tr>
</tbody>
</table>

A.3 Influence of the Number of Nanoparticles on PSF Repeatability

Although nanoparticles are not point sources, they are an excellent candidate for measuring the PSF of the SEM because they are close to identical in size and shape. The average nanoparticle in a calibration image is compared to a simulated high resolution reference particle to determine the PSF. Nanoparticles are small and generally spherical in shape, so simulating a reference image for them is relatively simple. A single nanoparticle can span multiple pixels at the magnifications of the images to be restored. Spanning multiple pixels is actually beneficial in this case, because if the nanoparticle were too small, it may be missed or not fully sampled and also give rise to a weak high noise signal. Single nanoparticles in a range of acceptable sizes (around the nominal size,
e.g., 19nm diameter) are averaged (stacked) to account for any variations in size and shape between nanoparticles. It was not known how many nanoparticles were necessary to create a repeatable PSF.

The data collected in Chapter 3 was used for this analysis. It was important to understand if the difference in support thickness (thin C film vs. thick Kapton substrate) would change the necessary number of nanoparticles for repeatable PSFs. It was also necessary to see if the number of nanoparticles differed depending on the beam voltage. At each voltage, the automatic minimum spot size was determined by the microscope software, which also automatically set the probe current. In general, as beam voltage decreased, so did the probe current. Less probe current means less electrons reaching the sample, which in turn means worse statistics for the signal received.

The following procedure was used for each region image at each voltage. An image was broken down into smaller squares and slices (Figure A-5). Each square and slice contained a different subset of the nanoparticles in the original image. Both squares and slices were used because the squares captured smaller subsets of nanoparticles and slices captured larger subsets, generally. A PSF was generated for the original image, each square, and each slice. Each PSF was fitted with a Gaussian for comparison (Appendix A.2). The goal was to determine at what number of nanoparticles the beam (PSF) spread stabilized.

With our setup, a repeatable PSF was achieved using twenty-five nanoparticles (Figure A-6). Using fewer nanoparticles increased the PSF noise and variation in shape, which may result in differing restoration results when applied for deconvolution. In general, including more nanoparticles resulted in a smoother PSF, although knowing that only twenty-five are required increases the practicality of this technique. A microscope user should be able to quickly find a suitable calibration field and then move on to image their samples-of-interest.
Figure A-5. Acquiring Nanoparticle Subsets through Image Breakdown. Left: The original calibration image is shown cut into squares. Two different sizes of squares were used, corresponding to a quarter (blue) of the original image and a sixteenth (yellow) of the original image. Right: The same calibration image is sliced into sizes shown by the brackets. Slicing was also done vertically. The subsets were overlapped intentionally. As more nanoparticles are included in a subset, the PSF should converge to the PSF found when using all nanoparticles.

Figure A-6. Determining the Number of Nanoparticles for PSF Repeatability. The 2kV analysis for 20nm Au nanoparticles on Kapton is shown here, and similar results were found at 20kV and for 19nm Au nanoparticles on C film. Top: PSFs generated using the number of nanoparticles corresponding to the x-axis of the plots below. Middle: The beam spreads were determined for each region’s subsets using Gaussian fitting. Regions are labeled as first region R1, second region R2, and so on. The beam spread for the y-axis is shown, and similar results were found for the x-axis. Bottom: The goodness of fit, R², is shown to support that the beam spread values are valid.
A.4 Visual Comparison of Low Voltage Image Restoration to High Resolution Imaging

The goal of PSF deconvolution is to restore the true image, \( I_\text{t} \), by knowing the original output image, \( I_\text{o} \), and the PSF, \( A \). A reference of the true image is helpful in determining the effectiveness of PSF deconvolution on low voltage SEM images, and to evaluate possible image artifacts introduced by the technique. High resolution imaging could provide suitable reference imagery, as well as a way to test the effectiveness of PSF deconvolution against hardware solutions for improved low voltage imaging. Possible high resolution imaging techniques include high voltage imaging and beam deceleration mode (BDM) at low voltage.

High beam voltages correspond to higher image resolutions. Collecting a high voltage reference image may restrict either the beam voltage or the sample choice to avoid sample charging and damage. Some aspects of image formation differ between beam voltages, so further study would be necessary to perform direct comparisons between a low and high voltage reference image. Even so, a high voltage image would make an appropriate visual reference.

BDM is a hardware development for improved low voltage SEM imaging. An electric potential is applied to the sample, which slows the beam electrons before impact. Basically, a higher beam voltage can perform low voltage imaging. The advantage of this technique is the improved resolution at low voltage. Installing BDM on a microscope can be costly, though. PSF deconvolution could provide a software solution to improved low voltage imaging, and may be able to match the performance of BDM.

The calibration sample (20nm Au nanoparticles on Kapton substrate) and a high resolution Au-on-C standard were imaged at 2kV in BSE mode. The same region on the sample was imaged at 20kV, and a similar region was imaged with BDM using 7kV electrons impacting the surface at 2kV. The same region could not be imaged with BDM because of high levels of contamination. These images are shown in Figure A-7.

Upon visual comparison, PSF deconvolution did improve image quality in terms of blur and noise. In general, the restored image appears much sharper and some new features are resolvable. The shades of gray seen in the 20kV reference are not readily apparent in the 2kV image, so it is not expected that they would appear in the restoration. In the restoration, very fine features were not restored, and there is some bridging between Au grains. The bridging may or may not actually be present. The restored image came close to the quality of the BDM image,
using visual inspection. Only once the images are magnified are the differences evident. The BDM image is free of the bridging seen in the restoration and may be somewhat sharper overall. The restored image is comparable though, which supports further study of PSF deconvolution as a solution for better low voltage imaging.

![Image Restoration at Low Voltage Compared to High Resolution Imaging](image)

Figure A-7. Image Restoration at Low Voltage Compared to High Resolution Imaging. A: Original 2kV image of Au-on-C, B: restoration of (A), C: 20kV reference of the same region in (A), D: BDM reference of a similar region. All images have the same scale bar.

**A.5 Comparison of PSF Deconvolution to Basic Image Enhancement Methods**

PSF deconvolution is unique in its ability to perform image restoration, as opposed to widely available processing methods which perform image enhancement. In theory, PSF deconvolution is able to restore information which was lost to blur and distortion by the imaging system. This is because PSF deconvolution uses the measured PSF in its processing. Other image enhancement methods like denoising, sharpening, and blind deconvolution can improve an image’s appearance, but cannot restore any information because they have no knowledge of the system. This work was used as proof-of-concept to determine if PSF deconvolution results provided more information.
than the results of widely available image processing methods, with a focus on low voltage SEM images. (Additional resources for in-depth comparison of PSF deconvolution to image enhancement methods given in Chapter 5.)

The 2kV BSE Au-on-C image and corresponding calibration image from Appendix A.4 were used in this analysis. The Au-on-C image was processed with different tools for comparison. The image was restored with PSF deconvolution using Aura. Denoising was performed in ImageJ, a free image processing and measurement tool commonly used in microscopy. Generic deconvolution was performed in RawTherapee, which is image processing freeware similar to Adobe Photoshop and is popular in photography. Deconvolution in photography software utilizes an estimated PSF based on a conventional camera, a simulated Gaussian in this case. The image enhancement methods could have been performed by other well-known programs, including MATLAB.

The original image, restoration, and enhancements were compared using line profiles of pixel intensity across Au-on-C feature boundaries (Figure A-8). While the image enhancements did improve the visual appearance, only PSF deconvolution was able to restore information. The image enhancement methods performed as expected. The denoised image suffered less from

![Figure A-8. Comparison of Image Restoration and Image Enhancement. Right: Intensity profiles depicted by the arrows in the images shown on the left. The border color of an image on the left matches the corresponding intensity profile color in the plot on the right. Left: A/blue: Original 2kV image of Au-on-C, B/red: restoration of (A) from Aura, C/green: generic deconvolution performed with arbitrary PSF in RawTherapee, D/magenta: denoised image from ImageJ. Images (A)-(D) have the same scale bar.](image)
intensity fluctuations due to noise, and the generic deconvolution provided some denoising and also smoothing. PSF deconvolution was the only technique of those tested which provided a clearly resolvable feature boundary. The feature boundary was not plainly visible in the original image, $I_o$, confirming that PSF deconvolution determined a better approximation of the true image, $I_t$. 
Appendix B: Chapter 3 Supplemental

B.1. Determination of the Relationship between $d_{25\%-75\%}$ and $d_{FWHM}$

This section (B.1) was contributed by Prof. Eric Lifshin. It contains a derivation that was referenced in the Results and Discussion subsection 3.3.2 Comparison of the New Method with the Knife Edge Test.

The point spread function (PSF) of a focused beam can be determined by scanning it across a sharp edge and measuring the profile of either the transmitted or emitted signal as a function of position. In the case of a transmitted beam, the measured intensity goes from 0% when the beam is fully occluded to 100% when it no longer strikes the edge surface, with values somewhere in between when the beam partially covers the edge. If a detector is above the beam monitoring an emitted signal, such as secondary or backscattered electrons in the case of an electron beam, it will measure the complement of the transmitted signal ranging from 100% when fully on the knife edge to 0% when no longer intersecting it. A typical profile is shown below:

![Profile of a focused beam](image)

A common metric for the beam size is to drop a vertical line from the point of 25% the maximum intensity to the x-axis (the scan direction) and second line from 75% the maximum intensity and then refer to distance between the two points of intersection as $d_{25\%-75\%}$. This value obtained in a one-dimensional scan is sometimes used as a surrogate for beam size, but it is not unambiguous because different beam shapes can give rise to the measurement of 50% (25%-75%) of the signal. For example, an elliptical beam will give one value if measured along its long axis.
and another in a direction perpendicular to it. Even if a beam is Gaussian and has circular symmetry to determine the FWHM, it is necessary to divide $d_{25\%-75\%}$ by 0.57. This relationship is mentioned in a paper by Kolosova et al. (2015) referenced in the main article. What follows is an independent derivation of the relationship between $d_{\text{FWHM}}$ and $d_{25\%-75\%}$ for the specific case of a two-dimensional Gaussian beam profile.

First consider the following:

The normalized PSF is given by:

$$PSF(x, y) = \frac{1}{2\pi\sigma^2} e^{-\frac{(x^2+y^2)}{2\sigma^2}}$$ \hspace{1cm} (1)

The error function is defined as:

$$erf(\eta) = \frac{2}{\sqrt{\pi}} \int_0^\eta e^{-t^2} dt$$ \hspace{1cm} (2)

Imagine a knife edge blocking 75% of the beam whose center is at (0,0). If a backscatter or secondary electron detector measures a signal above the knife edge, then the signal will be emitted from 75% of the area. If a transmission detector is placed below the knife edge, then it will see 25% of the signal. The first part of the problem is to determine the value of $x'$ such that this can happen for a Gaussian beam whose total intensity has been normalized to 1. It is first noted that 50% of the beam’s footprint extends from $x = -\infty$ to $x = 0$ and 25% from $x = 0$ to $x = x'$. This also means that 25% of the beam corresponds to the distance from $x = 0$ to $-x'$ or $d_{25\%-75\%} = 2x'$ as a result of symmetry about the y-axis.
The problem is therefore to find \(2x'\) and relate it to the FWHM of the beam. Using equation (1), the total area \(A\) of the beam between \(x = 0\) to \(x = x'\) is given by:

\[
A = \int_{0}^{x'} \int_{-\infty}^{+\infty} \frac{1}{2\pi\sigma^2} e^{-\frac{(x^2+y^2)}{2\sigma^2}} \, dy \, dx
\]

(3)

\[
A = \frac{1}{2\pi\sigma^2} \int_{0}^{x'} \int_{-\infty}^{+\infty} e^{-\frac{x^2}{2\sigma^2}} \, dx \int_{-\infty}^{+\infty} e^{-\frac{y^2}{2\sigma^2}} \, dy
\]

(4)

Both the integrals have a form similar to that of the error function. Starting with the integral in \(y\):

\[
\int_{-\infty}^{+\infty} e^{-\frac{y^2}{2\sigma^2}} \, dy
\]

Let \(t = \frac{y}{\sigma\sqrt{2}}\) then \(t^2 = \frac{y^2}{2\sigma^2}\) and \(dt = \frac{dy}{\sigma\sqrt{2}}\) or \(dy = \sigma\sqrt{2} \, dt\) then:

\[
\int_{-\infty}^{+\infty} e^{-\frac{y^2}{2\sigma^2}} \, dy = \int_{-\infty}^{+\infty} e^{-t^2\sigma\sqrt{2}} \, dt = \sigma\sqrt{2} \frac{\sqrt{\pi}}{2} \int_{-\infty}^{+\infty} e^{-t^2} \, dt
\]

(5)

\[
\int_{-\infty}^{+\infty} e^{-\frac{y^2}{2\sigma^2}} \, dy = \sigma\sqrt{2} \frac{\sqrt{\pi}}{2} \left( \int_{-\infty}^{0} e^{-t^2} \, dt + \int_{0}^{+\infty} e^{-t^2} \, dt \right)
\]

(6)

\[
\frac{2}{\sqrt{\pi}} \int_{-\infty}^{0} e^{-t^2} \, dt = -\frac{2}{\sqrt{\pi}} \int_{0}^{-\infty} e^{-t^2} \, dt = -\text{erf}(-\infty) = 1
\]

(7)

Also:

\[
\frac{2}{\sqrt{\pi}} \int_{0}^{+\infty} e^{-t^2} \, dt = \text{erf}(\infty) = 1
\]

(8)

Therefore:

\[
\int_{-\infty}^{+\infty} e^{-\frac{y^2}{2\sigma^2}} \, dy = \sigma\sqrt{2} \frac{\sqrt{\pi}}{2} (1 + 1) = \sigma\sqrt{2\pi}
\]

(9)

In the case of the integral in \(x\):

\[
\int_{0}^{x'} e^{-\frac{x^2}{2\sigma^2}} \, dx
\]
Let \( t = \frac{x}{\sigma \sqrt{2}} \) then \( t^2 = \frac{x^2}{2\sigma^2} \) and \( dt = \frac{dx}{\sigma \sqrt{2}} \) or \( dx = \sigma \sqrt{2} dt \) then:

\[
\int_0^{x'} e^{-\frac{x^2}{2\sigma^2}} dx = \int_0^{t'} e^{-t^2} \sigma \sqrt{2} dt = \sigma \sqrt{2} \frac{\sqrt{\pi}}{2} \int_0^{t'} e^{-t^2} dt = \frac{\sqrt{2\pi}}{2} \text{erf}(t') \quad (10)
\]

Combining equations (9) and (10) with (4) gives:

\[
A = \frac{1}{2\pi \sigma^2} \left[ \sqrt{2\pi} \sigma \right] \frac{\sqrt{2\pi} \sigma}{2} \text{erf}(x') = \frac{\text{erf}(t')}{2} \quad (11)
\]

For \( \sigma = 0.25 \), that is 25%

\[
A = 0.25 = \frac{\text{erf}(t')}{2} \quad (12)
\]

Therefore:

\[
\text{erf}(t') = 0.50 \text{ which from the error function table}
\]

\[
t' = \frac{x'}{\sigma \sqrt{2}} = 0.4770 \quad (13)
\]

\[
x' = 0.447\sigma \sqrt{2} \quad (14)
\]

From \( d_{25\%-75\%} = 2x' \)

\[
d_{25\%-75\%} = 2x' = 2(0.4770\sigma \sqrt{2}) \quad (15)
\]

To find \( \sigma \), we start from equation (1):

\[
PSF(x,y) = \frac{1}{2\pi \sigma^2} e^{-\frac{(x^2+y^2)}{2\sigma^2}}
\]

In polar coordinates, it is:

\[
PSF(r) = \frac{1}{2\pi \sigma^2} e^{-\frac{r^2}{2\sigma^2}} \quad (16)
\]
since

\[ r^2 = x^2 + y^2 \]  \hspace{1cm} (17)

It has a maximum value when \( r = 0 \) given by:

\[ PSF(\text{Maximum}) = \frac{1}{2\pi\sigma^2} \]  \hspace{1cm} (18)

Half of this value is

\[ PSF(\text{Half Maximum}) = \frac{1}{4\pi\sigma^2} \]  \hspace{1cm} (19)

The corresponding value of \( r = r' \) is therefore given by:

\[ \frac{1}{4\pi\sigma^2} = \frac{1}{2\pi\sigma^2} e^{-\frac{(r'^2)}{2\sigma^2}} \]  \hspace{1cm} (20)

or

\[ \frac{1}{2} = e^{-\frac{(r'^2)}{2\sigma^2}} \]  \hspace{1cm} (21)

\[ -\ln(2) = -\frac{(r'^2)}{2\sigma^2} \]  \hspace{1cm} (22)

\[ r' = \sigma\sqrt{2\ln(2)} \]  \hspace{1cm} (23)

\[ d_{FWM} = 2r' = 2\sigma\sqrt{2\ln(2)} \]  \hspace{1cm} (24)

\[ \sigma = \frac{d_{FWM}}{2\sqrt{2\ln(2)}} \]  \hspace{1cm} (25)

Inserting this value of \( \sigma \) into equation (15) yields:

\[ d_{25\% - 75\%} = 2 \left( 0.447 \frac{d_{FWM}}{2\sqrt{2\ln(2)}} \right) = \frac{0.4770}{\sqrt{\ln(2)}} d_{FWM} \]  \hspace{1cm} (26)

\[ d_{25\% - 75\%} = 0.5729d_{FWM} \]  \hspace{1cm} (27)
B.2 Effect of Working Distance

This section (B.2) includes figures referenced in the Results and Discussion subsection 3.3.3 Effect of Working Distance.

Figure B-1. Working Distance Series PSF FWHM Contours at 10kV and 20kV. The 10kV contours are shown on the left, and the 20kV contours are shown on the right. The best focus image of the calibration sample was found at multiple working distances (WD). Probe current was held constant at all WD. PSF size increases as WD increases, which matches probe size expectations. The effect is more noticeable at lower beam voltages.
Figure B-2. *Focal Point Diagram for Through Focus Series.* The stage was held at a constant height, and the beam was moved through focus to simulate a change in working distance. Changing the beam afforded more accurate focal intervals than changing the stage height.
Figure B-3. *Through Focus Series*. Top to bottom, the images on the left side correspond to the contours on the right side, respective with the following order. From outer contour to inner contour, the red contours represent -24μm, -12μm, -6μm, and 0μm (best focus). From inner contour to outer contour, the blue contours represent 0μm (best focus), +6 μm, +12 μm, and +24 μm. This figure was created by Matthew Zotta.
B.3 Characterization of Astigmatism

This section (B.3) includes figures referenced in the Results and Discussion subsection 3.3.4 Characterization of Astigmatism.

Figure B-4. Astigmatism Series at 2kV. A box containing a contour plot is shown for each point spread function (PSF). Each box has size 85nm x 85nm. The contours shown are $0.9I_{\text{max}}$ (inner contour), $0.5I_{\text{max}}$ (middle contour), and $\sim0.1I_{\text{max}}$ (outer contour), where $I_{\text{max}}$ is the maximum intensity for the PSF within the box.
Figure B-5. Astigmatism Series at 5kV. A box containing a contour plot is shown for each PSF. Each box has size 85nm x 85nm. The contours shown are $0.9I_{\text{max}}$ (inner contour), $0.5I_{\text{max}}$ (middle contour), and $\approx 0.1I_{\text{max}}$ (outer contour), where $I_{\text{max}}$ is the maximum intensity for the PSF within the box. Minor artifacts from the PSF determination process were minimized in the outer contours of a small number of PSFs to avoid distraction from the series as a whole.
Figure B-6. Astigmatism Series at 20kV. A box containing a contour plot is shown for each PSF. Each box has size 85nm x 85nm. The contours shown are $0.9I_{\text{max}}$ (inner contour), $0.5I_{\text{max}}$ (middle contour), and $0.1I_{\text{max}}$ (outer contour), where $I_{\text{max}}$ is the maximum intensity for the PSF within the box. Minor artifacts from the PSF determination process were minimized in the outer contours of a small number of PSFs to avoid distraction from the series as a whole.
B.4 Restoration of Astigmatic Images

This section (B.4) includes figures referenced in the Results and Discussion subsection 3.3.6 Image Restoration.

Figure B-7. Observed Images of Gold-on-Carbon from 10kV Astigmatism Series. Two regions were imaged: one region depicted in the central cross, the other region in the four quadrant images. For each tested stigmator combination, the calibration sample and gold-on-carbon sample were imaged using the same beam shape. The corresponding PSFs for these images are shown in Figure 3-7 in Chapter 3. The restorations of these images are shown in Figure B-8. Scale bar in central (best focus) image is of length 100nm.
Figure B-8. *Restored Images of Gold-on-Carbon from 10kV Astigmatism Series.* These restorations correspond to the observed images in Figure B-7. Each observed image was restored using the PSF with corresponding stigmator settings (Figure 3-7 in the main article). Scale bar in central (best focus) image is of length 100nm.
Appendix C: Chapter 4 Supplemental

C1. ImageJ Measurements of Particle Size

This section (C.1) contains a table and figures that were referenced in the Materials and Methods subsection 4.2.4 Particle Sizing and Profiling in the SEM in the article.

The following measurements were performed during the initial stages of this experiment. Only the plasma-cleaned particles on Kapton were measured (June 2018 data). The particles were re-cleaned and reimaged for the data presented in the article (January 2019 data).

Table C-1. Particle Diameters for BSE and SE at Multiple Voltages (measured in ImageJ).

<table>
<thead>
<tr>
<th>Voltage (kV)</th>
<th>Signal Type</th>
<th>Particle Diameter (nm)</th>
<th>COV (%)</th>
<th>Number of Particles Measured</th>
<th>Difference of SEa and BSE Mean Sizes (nm)</th>
<th>SE sizeb/BSE size</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>BSE</td>
<td>24.6 ± 1.8</td>
<td>8</td>
<td>100</td>
<td>2.7</td>
<td>1.11</td>
</tr>
<tr>
<td>20</td>
<td>SE</td>
<td>27.3 ± 2.0</td>
<td>7</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>BSE</td>
<td>25.8 ± 2.0</td>
<td>8</td>
<td>100</td>
<td>2.3</td>
<td>1.09</td>
</tr>
<tr>
<td>10</td>
<td>SE</td>
<td>28.1 ± 2.6</td>
<td>8</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>BSE</td>
<td>25.6 ± 2.4</td>
<td>9</td>
<td>100</td>
<td>1.5</td>
<td>1.06</td>
</tr>
<tr>
<td>5</td>
<td>SE</td>
<td>27.1 ± 2.1</td>
<td>8</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>BSEc</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2</td>
<td>SE</td>
<td>28.0 ± 2.6</td>
<td>9</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aThe mean SE particle size is consistently larger than the mean BSE particle size.

bSE size/BSE column shows the size ratio between the two signal types.

c2kV BSE was not measured due to poor contrast.
Figure C-1. *Histogram of SE Particle Diameters Measured in ImageJ*. When compared to BSE in Figure C-2, notice that the SE distributions are shifted to the right, indicating larger SE diameters. Size measurements were rounded to the nearest 1nm.

Figure C-2. *Histogram of BSE Particle Diameters Measured in ImageJ*. When compared to SE in Figure C-1, notice that the BSE distributions are shifted to the left, indicating smaller BSE diameters. Size measurements were rounded to the nearest 1nm.
C.2 ISO Measurements of Particle Size

This section (C.2) contains figures that were referenced in the Materials and Methods subsection 4.2.4 Particle Sizing and Profiling in the SEM in the article.

The following measurements were performed during the initial stages of this experiment. Only the plasma-cleaned particles on Kapton were measured (June 2018 data). The particles were re-cleaned and reimaged for the data presented in the article (January 2019 data).

Measurements were made by following the protocol given in ISO 13322-1:2014 – Particle size analysis – Image analysis methods – Part 1: Static image analysis methods.

For 20kV, 10kV, and 5kV (Figs. C-3, C-4, and C-5, respectively), the BSE and SE distributions were proven to have different means ($\mu_{\text{BSE}} \neq \mu_{\text{SE}}$) using two-tailed t-tests ($\alpha = 0.001$). The problem with this analysis is that the particle size distributions would shift depending on the intensity threshold chosen during the sizing process. This became a problem when comparing the particle sizes between grid and Kapton (Fig. C-6). The mean sizes between grid and Kapton particles would show a statistically significant difference for certain thresholds and not others. For this reason, particle profiling was used as a direct comparison of the original image data and is possibly more meaningful than reporting measured particle sizes.

Figure C-3. Histogram of BSE and SE Particles at 20kV Measured According to ISO Protocol. Histogram bin sizes = 0.5nm.
Figure C-4. *Histogram of BSE and SE Particles at 10kV Measured According to ISO Protocol.* Histogram bin sizes = 0.5nm.

Figure C-5. *Histogram of BSE and SE Particles at 5kV Measured According to ISO Protocol.* Histogram bin sizes = 0.5nm.
Figure C-6. *Histogram of Grid and Kapton BSE Particle Sizes at 20kV Measured According to ISO Protocol.* By slight changes to threshold selection, the particle size distributions would shift enough to change the result of the two-tailed $t$-test regarding their mean size ($\mu_{BSE} \neq \mu_{SE}, \alpha = 0.001$). Histogram bin sizes = 0.5nm.
C.3 PSF FWHMs for a Range of K and Background Correction

These plots (C.3) represent all tested voltage-signal-support go along with the Results and Discussion subsection 4.3.1.2 K and Background Correction and were made in the same manner as Figure 4-7 (in the article).

Figure C-7. 20kV PSF FWHMs over a Range of K and Background Correction. The best representations of the PSFs were estimated at: Grid BSE, $K = 3$, background correction (“BC”) = 0.12; Grid SE, $K = 100$, BC = 0.35; Kapton BSE, $K = 31$, BC = 0.12; Kapton SE, $K = 31$, BC = 0.35. Note that the vertical axis is linear, while the horizontal axis is logarithmic.
Figure C-8. 10kV PSF FWHMs over a Range of K and Background Correction. The best representations of the PSFs were estimated at: Grid BSE, $K = 10$, background correction (“BC”) = 0.20; Grid SE, $K = 100$, BC = 0.30; Kapton BSE, $K = 31$, BC = 0.20; Kapton SE, $K = 100$, BC = 0.30. Note that the vertical axis is linear, while the horizontal axis is logarithmic.
Figure C-9. *5kV PSF FWHMs over a Range of K and Background Correction.* The best representations of the PSFs were estimated at: Grid BSE, $K = 100$, background correction (“BC”) = 0.25; Grid SE, $K = 316$, BC = 0.30; Kapton BSE, $K = 100$, BC = 0.25; Kapton SE, $K = 100$, BC = 0.30. Note that the vertical axis is linear, while the horizontal axis is logarithmic.
Figure C.10. 2kV PSF FWHMs over a Range of K and Background Correction. The best representations of the PSFs were estimated at: Grid BSE, $K = 1000$, background correction (“BC”) = 0.35; Kapton SE, $K = 1000$, BC = 0.40. Grid SE and Kapton BSE image contrasts were too poor for reliable measurement. Note that the vertical axis is linear, while the horizontal axis is logarithmic.
C.4 Profiles of Simulated Particles for Comparison of BSEvsSE and GridVsKapton

This section (C.4) contains figures that were referenced in the Results and Discussion subsections 4.3.2.1 Data Collection Parameters: Signal Type and 4.3.2.2 Data Collection Parameters: Particle Support Material in the article.

Figure C-11. Signal Comparison using Simulated Particle Profiles. The legend in the top left plot (20kV Grid) applies to all plots. BSE and SE modes are compared at multiple voltages on both TEM carbon grid and Kapton substrate supports.
Figure C-12. *Support Material Comparison using Simulated Particle Profiles.* The legend in the top left plot (20kV BSE) applies to all plots. TEM carbon grid and Kapton substrate supports are compared at multiple voltages for both BSE and SE modes.
C.5 Single Particle Images and Average Profiles for Comparison of Experimental and Simulated Data on the TEM Grid

This figure in this section (C.5) was referred to in the Results and Discussion subsection 4.3.2.2 Data Collection Parameters: Particle Support Material in the article. The figure shown here is similar to Figure 4-10 in the article, which shows the experimental and simulation comparison for Kapton.

Figure C-13. Observed and Simulated Single Particle Images and Average Profiles on Grid. Observed and simulated images for single particles are shown for each voltage-signal case to provide visual comparison of what a calibration particle would look like while imaging. The average profiles are shown to compare observation and simulation, while taking advantage of lower noise levels for simpler comparison. The solid line (−) represents the observed data, and the star line (*) represents the simulated data.
Appendix D: Chapter 5 Supplemental

D.1 Images and Curves for a Range of $\lambda$

This section (D.1) contains figures that were referenced in the Results and Discussion subsection 5.3.1.1 Restoration-specific Parameters: $\lambda$ in the article.

Figure D-1. Restorations for a Range of $\lambda$ Values. The observed image (1kV SE tin balls region-of-interest (ROI)) is given in the top left of this figure for comparison. The $\lambda$ for each restoration is given in the top left corner of each image. Low $\lambda$ correspond to low levels of regularization (denoising) and better edge preservation, while high $\lambda$ to high levels of smoothing and worse edge preservation. Scale bar = 100nm.
Figure D-2. Frequency Content Analysis of Restorations for a Range of $\lambda$ Values. The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the central horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI, and the rainbow of colors (i.e., red, orange, yellow, green, blue, violet, and then dark red through dark green) represent the ROI restorations from lowest to highest $\lambda$ (i.e., $\lambda = 0, 7, 17, 27, 37, \ldots, 97$). Note that the y-axes are plotted on log scale.
D.2 Images and Curves for Reference Particle Size Comparison ($\lambda = 17, 57, 77$)

This section (D.2) contains figures that were referenced in the Results and Discussion subsection 5.3.2.1 PSF Calculation Parameters: Reference Particle Size in the article.

![Restorations with a Selection of Reference Particle Sizes for Different $\lambda$.](image)

From left to right: 1kV SE observed ROI of tin balls and restored ROI with reference particle sizes = 27.6nm, 28.5nm, and 29.4nm to show the $2\sigma$ range. From top to bottom, the restorations were performed with $\lambda = 17, 57, 77$ (not applied to observed image, which was not restored). Scale bar = 100nm.
Figure D-4. Frequency Content Analysis of Restorations for a Range of Reference Particle Sizes ($\lambda = 17$). The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI, and the rainbow of curve colors from red to purple represents the restored ROI with reference particle diameters of 27.6nm to 29.4nm in increments of 0.3nm, respectively. The data shown here were calculated from the $\lambda = 17$ restorations. Note that the y-axes are plotted on log scale.
Figure D-5. *Frequency Content Analysis of Restorations for a Range of Reference Particle Sizes ($\lambda = 57$).* The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI, and the rainbow of curve colors from red to purple represents the restored ROI with reference particle diameters of 27.6nm to 29.4nm in increments of 0.3nm, respectively. The data shown here were calculated from the $\lambda = 57$ restorations. Note that the y-axes are plotted on log scale.
Figure D-6. Frequency Content Analysis of Restorations for a Range of Reference Particle Sizes ($\lambda = 77$). The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI, and the rainbow of curve colors from red to purple represents the restored ROI with reference particle diameters of 27.6nm to 29.4nm in increments of 0.3nm, respectively. The data shown here were calculated from the $\lambda = 77$ restorations. Note that the y-axes are plotted on log scale.
D.3 Images and Curves for K Comparison ($\lambda = 17, 57, 77$)

This section (D.3) contains figures that were referenced in the Results and Discussion subsection 5.3.2.2 PSF Calculation Parameters: $K$ in the article.

Figure D-7. Restorations with a Selection of $K$ Values for Different $\lambda$. From left to right: 1kV SE observed ROI of tin balls and restored ROI with $K = 316, 3162, \text{ and } 31622$. From top to bottom, the restorations were performed with $\lambda = 17, 57, \text{ and } 77$ (not applied to observed image, which was not restored). Scale bar = 100nm.
Figure D-8. Frequency Content Analysis of Restorations with a Selection of K Values ($\lambda = 17$). The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The data shown here were calculated from the $\lambda = 17$ restorations. Note that the y-axes are plotted on log scale.
Figure D-9. *Frequency Content Analysis of Restorations with a Selection of K Values (λ = 57).* The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The data shown here were calculated from the λ = 57 restorations. Note that the y-axes are plotted on log scale.
Figure D-10. *Frequency Content Analysis of Restorations with a Selection of K Values ($\lambda = 77$).* The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The data shown here were calculated from the $\lambda = 77$ restorations. Note that the y-axes are plotted on log scale.
D.4 Images and Curves for PSF Background Correction Comparison ($\lambda = 17, 57, 77$)

This section (D.4) contains figures that were referenced in the Results and Discussion subsection 5.3.2.3 PSF Calculation Parameters: Background Correction in the article.

Figure D-11. Restorations with a Selection of PSF Background Corrections for Different $\lambda$. From left to right: 1kV SE observed ROI of tin balls and restored ROI with background correction (“bc”) = 0.00, 0.13, 0.23, and 0.33. From top to bottom, the restorations were performed with $\lambda = 17, 57, and 77$ (not applied to observed image, which was not restored). Scale bar = 100nm.
Figure D-12. *Frequency Content Analysis of Restorations with a Selection of PSF Background Corrections (λ = 17)*. The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI and the rainbow of curve colors from red to purple represents the restored ROI with background correction = 0.00, 0.13, 0.18, 0.23, 0.28, 0.33, respectively. The data shown here were calculated from the $\lambda = 17$ restorations. Note that the y-axes are plotted on log scale.
Figure D-13. Frequency Content Analysis of Restorations with a Selection of PSF Background Corrections ($\lambda = 57$). The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI, and the rainbow of curve colors from red to purple represents the restored ROI with background correction = 0.00, 0.13, 0.18, 0.23, 0.28, 0.33, respectively. The data shown here were calculated from the $\lambda = 57$ restorations. Note that the y-axes are plotted on log scale.
Figure D-14. *Frequency Content Analysis of Restorations with a Selection of PSF Background Corrections ($\lambda = 77$).* The FFT from the ROI is shown in the top row, the FFT from the shifted-ROI in the middle row, and the cross-correlation of the FFTs in the bottom row. The left and right columns show the horizontal and vertical intensity profiles (respectively) of the FFT or cross-correlation. The black curve represents the observed ROI and the rainbow of curve colors from red to purple represents the restored ROI with background correction = 0.00, 0.13, 0.18, 0.23, 0.28, 0.33, respectively. The data shown here were calculated from the $\lambda = 77$ restorations. Note that the y-axes are plotted on log scale.
Appendix E: Image Restoration Examples

E.1 Carbon Nanotubes

Figure E-1. *PSF for 1kV Carbon Nanotubes.* PSF determined from 1kV SE calibration image of 28.5nm particles on Kapton substrate with working distance = 2mm. \( K = 1000 \), background correction = 0.26. Scale 1nm/px. Scale bar = 10nm.

Figure E-2. *PSF for 2kV Carbon Nanotubes.* PSF determined from 2kV SE calibration image of 28.5nm particles on Kapton substrate with working distance = 6mm. \( K = 1000 \), background correction = 0.23. Scale 1nm/px. Scale bar = 10nm.
Figure E-3. *Observed and Restored Images of Carbon Nanotubes (>50nm).* Images of >50nm carbon nanotubes restored using the PSFs in Figure E-1 for 1kV (top row, $\lambda = 67$) and Figure E-2 for 2kV (bottom row, $\lambda = 77$). Different fields were imaged for 1kV and 2kV. 1kV working distance = 2mm, 2kV working distance = 6mm. Scale = 1nm/px. Scale bar = 100nm.
Figure E-4. Observed and Restored Images of Carbon Nanotubes (20-30nm). Images of 20-30nm carbon nanotubes restored using the PSFs in Figure E-1 for 1kV (top row, $\lambda = 72$) and Figure E-2 for 2kV (bottom row, $\lambda = 62$). Different fields were imaged for 1kV and 2kV. 1kV working distance = 2mm, 2kV working distance = 6mm. Scale = 1nm/px. Scale bar = 100nm.
Figure E-5. *Observed and Restored Images of Carbon Nanotubes (8-15nm).* Images of 8-15nm carbon nanotubes restored using the PSFs in Figure E-1 for 1kV (top row, \( \lambda = 72 \)) and Figure E-2 for 2kV (bottom row, \( \lambda = 67 \)). Different fields were imaged for 1kV and 2kV. 1kV working distance = 2mm, 2kV working distance = 6mm. Scale = 1nm/px. Scale bar = 100nm.
Figure E-6. Observed and Restored Images of Carbon Nanotubes (<8nm). Images of <8nm carbon nanotubes restored using the PSFs in Figure E-1 for 1kV (top row, $\lambda = 67$) and Figure E-2 for 2kV (bottom row, $\lambda = 72$). Different fields were imaged for 1kV and 2kV. 1kV working distance = 2mm, 2kV working distance = 6mm. Scale = 1nm/px. Scale bar = 100nm.
E.2 Mouse Brain

Figure E-7. **PSF for 2kV Osmium-stained Mouse Brain.** PSF determined from 2kV BSE calibration image of 52nm particles on carbon film TEM grid with working distance = 8.5mm. $K = 100$, background correction = 0.14. Scale 7nm/px. Scale bar = 20nm.

Figure E-8. **PSF for 1kV Osmium-stained Mouse Brain.** PSF determined from 1kV SE calibration image of 52nm particles on carbon film TEM grid with working distance = 8.5mm. $K = 31$, background correction = 0.18. Scale 7nm/px. Scale bar = 20nm.
Figure E-9. **TEM Comparison to Observed and Restored Images of Osmium-stained Mouse Brain at 2kV.** BSE images restored using the PSF in Figure E-7 for 2kV and $\lambda = 72$. Working distance = 8.5mm. Scale = 7nm/px. TEM image courtesy of Prof. Richard Hailstone.

Figure E-10. **Overview 2kV BSE Images of Osmium-stained Mouse Brain.** Two different fields are shown on left and right, with white boxes indicating magnified regions in Figure E-11 (left) and Figure E-12 (right). Scale = 7nm/px.
Figure E-11. Observed and Restored Images of Osmium-stained Mouse Brain at 2kV (Regions A&B). BSE images restored using the PSF in Figure E-7 for 2kV and \( \lambda = 72 \). Working distance = 8.5mm. Scale = 7nm/px. Scale bar = 500nm.
Figure E-12. Observed and Restored Images of Osmium-stained Mouse Brain at 2kV (Regions C&D). BSE images restored using the PSF in Figure E-7 for 2kV and $\lambda = 72$. Working distance = 8.5mm. Scale = 7nm/px. Scale bar = 500nm.
Figure E-13. *Observed and Restored Images of Osmium-stained Mouse Brain at 1kV.* SE images restored using the PSF in Figure E-8 for 1kV and $\lambda = 67$. Working distance = 8.5mm. Scale = 7nm/px.
E.3 Carbon Fiber

Figure E-14. Carbon Fiber Images, Restorations, and PSFs at Different Scales with High Voltage Reference. Calibration sample comprised of 18.5nm particles on graphene TEM grid. 0.5nm/px PSF is in 91px x 91px box, and 1nm/px PSF is in 45px x 45px box. The same field was imaged at both scales for comparison. Working distance = 9mm. Note that no PSF background correction was employed for these restorations, as this analysis was performed before that was standard practice. Including a background correction is expected to improve the restoration quality.
E.4 Mixed Particles

Figure E-15. Mixed Particles Images, Restorations, and PSFs at Different Scales with High Voltage Reference. Calibration sample comprised of 18.5nm particles on graphene TEM grid. 0.5nm/px PSF is in 91px x 91px box, and 1nm/px PSF is in 45px x 45px box. The same field was imaged at both scales for comparison. Working distance = 9mm. Note that no PSF background correction was employed for these restorations, as this analysis was performed before that was standard practice. Including a background correction is expected to improve the restoration quality.
Figure E-16. More Mixed Particles Images, Restorations, and PSFs at Different Scales with High Voltage Reference. Calibration sample comprised of 18.5nm particles on graphene TEM grid. 0.5nm/px PSF is in 91px x 91px box, and 1nm/px PSF is in 45px x 45px box. The same field was imaged at both scales for comparison. Working distance = 9mm. Note that no PSF background correction was employed for these restorations, as this analysis was performed before that was standard practice. Including a background correction is expected to improve the restoration quality.