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Design and Testing of a New Conical Beam Precession System for High-throughput Electron Tomography

A thesis submitted in partial satisfaction of the requirements for the Degree Master of Science in Bioengineering

by Richard James Giuly

Committee in charge:
Professor Mark H. Ellisman, Chair
Professor Gabriel A. Silva, Co-Chair
Professor Michael W. Berns
Professor Thomas R. Nelson

2008
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Co-Chair

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Chair

University of California, San Diego

2008
Dedication

This thesis is dedicated to my parents.
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ABSTRACT OF THE THESIS

Design and Testing of a New Conical Beam Precession System for
High-throughput Electron Tomography

by

Richard James Giuly

Master of Science in Bioengineering
University of California, San Diego, 2008

Professor Mark H. Ellisman, Chair
Professor Gabriel A. Silva, Co-Chair

Three dimensional imaging at multiple length scales is of great importance in biological research [4]. Conventional electron tomography addresses the length scale of 50nm$^3$ to 50μm$^3$, which lies between X-ray crystallography and light microscopy. A desirable technological advance is the scaling up of electron tomography to address large volumes on the order of 0.5mm$^3$ at high resolution, and a major challenge in achieving such large scale reconstructions is reducing the time of acquisition. Electron tomography is typically a slow and tedious process. Recent advances in automated
data acquisition using predictions of stage movement have lead to significant improvement [14] in acquisition speed. However, for very large scale high resolution imaging projects such as serial section tomography applied to brain circuit reconstruction [2], faster tomographic acquisition systems will be necessary.

In this work, a new conical electron beam control system was designed and tested on the following 3D imaging applications: high speed tomographic data acquisition without need for specimen tilt, enhancement of single axis tilt tomography with additional beam tilting, and object precession. Since tilting the beam requires only modification of current to deflection coils, the speed of beam tilt far exceeds mechanical tilting. Beam deflection also gives higher precision of changes in angle than mechanical tilt. Results are presented for each imaging application.
Introduction

Detailed maps of tissue that reveal structure-function relationships will have applications throughout biology, especially in the field of neuroanatomy [2]. Electron tomography has emerged as an important tool for high resolution structure-mapping, filling a gap between the length scales of atomic structure analysis, which is typically studied with X-ray crystallography, and cell architecture, which is often studied with light microscopy [13][7]. Over the past 30 years electron tomography has progressed from a labor intensive process to an almost fully automatic process. While early work was done with custom software and manual tilting of the sample, fully developed free software is now available for single axis reconstruction and microscopes are built with automated mechanical tilt systems. Although much improvement has been made, a massive increase in throughput will still be necessary to map out larger millimeter sized 3D volumes of biological tissue with resolution on the order of 10 nm.

Electron tomography is typically performed by rotating a stage mechanically and acquiring views with a fixed beam. When a mechanical stage is tilted to each angle, slight imperfection in movement mean that features in the image often must be re-centered, and focus has to be changed to adjust for changes in vertical (Z) height. Adjustments necessary to compensate for inaccurate rotation of the physical stage are referred to as "tracking." A recent tracking system developed by Mastronarde requires an average of 1-4.5 seconds of tracking and mechanical stage rotation for each change in angle. While automatic systems yield higher performance that original manual systems, significantly greater throughput methods will still be necessary to
address large scale reconstruction projects.

An example that demonstrates the magnitude of the throughput problem is reconstruction of the rat barrel cortex with dimensions .66mm .66mm 1.55 mm at approximately 10nm resolution [20] (see Appendix A for details). Time spent on physical stage movement for all tomograms (tiled across sections) would be approximately 15,000 hours. To address such large scale imaging projects with tomography new higher throughput technology is clearly desirable. Although this thesis does not address this particular whisker barrel circuit reconstruction project, data is presented as a proof or principle that tomography based on beam precession can deliver significant increases in acquisition speed.

Volumetric imaging throughput depends on multiple factors such as sectioning, reconstruction time, and data transfer rates. This thesis focuses on one component of the throughput problem, the time required for tilting. A new electric beam precession system is introduced that provides the requisite views of the specimen without the need for mechanical tilting. Beam tilt has advantages of speed, accuracy and flexibility. The speed and accuracy increases are due to the fact that the current of an electromagnet can be changed more accurately and more quickly than a stage can be rotated. Beam tilt has flexibility in the since that the angular changes are not limited to rotation about a single axis.

Conical beam precession systems for various applications have been designed previously [9][8][18][21], but these systems do not meet the requirements of electron tomography. The key requirements were that during beam precession (1) the diffraction image must remain stationary in the back focal plane, and (2) the image
must remain stationary on the CCD camera. A new system was designed to meet these requirements. A mathematic model of the beam deflection is presented in Chapter 2 to describe in detail how sinusoidal signals are sufficient for the beam control system, when assuming small beam deflection angles. A thorough calibration procedure was also developed to set parameters of this control system. Care was taken to ensure that the calibration procedure for the formation of the conical beam could be performed in a reasonable amount of time. (Alignment of transmission electron microscopes tends to drift over time so calibration and alignment are part of the daily use of the microscope and can be a significant time sink.) The precession system was tested for both beam angle accuracy and image positioning accuracy, and results are reported in chapter 2.

In addition to developing the conical precession system, a major part of this work was generation of preliminary data for multiple 3D imaging applications based on conical beam precession, and results are presented for each application. Two modes of high speed volumetric reconstruction (electron optical sectioning and backprojection) were tested by reconstruction of a high contrast spherical gold bead. The reconstructed volumes have higher resolution in X-Y plane than in Z, which is expected from theory presented in Appendix B. The volumetric methods were also tested on biological samples (see Figure 8) and results show that backprojection performs best for the purpose of bringing a specific plane into focus. An alternative application of conical beam precession presented is enhancement of mechanical tilt with beam tilt; results show that backprojection artifacts can be reduced with this method.
1. New Applications of Conical Beam Precession

Tomographic reconstruction relies on collecting different views of the specimen at various angles. In electron tomography, typically this is accomplished by a time consuming process of physically tilting the specimen around an axis and acquiring images with the beam fixed. This thesis introduces a new conical beam precession system which can generate multiple views of the specimen quickly using electrically controlled beam tilt alone, without need to rotate the specimen. With electrical control of the beam, the azimuthal angle of the beam $\phi$ (see Figure 3) can be changed in less than 10 milliseconds. This is two orders of magnitude faster than physical tilt of a specimen, which requires approximately 1-4.5 seconds per change in angle using advanced automatic control [14] and more time if performed manually.

This chapter covers the theory and results of several 3D imaging modalities based on conical beam precession. Applications that the system was applied to are electron optical sectioning (EOS), conical beam backprojection, hybrid acquisition, and object precession video, each of which is fully explained in sections that follow.

Figure 1 shows a schematic view of the beam precession system. This system is fully described in Chapter 2; the following is a brief description. Conical scanning of the beam is performed with coil sets 3 and 4. On a conventional TEM, the objective aperture performs a key role in the formation of amplitude contrast [7]. It allows undeflected electrons to pass and blocks electrons that have been deflected from interactions with atoms in the specimen. To enable conical beam precession imaging, the objective aperture is removed as it would block the beam (see Figure 1). Another
aperture is needed to produce amplitude contrast, so the selective area (SA) aperture is used in place of the objective aperture; this requires that the microscope be in a special "B-mode" described in [6] which ensures that the back focal plane of the objective lens is located at the SA aperture. Deflector coil sets 5 and 6 are used to ensure that (1) the electron beam at the back focal plane is centered on the SA aperture and that (2) the beam is returned to the optical axis.
Figure 1: Electron beam path during conical precession.
Figure 2: Theoretical value for Z resolution compared to resolution in the X-Y plane for conical tomography. The optimum factor is 1, which is achieved approximately as the angle approaches 90°. The microscope used for this work limits the zenith angle $\theta_0$ to 1.7°, which corresponds to an elongation factor of 41. (see Equation 162)

Z resolution depends on the zenith angle $\theta_0$

With conical tomography, which includes both electron optical sectioning (EOS) and backprojection, the theoretical X-Y resolution is equal to the resolution of the individual projections collected with the CCD camera (see Appendix B). However, the theoretical Z resolution depends heavily on the zenith angle $\theta_0$ as shown in Figure 2. As $\theta_0$ is increased, Z resolution improves. The optimum angle $\theta_0$ is theoretically 90°, but the effective thickness of typical specimens limit the angle to approximately 60°. Furthermore, the deflector performance of the JEOL JEM-3200 transmission electron microscope (TEM) used for this work limited the angle $\theta_0$ to 1.7°. Due to this limitation, all data presented uses a zenith angle of 1.7° or less.
Acquisition Speed

For reduction in overall speed of tomographic acquisition, both the time required for tilting and for camera data acquisition should be optimized. As modern computers and CCD camera increase in speed, physical tilt of the specimen performed in conventional electron tomography has become a significant bottleneck. A focus of this study is to show feasibility of beam precession as a higher speed alternative to mechanical stage tilt. Optimizations were not performed to ensure that the beam is precessed at the maximum possible rate, therefore it should be noted that with optimizations of beam precession speed, it is likely that tomography could be performed even faster than reported here.

Table 1 gives the approximate time required to acquire the datasets shown in the following sections. Results show that use of electric beam tilt reduces the time required for tilting to a negligible amount, so the acquisition time is greatly dependent on camera acquisition speed.
The depth of field of a TEM is typically so great that the full sample, on the order of 1 micron thick, is in focus. The images formed are essentially projections, which show all X-Y planes of information simultaneously. With a light microscope it is often possible to reduce the depth of field by using a larger objective aperture, and this is useful for viewing specific planes of the specimen. However, in transmission electron microscopy a small objective aperture is necessary for creating contrast, especially when imaging thick biological specimens [6]. The technique of single slice electron optical sectioning (EOS) simulates a reduction in the depth of focus by means of conical beam precession rather than increase in aperture size. This allows one to focus on a particular X-Y plane, as in light microscopy. This function gives the user a quick

---

**Table 1: Summary of speed performance for each application presented in this work. Note that use of a higher speed CCD camera could significantly reduce time. (CCD cameras with speeds of 0.083 s per frame are currently commercially available.)**

<table>
<thead>
<tr>
<th>Imaging Application</th>
<th>Result shown in Figure</th>
<th>Physical Tilts (1 sec)</th>
<th>Electric Tilts (10 ms)</th>
<th>Piezo driven Z stage movements, 24nm steps (1 s)</th>
<th>Motor driven Z stage movements, 0.3(\mu)m steps (1 s)</th>
<th>Image Acquisitions at 1024X1024 (2 s)</th>
<th>Total time for camera used in this work (2 s per frame)</th>
<th>Total time if high speed camera were used (0.083 s per frame)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Slice EOS</td>
<td>3B</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Single slice, time exposure</td>
<td>5C</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Volumetric EOS, using Z-tilt</td>
<td>8</td>
<td>-</td>
<td>396</td>
<td>33</td>
<td>-</td>
<td>396</td>
<td>799</td>
<td>40</td>
</tr>
<tr>
<td>Volumetric EOS, using mechanical Z stage</td>
<td>8A-D</td>
<td>-</td>
<td>432</td>
<td>-</td>
<td>12</td>
<td>432</td>
<td>880</td>
<td>52</td>
</tr>
<tr>
<td>Conical Backprojection</td>
<td>8E-F</td>
<td>-</td>
<td>36</td>
<td>-</td>
<td>-</td>
<td>36</td>
<td>72</td>
<td>3</td>
</tr>
<tr>
<td>Conical Backprojection</td>
<td>11</td>
<td>-</td>
<td>360</td>
<td>-</td>
<td>-</td>
<td>360</td>
<td>724</td>
<td>34</td>
</tr>
<tr>
<td>Single Axis Tilt with Conical Backprojection</td>
<td>12C,D,13B</td>
<td>31</td>
<td>1116</td>
<td>-</td>
<td>-</td>
<td>1116</td>
<td>2274</td>
<td>135</td>
</tr>
<tr>
<td>Object Precession</td>
<td>16</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>24</td>
<td>1</td>
</tr>
</tbody>
</table>

* For the single slice time exposure, the beam is precessed continuously around the cone at approximately 2 cycles per second.

---

1.1 Application: Electron Optical Sectioning

The depth of field of a TEM is typically so great that the full sample, on the order of 1 micron thick, is in focus. The images formed are essentially projections, which show all X-Y planes of information simultaneously. With a light microscope it is often possible to reduce the depth of field by using a larger objective aperture, and this is useful for viewing specific planes of the specimen. However, in transmission electron microscopy a small objective aperture is necessary for creating contrast, especially when imaging thick biological specimens [6]. The technique of single slice electron optical sectioning (EOS) simulates a reduction in the depth of focus by means of conical beam precession rather than increase in aperture size. This allows one to focus on a particular X-Y plane, as in light microscopy. This function gives the user a quick
way to gather information about the 3D structure of the specimen without any need for time consuming mechanical tilting. Furthermore, by stacking images generated by single slice EOS, volumetric reconstructions can be generated. These two modes of EOS, single slice and volumetric, are described in the following sections and preliminary data for each are shown.

1.1.1 Single slice electron optical sectioning

Figure 3: Illustration of beam tilted by zenith angle $\theta_0$ and azimuthal angle $\phi$.
During EOS imaging, the beam circumscribes a cone (Figure 3) with an apex at a plane of the specimen. As shown in Figure 4, one plane is reinforced as the beam precesses while others have an apparent circular motion during beam precession and become blurred. (The operating principle is similar to that of the circular polytome built for X-ray tomography [3].)

**Results for single slice electron optical sectioning**

Figure 5A demonstrates the concept of depth discrimination with EOS. A single projection image of a 1µm thick specimen was acquired with the beam untilted
(Figure 5A). The specimen has beads on its top and bottom surface separated by approximately 1 micron. Notice that all of the beads are visible. For comparison, Figure 5B shows an image acquired by the method of EOS. To generate this image, a series of views were acquired at zenith angle $\theta_0 = 0.8^\circ$ and with azimuthal angles $\phi = 0^\circ$ to $330^\circ$ in $30^\circ$ degree intervals and then averaged. In this image it is evident that some of the beads are at one Z plane while others are at another; beads near the Z level of the apex of the cone remain visible, while beads at another Z level are blurred and no longer visible. This demonstrates the principle of depth discrimination with EOS.

As an alternative to acquiring a set of images as the beam precesses in steps, a "time exposure" method of single slice EOS involves acquisition of a single image while the beam precesses continuously around the cone (with CCD camera shutter open). This method of acquisition allows the EOS image be acquired as a single CCD camera acquisition rather than a series of acquisitions, which reduces the overall time required. Figure 5C shows an image in which the beam was precesses completely around the cone at 2 Hz while the CCD camera shutter remained open for 2 seconds. The similarity of Figures 5B and 5C indicates that the faster acquisition method achieves depth discrimination, and, as expected, gives similar results as the averaged series of views.
Figure 5: Single plane electron optical sectioning results. The zenith angle used for conical precession was 0.8 degrees. The scale bar is 100nm in length.

(A) A typical projection image with the full depth of the sample in focus. (B) Average of a series of views taken at 12 different angles, $\varphi = 0^\circ$ to $330^\circ$ in $30^\circ$ increments. The beads indicated with arrows are far from the focus plane and have been blurred out. Other beads near the focus plane remain visible. (C) Single image taken with the CCD camera shutter remaining open as the bead precesses completely around the cone. The similarity of (B) and (C) indicates the 2 Hz precession of the beam is precise enough to generate virtually the same image as a set of superimposed individual shots at various azimuthal ($\varphi$) angles.
1.1.2 Volumetric electron optical sectioning

To generate a volumetric image, the technique of single slice EOS is performed repetitively with the specimen position at a series of Z levels. The single slice EOS images acquired at each Z level are stacked to form a volume. This is much like the optical sectioning technique described in [1]. The resultant stack of images represents a volumetric image, \( i \), containing the object, \( o \), convolved with the 3D point-spread-function (PSF) of the system, \( h \), so

\[
i = o \ast h
\]

or equivalently,

\[
i(x, y, z) = \int \int \int o(x', y', z') h(x-x', y-y', z-z') dx' dy' dz'.
\]

Results for volumetric electron optical sectioning

Figure 6A shows an experimental characterization of the 3D point spread function of volumetric EOS. To produce this plot, a conical image set was acquired at each of 33 Z levels at 24 nm spacing. (The Z level was set with a piezo driven stage). Each conical set consisted of 12 projections at \( \phi = 0^\circ \) to \( 330^\circ \) in \( 30^\circ \) increments.

Bead positions were determined using tracking tools of the IMOD software package. The bead positions trace a circular path at each Z level at the beam precesses. As expected, at levels far from the optical section focus, the radius of the circular path is large, while at the Z level near the cone apex (Z=480nm) the circular path radius
reduces to approximately zero. This illustrates the principle of reinforcement near the cone apex plane and blurring far from the cone apex. Note that the PSF shown in 6A is approximately conical but imperfect as it is warped and tilted off of the Z axis. It is likely that warping of the cone and misalignment with the Z axis result from the inherent curvature of electron trajectories and misalignment of the Z stage motion with the optical axis of the objective lens, respectively.
Deconvolution

Deconvolution is a technique used to recover the original object $o$ from a convolved image $i$. It is convenient to perform deconvolution in Fourier space.

Figure 6: Three-dimensional point-spread function of electron optical sectioning
and volumetric image of a spherical gold bead. (X, Y, and Z axis are in units of
nanometers.) X-Y pixel size of individual views is 1.7nm. The zenith angle used
for conical precession was 0.8 degrees.

(A) Experimental characterization of 3D point spread function. Each 24 nm step
in the Z direction corresponds to 1 pixel in the Z direction. (B) X-Z plane of the
reconstruction at Y=44. (C) X-Y plane of the reconstruction at Z=624. (D) X-Y plane
of the reconstruction at Z=360. (E) X-Y plane of the reconstruction at Z=96.
Taking the Fourier transform of both sides of Equation 1 and using the convolution theorem gives:

\[ I(u,v,w) = H(u,v,w)O(u,v,w) \]

Where \( H(u,v,w) \), \( I(u,v,w) \), and \( O(u,v,w) \) are the Fourier transforms of the PSF, the image, and object respectively. \( H(u,v,w) \) is commonly referred to as the contrast transfer function (CTF). The object can then be deconvolved with the following equation:

\[ O(u,v,w) = \frac{I(u,v,w)}{H(u,v,w)} \text{ where } H(u,v,w) \neq 0 \]  \hspace{1cm} (3)

The inverse Fourier transform of \( O \) gives \( o \), which is a volumetric image of the object, ideally without blur. However, since some values of \( H \) are typically zero, a modified filter, \( H' \), with values less than a threshold \( C_t \) replaced, is used instead:

\[ O'(u,v,w) = \frac{I(u,v,w)}{H'(u,v,w)} \]  \hspace{1cm} (4)

where

\[ H' = C_t \text{ if } H < C_t \]

\[ H' = H \text{ if } H \geq C_t . \]

The inverse Fourier transform of \( O' \) generates the approximation of \( o \) used in
the results that follow. (Use of a threshold when filtering in Fourier space is common in filtered backprojection and described in [7].)

**Deconvolution Results**

Deconvolution was performed with a custom MATLAB script. To generate the 3D array representing the PSF $h$, first a bead was tracked throughout a Z stack as described earlier (an example is shown in Figure 6A). The 3D array was created by initializing the array to zero, and then incrementing the pixels at X, Y, Z locations designated by tracking a single bead throughout the Z stack. Deconvolution was then performed as described above. The threshold value $C_t$ was chosen to be large enough to suppress excessive noise in the deconvolved image. Results are shown in Figure 8C-D. There was no beneficial effect of deconvolution (compare to 9A-B, which show the data before deconvolution). Reasons for this could be (1) 3D clipping, (2) error introduced by use of a threshold, and (3) slight misrepresentation of the point spread function, all of which are explored in appendix G. Results in Appendix G imply that a larger zenith angle $\theta_0$ would improve reconstruction quality and deconvolution performance.

1.2 Application: Reconstruction by Backprojection

Backprojection is a 3D reconstruction method which is commonly used in
electron tomography. This section presents backprojection results based on conical beam precession, where tilt of the beam was used to produce different views. (In conventional electron tomography, the beam is held fixed and the sample is rotated.) Beam precession can be used by itself or in combination with physical tilt of the sample. Beam precession without physical tilt (conical backprojection) is particularly suited for fast high-throughput tomography, but currently lacks the Z resolution achievable with mechanical tilt. A hybrid approach combining beam precessing and physical tilting allows one to exceed the resolution given by a typical mechanical tilt series with only a small increase in acquisition time. Both pure beam tilt and hybrid results are presented in the following text.

1.2.1 Conical Backprojection

To acquire data for conical backprojection, the beam is precessed in a conical fashion, acquiring images at intervals and pausing at each acquisition. (The acquisition scheme is the same as described in the section above regarding determination of the PSF, except that only one cycle around the cone is used, i.e. no stepping in the Z direction is required.) Precise beam trajectory information for each acquired image must be known to produce results with backprojection. Although the beam follows a trajectory that circumscribes a cone approximately, beam trajectories are not precisely known, so a technique called bundle adjustment was used to determine the trajectories from the images.
Bundle adjustment is a technique commonly used in electron tomography. It will be described briefly in the following text; for a more complete description see [11]. The coordinates \( X, Y, \) and \( Z \) refer to points on the 3D object being imaged. The coordinates \( x \) and \( y \) refer to a point a 2D projection image (view) of the object. Mathematically the imaging process of the transmission electron microscope, which is modeled as a projection, has this effect on 3D points of the object being imaged:

\[
\begin{pmatrix}
  x \\
  y
\end{pmatrix} = P(G \begin{pmatrix}
  X \\
  Y \\
  Z
\end{pmatrix} + t)
\]  
(5)

where \( P = \begin{pmatrix}
  1 & 0 & 0 \\
  0 & 1 & 0 \\
  0 & 0 & 0
\end{pmatrix} \). The nonsingular transformation \( G \) is a nonsingular 3 by 3 matrix; its main purpose it to represent rotation of the specimen relative to the beam. The vector \( t \) describes the 3D translation of the object relative to the beam.

Assume each fiducial mark on the specimen (usually a spherical gold bead) is labeled with an index \( i \). The 3D location of the bead is \( (X_i, Y_i, Z_i) \). In a particular view, indexed by \( j \), the location of bead \( i \) is represented as \( (x_{ij}, y_{ij}) \). Tracking of beads, which was done semi-automatically in IMOD [15] was the process of locating the beads in every image, i.e. determining \( x_{ij} \) and \( y_{ij} \) for each bead in each image. Applying Equation 5 to each bead in each view gives a set of equations of the form:
\[
\begin{pmatrix}
    x_{ij} \\
    y_{ij}
\end{pmatrix}
= P \begin{pmatrix}
    X_i \\
    Y_i \\
    Z_i
\end{pmatrix} + t_j
\] (6)

The values \( x_{ij} \) and \( y_{ij} \) are known from tracking. An optimization procedure described in [11] is used to find values of \( G_j \), \( X_i \), \( Y_i \), \( Z_i \), \( t_j \) that are approximately consistent with the set of equations represented by Equation 6. \( G_j \) and \( t_j \), which describe the beam trajectory for every view \( j \), are then used to perform backprojection.

**Results for conical backprojection applied to a gold bead**

Reconstruction results in this section were generated with a current version of TxBR [11], which was recently updated by Dr. Sebastien Phan and Dr. Albert Lawrence to accept conical tomography image sets. Figure 7 shows the correspondence between the expected 3D point-spread function (PSF) from the backprojection operation and the observed effect of the PSF on a spherical bead. Figure 7A shows a representation of the expected PSF, \( h \), of the backprojection operation. A line is drawn in the direction of the beam for each view that was acquired. The \( G_j \) transformations were derived from bundle adjustment as described earlier. (Only one bead of the set used for bundle adjustment is shown in this figure). Figure
7B is an X-Z cross-sectional view of a 3D reconstruction of the bead. As expected, the reconstruction shows the shape of a spherical beam convolved with an approximate hollow cone. The Z resolution is lower than resolution in the X-Y plane, so the spherical bead appears elongated in the Z direction.
Figure 7: Beam trajectories and resulting backprojection reconstruction of a gold bead. B-E show a volume of a bead reconstructed by backprojection assuming beam trajectories depicted in A. The scale of X, Y, and Z axes match in A and B for comparison. In B-E, axes are labeled in pixels. In X and Y directions one pixel corresponds to 11 nm. In the Z direction, one pixel corresponds to approximately 18 nanometers. The zenith angle used for conical precession was 1.4 degrees.

(A) Beam trajectory directions; (B) X-Z plane at Y=28; (C) X-Y plane at Z=61; (D) X-Y plane at Z=41; (E) X-Y plane at Z=21
Volume reconstruction results for EOS and conical backprojection

EOS and backprojection both create a 3D reconstruction that is approximately the original object $o$ convolved with a conical PSF $h$. Because a point in the object is convolved with a hollow cone whose axis is approximately aligned with the Z axis, an X-Y slice near the cone apex will show the point sharply and an X-Y slice a distance $Z_s$ from the apex will show the point blurred (convolved with a circle of radius $Z_s \tan(\theta_0)$). This effect creates a virtual "focus" so that objects at the plane of the apex are in focus and objects far from the apex are out of focus. (Note that this differs from the "focus" of the objective lens, which typically includes the full depth of the sample as the depth of field is large.)

The reconstructions shown in Figure 8 demonstrate EOS without deconvolution, EOS with deconvolution, and conical filtered backprojection. To acquire data for these reconstructions a motor driven Z stage was stepped in 0.3 micron increments. At each Z level, 36 images from azimuthal angles of 0 to 350 degrees were acquired at 10 degree intervals. Images (views) acquired at a plane were averaged and these average images were stacked to generate a volumetric electron optical sectioning reconstruction. The 36 views associated with only one Z plane were used to generate a conical backprojection reconstruction.

Qualitative examination of Z resolution for depth sectioning:

In Figure 8, boxes are draw around spines to identify them as spine 1 and spine 2.
The spines are at two distinct Z levels separated by approximately 0.9 microns. (Z levels of the volume were chosen by visually checking the stack for the slice that shows structure 1 or structure 2 most sharply.) The left side of Figure 8 shows slices at the plane of spine 1 and the right side shows slices of the reconstruction at the plane of spine 2. EOS without deconvolution (Figure 9A and 9B) shows an expected result: in 8A spine 1 is sharper than spine 2, and in 8B spine 2 is sharper than spine 1, although the effect is subtle. Figures 8B and 8C show slices from the method of EOS with deconvolution. The intended effect of deconvolution is to enhance sharpness, although with EOS, there is no perceivable benefit from deconvolution in these results (reasons for this are discussed in Appendix B and Appendix G). Figures 8E and 8F show slices from filtered conical backprojection reconstructions. Filtered backprojection shows the best Z resolution of the methods shown: there is a strong perceivable difference between sharpness of spine 1 and spine 2.

Characterization of Z resolution:

The bandwidth of the signal in the Z direction at Y=9/\mu m^{-1} was used as an indication of Z resolution. The threshold to determine bandwidth was chosen to be 28\% of the log of the maximum value in the power spectrum (see Figure 9). (Y=0 was not used because it lies in the missing cone of information; the power along the Z axis at Y=0 is noise rather than signal.) The bandwidths for deconvolved EOS, EOS (not deconvolved), and filtered backprojection, were 1.6, 1, and 2, respectively. For EOS and filtered backprojection, the results agree with qualitative results above in that the
larger bandwidth is associated with higher Z resolution and therefore more pronounced depth sectioning. Deconvolution of the EOS data gave a larger bandwidth but examining Figure 8 indicates no significant improvement in Z depth sectioning.

**Characterization of X-Y resolution:**

Radially averaged power spectrum plots were used to characterize X-Y resolution. Figure 10D shows that filtered backprojection gives more signal in a higher frequency range compared to EOS, and images show a corresponding increase in resolution. Figure 10B shows deconvolved EOS gives more signal at a higher frequency range than EOS, but the image resolution is not enhanced according to visual inspection (see Figure 8).

The higher performance of filtered backprojection compared to other techniques for X, Y, and Z resolution is most likely due better alignment (from bundle adjustment) and use of a deconvolution technique that avoids the 3D clipping problem (see Appendix G). The 3D clipping problem is avoided with backprojection because the deconvolution (also known as filtering) operation is performed in 2D for each view before backprojection is performed. (The technique of filtering before backprojection is described in [7].)
Figure 8: Comparison of EOS (A-B), EOS with deconvolution (C-D) and filtered backprojection (E-F). Figures on the left (A,C,E) are at the spine of the spine labeled 1. Figures on the right (B,D,F) are at the plane of the spine labeled 2. The Z separation between the slices shown on the left (A,C,E) and the right (B,D,F) is approximately 0.9 microns. The scale bar is 1 micron in length. The zenith angle used for conical precession was 1.4 degrees.
Figure 9: Plots on the right show power spectrum of a Y-Z planes (the arrow marks \( Y = 9 \) m\(^{-1}\)). Plots on the left show the power spectrum along the Z direction (Z is vertical in the plot) at \( Y = 9 \) m\(^{-1}\). In (a), (c), and (e), the red line shows the threshold (28% of maximum) used to determine bandwidth. All plots use a log base 10 scale. For each plot, a constant offset was added to make the minimum value zero. (Figures 42-44 also show power spectrums for this volume.) (a) Deconvolved EOS reconstruction power spectrum. (b) Deconvolved EOS power spectrum divided by EOS (without deconvolution) power spectrum. (c) EOS reconstruction power spectrum. (d) Filtered backprojection reconstruction power spectrum divided by EOS power spectrum. (e) Filtered backprojection power spectrum.
Figure 10: Radially averaged power spectrums of X-Y slices. Plots are on a log base 10 scale. (A) Power spectrum of deconvolved EOS reconstruction. (B) Power spectrum of deconvolved EOS reconstruction divided by power spectrum of EOS reconstruction. (C) Power spectrum of EOS reconstruction. (D) Power spectrum of filtered backprojection reconstruction divided by power spectrum of EOS reconstruction. (E) Power spectrum of filtered backprojection reconstruction.

Results for conical backprojection for a three-layer test sample

Figure 11 shows individual X-Y slices derived from a conical backprojection reconstruction. As with the reconstruction in Figures 8E and 8F, the purpose of this reconstruction is to show a plane in focus at a particular Z position and other planes out of focus. However, with this experiment was designed to give more dramatic results, as a proof of principle, and not intended to reveal any biological structure. A sample with three layers was prepared; three standard TEM copper mesh grids with a specimen on each were stacked to form the sample. A large separation between three layers was desirable so that one plane could be focused on with little perceivable effect
from the layers above and below.

Figure 11B shows an example of a single view of the specimen, note that all layers are superimposed. Figure 11C shows a conical backprojection reconstruction based on 360 views taken at azimuthal angles from 0 to 359 in 1 degree increments. The X-Y slices in Figure 11C show that each of the three layers were successfully extracted from the backprojection reconstruction.
Figure 11: Conical backprojection used to reconstruct a three-layer test specimen; The zenith angle used for conical precession was 1.7 degrees.
1.2.2 Hybrid approach for artifact reduction

The application of beam precession presented in this section is a hybrid approach where mechanical tilting is augmented with beam precession that is performed at each mechanically set angle. When discussing resolution, it is useful to consider the tomographic imaging process in Fourier space. The "central section theorem" [10] states that a projection image represents a slice of Fourier space oriented orthogonal to the viewing direction. Therefore this hybrid application acquires more unique planes of Fourier space and in this way samples Fourier space with higher resolution. An advantage of this technique is that it gives a reduction of artifacts with very little extra time required, assuming that a fast CCD camera is used.

Results for hybrid approach

To test the hybrid approach, a specimen was physically tilted from -60° to +60° in increments of 4°. At each physical tilt a set of 36 images were acquired, with the beam precessing conically at azimuthal angle increments of 10 degrees. Also at each mechanical tilt position, one image was acquired with no tilt of the beam. Two reconstructions were generated. The first reconstruction was generated from the set of 31 images taken with mechanical single axis tilt from -60 to +60 and no beam tilt, which represented a conventional mechanical tilt series. The second reconstruction was generated from all of the images acquired with conical precession, a total of 1116 images (36 images at each of 31 physical tilt angles).

Figures 12 and 13 shows the results for the two reconstructions, both of which
were performed with backprojection. Figure 12 shows slices through the X-Y plane and X-Z planes. Figure 13 also show another slice of the volumes, which cuts perpendicular to the mechanical tilt axis. As expected the reconstruction performed with the hybrid image set shows a reduction in backprojection artifacts (which appear as texture). The difference between the power spectrum of the tilt series and the hybrid series (shown in Figure 14B) shows the region of Fourier space attenuated by using the hybrid method occupies an elliptical band in the Z-Y plane. This region apparently represents the texture that is removed in the direct space images. Figure 15 shows attenuation of higher frequencies (approximately 15/μm and higher) in the X-Y plane, which apparently represent the texture artifact in direct space. (Power spectrums for these two reconstructions are also shown in Figures 46 and 47.)
Figure 12: Single axis tilt series (left) compared to hybrid reconstruction (right). The hybrid reconstruction shows a reduction in texture caused by backprojection artifacts. (The zenith angle used for conical precession was 1.7 degrees.) The scale bar is one micron in length.

(A) X-Z slice of single axis reconstruction. The yellow cross-hair in B specifies the Y location of this slice; (B) X-Y slice of single axis reconstruction. The yellow cross-hair in A specifies the Z location of this slice; (C) X-Z slice of hybrid reconstruction. The yellow cross-hair in D specifies the Y location of this slice; (D) X-Y slice of single axis reconstruction. The yellow cross-hair in C specifies the Z location of this slice.
Figure 13: Slice through the U-Z plane of the data volume shown in Figure 10. In this figure the Z axis is horizontal. The single axis reconstruction is on the left (A) and the hybrid reconstruction in on the right (B). The hybrid reconstruction shows less texture (throughout the image) and less streaking (shown in the dotted box), both of which are caused by backprojection artifact.

U is defined as a coordinate axis in the X-Y plane. It is 45.7 degrees clockwise of the +Y direction. The U-Z plane was chosen for this figure because it is approximately perpendicular to the physical axis of rotation, so the backprojection artifacts are more easily identified. The horizontal scale bar is 1 micron in the Z direction. The vertical scale bar is 1 micron in the U direction.
Figure 14: Power spectrums of Y-Z slices. (A) Power spectrum for single axis tilt series. (B) Power spectrum for hybrid method divided by power spectrum for single axis method. (C) Power spectrum for hybrid method. All images are plotted on a log base 10 scale. For each plot, a constant offset was added to make the minimum value zero.
Figure 15: Radially averaged power spectrums of X-Y slices. Plots are log base 10 scale. (A) Power spectrum of single axis tilt reconstruction. (B) Power spectrum of hybrid reconstruction divided by power spectrum of single axis tilt reconstruction. (C) Power spectrum of hybrid reconstruction.

1.3 Application: Object precession video

Before performing a full tomographic acquisition, a significant amount of time is often spent searching for a site on the specimen that has the structures of interest and well distributed fiducial marks (such as gold beads). The three-dimensional distribution of fiducial marks is important for accuracy of bundle adjustment and reconstruction. Since projection views of a 3D specimen are inherently ambiguous, it can be difficult to identify biological structures and ensure that fiducial beads are well distributed in 3D. Typically a tedious process of mechanical tilting of the stage is used to form parallax and give some 3D information, but the "object precession" method, described in the following text, offers a more convenient approach.

Object precession involves acquiring a set of images using conical beam precession and viewing them in a video. This provides a quick way of using parallax
effects to observe the 3D nature of the specimen. Object precession uses the same input data as conical backprojections, a set of images acquired at azimuthal angles stepped in increments from 0 to 360 degrees. The 3D nature of the specimen becomes apparent when images are shown in the form of a video at approximately 15 frames per second as the sample appears to precess smoothly.

Figure 16 shows frames of such a video. Although these still images do not produce the same effect as a video, some differences in the images are apparent from parallax. Since commercially available CCD cameras are capable of more than 15 frames per second, this imaging technique could be performed in real-time and significantly reduce time spent searching for desired biological structures and well distributed fiducial marks. In this way the object precession method would compliment full volumetric reconstruction approaches such as standard single axis reconstruction, volumetric EOS and conical backprojection. It would allow for quick identification of an appropriate area of a specimen, before performing a complete tomographic acquisition and reconstruction.
Figure 16: Example frames from a video that shows views of specimen as the beam precesses. When shown in quick succession, the sample appears to precess and some 3D features become apparent. The views shown (A-L) are at an azimuthal angle of $0^\circ$ to $330^\circ$ in $30^\circ$ degree increments. The scale bar is 1 micron in length. (The zenith angle used for conical precession was 1.4 degrees.)
2. Beam Control

2.1 Introduction

This chapter presents a control system and a calibration procedure for a new conical precession system designed for 3D imaging applications. Conical beam precession systems for other applications have been designed previously [9][8][18][21], but these systems do not meet the requirements of tomography. Key requirements are that during precession the diffraction image must remain stationary in the back focal plane, and the image must remain stationary on the CCD camera. The control system presented here uses sinusoidal current signals to produce the beam precession and bring the beam back onto the optical axis. A beam trajectory model is presented to show that sinusoidal currents are appropriate for the scanning and de-scanning. The model covers the existing JEM-3200 compensation system and the new de-scan system implemented on coil sets 5 and 6. Also, an iterative process is described to calibrate the beam control system. The final portion of this chapter gives quantitative performance results for the control system.

With the current system, calibration must be re-performed if settings such as zenith angle, objective lens focus, or specimen z position are changed. For future work, a useful improvement would be use of the beam trajectory model to maintain proper de-scan calibration when settings are altered. If the model was fully incorporated into the control system so that de-scan calibration for the various settings could be determined from the model, time needed for re-calibration could be reduced.
Outline of Calibration Procedure

The full conical precession calibration procedure consists of three steps described fully in the sections that follow. In summary, the steps are:

1. Align the beam with the objective lens, the objective mini lens, and the first intermediate lens (as described in section 2.3). This first step establishes a center around which the beam will precess.

2. Calibrate the balance between deflector coil sets 3 and 4 so that a pivot point is formed at the specimen (as described in section 2.5.6).

3. Calibrate the control system for coil sets 5 and 6 to ensure that the beam is returned to the optical axis before it goes through the objective mini lens and other lower lenses (as described in section 2.6.4).

After calibration is complete, the beam precesses around a conical path and images are collected. Images are collected either individually, pausing at each azimuthal angle, or as one average image with the shutter open while the beam precesses around the full cone. The calibration is approximately stable over time, but has to re-performed periodically. (Periodic calibration is required because conditions within the microscope change over time, for example, magnetic fields from the soft iron components of the lenses depend on the history of nearby magnetic fields [5]).
Figure 17: Lenses and deflector coils of JEOL JEM-3200 TEM. Deflector coil sets are numbered from 1 to 11 on the right.
2.2 Formal description of deflector coil control system

An simplifying assumption in this document is that the deflector coils are solenoids; the precise geometry is defined by the manufacturer of the microscope. Coil $i$ refers to a pair of solenoids on either side of the column that are aligned on the same axis to produce a deflecting magnetic field near the center of the column. Coil set $i$, refers to two coils, one aligned with the x axis and one aligned with the y axis.

![Figure 18: Illustration showing the 3D arrangement of two sets of deflector coils.](image)

The variables $d_{ix}$ and $d_{iy}$ refer to the current for the x and y coils numbered i; the coils are approximately orthogonal as shown in Figure 18. A boldface subscripted variable such as $d_i$ refers to a vector with first component $d_{ix}$ and second component $d_{iy}$. The scalar constants written $C_{a,b}$ define a weighting of parameter $a$ when calculating the current of coil $b$, as shown in Equations 7-10. As the beam sweeps around a cone, the azimuthal angle $\phi$ changes from 0 to $2\pi$, either continuously or in steps, depending on the imaging mode.
The current of each coil in Figure 17 is set as follows:

- Gun deflector coil currents $d_1$ and $d_2$ are configured according to a standard alignment procedure documented in the user manual of the microscope.

- Currents $d_3$ and $d_4$ are set as a function of the azimuthal angle $\phi$ to create tilt in the beam before it enters the specimen. Rather than setting $d_3$ and $d_4$ values directly, the values of parameters $p_t$ and $p_s$ are set and $d_3$ and $d_4$ are determined by Equations 7-10. (Ideally the parameters $p_t$ and $p_s$ represent pure shift and pure tilt of the beam, respectively.)

The constants $C_{tx,3x}$, $C_{ty,3y}$, $C_{by,4x}$, $C_{sx,4x}$, $C_{tx,4y}$, $C_{sy,4y}$ must be set properly to ensure shift and tilt purity. A custom process, described in section 2.5.6 was required to determine these values because the standard procedure is does not scale well to zenith angles larger than 1.5°.

- Currents $d_5$ and $d_6$ are configured so that coil sets 5 and 6 will return the beam to the optical axis. A custom control system and calibration procedure was designed to accomplish this; it is described in section 2.6.

- Currents $d_7$ and $d_8$ control coil sets 7 and 8, which align the beam with the intermediate lenses. It was determined experimentally that coil set 7 alone is sufficient to return the beam approximately to the axis of the intermediate lenses, so coil set 8 is unused, i.e. $d_{8x}$ and $d_{8y}$ are set to zero.

- Currents $d_9$ and $d_{10}$ are configured according to a standard alignment
procedure documented in the user manual of the microscope.

- Coil set 11 is unused, so $d_{11x}$ and $d_{11y}$ are set to zero.

- $p_{sx}$ and $p_{sy}$ are parameters for controlling the amount of beam shift

- $p_{tx}$ and $p_{ty}$ are parameters for controlling the amount of beam tilt

The dynamic control system sets the coil currents $d_3$, $d_4$, $d_5$, and $d_6$, as described in equations 7-14. Equations 7-10 are built into the JEM-3200 microscope control system, the reasoning behind them is explained in the section 2.5. Equations 11-14 were implemented in custom software to bring the beam back onto the optical access; they are explained in the section 2.6.

\[
d_{3x}(\phi) = p_{sx} + C_{tx,3x} p_{tx}(\phi)
\]
\[
d_{3y}(\phi) = p_{sy} + C_{ty,3y} p_{ty}(\phi)
\]
\[
d_{4x}(\phi) = p_{tx}(\phi) + C_{ty,4x} p_{ty}(\phi) + C_{sx,4x} p_{sx} + C_{sy,4x} p_{sy}
\]
\[
d_{4y}(\phi) = p_{ty}(\phi) + C_{tx,4y} p_{tx}(\phi) + C_{sy,4y} p_{sy} + C_{sx,4y} p_{sx}
\]
\[
d_{5x}(\phi) = A_{5x} \sin(\phi + \sigma_{5x}) + C_{5x}
\]
\[
d_{5y}(\phi) = A_{5y} \sin(\phi + \sigma_{5y}) + C_{5y}
\]
\[
d_{6x}(\phi) = A_{6x} \sin(\phi + \sigma_{6x})
\]
\[
d_{6y}(\phi) = A_{6y} \sin(\phi + \sigma_{6y})
\]

where:

\[
p_{tx}(\phi) = A_{tilt} \cos(\phi) + C_{tilt}
\]
\[
p_{ty}(\phi) = A_{tilt} \sin(\phi) + C_{shift}
\]
2.3 Initial column alignment

After performing a standard alignment of the microscope according to the manufacturer's instructions and fully calibrating the scan and de-scan systems as described in sections 2.5.6 and 2.6.4, images at azimuthal angle near 130° appeared blurry while images near azimuthal angle 300° had higher quality. It was determined that the reason for this asymmetry was slight misalignment with the objective mini lens and the intermediate lenses. With conventional microscope usage these misalignments may have gone unnoticed, but cone beam imaging is more sensitive to beam misalignment. Even though ideally the control system should bring the beam precisely onto the optical axis before it enters the lens below, the system is imperfect so there are slight deviations. These slight deviations are much more problematic if the the axis of the cone is misaligned with the lenses.
Figure 19: Exaggerated illustration of lens misalignment and correction with beam deflection. Alignment with the optical axis requires that (1) the beam angle is aligned with the lens axis and (2) the beam position is centered on the lens axis. (A) Beam is not aligned with the optical axis of the lenses. (B) Deflectors 3, 4, 5, and 7 are used to ensure that the beam enters each lens approximately on its optical axis. (C) Deflectors 3, 4, 5, 6, 7, and 8 are used to ensure the beam enters each lens precisely on its optical axis.

To address this misalignment problem, current centering was performed on the objective lens, the objective mini lens, and the first intermediate lens. (In the alignment procedure suggested by the manufacturer, only the objective lens is current-centered.) These alignments are performed with the zenith angle $\theta_0$ set to zero, so the beam is not precessing around a cone. Rather, the beam is traveling down the axis of the cone that will be formed when the beam is precessed. "Current-centering" is the process of changing the lens current and adjusting the beam trajectory above the lens so the beam enters the lens at the center of the lens and aligned with the optical axis of the lens. If the lens and beam are properly aligned the image will expand and rotate about the center of the screen as the lens current changes. If the lens and beam are misaligned, the image will rotate around some other off-center point as the lens current changes. The JEM-3200 electronics include an "Objective Wobble" control.
that feeds a sinusoidal current to the objective lens to aid in current centering. Since the objective mini lens and first intermediate lens do not have wobble controls, their current was varied manually.

The procedure for aligning the objective lens, the objective mini lens, and the first intermediate lens is as follows:

1. The "condenser tilt coils" (coil sets 3 and 4) are used to adjust the tilt of the beam entering the objective lens. When the beam angle is correct, the illuminated beam spot on the viewing screen expands about the center of the screen as the objective lens current changes. (The compensation system for coils 3 and 4 allows the microscope user to set the shift and tilt of the beam rather than setting the coil currents directly.)

2. Coil set 5 is used to correct the angle of the beam before it enters the mini-objective lens.

3. Coil set 7 is used to correct the angle of the beam before it enters the first intermediate lens.

4. The "condenser shift coils" (coil sets 3 and 4) are used to correct the position of the beam on the viewing screen. This step may also be performed after step 1 or step 2.

At each lens, ideally both the translation error (centering) and the angle of the beam should be adjusted. This requires two deflections above every lens in the worst case (Figure 19c). In practice, it was determined that using coils 3, 4, 5 and 7 alone is sufficient, rather than 3, 4, 5, 6, 7, and 8. Technically, this simplification could create a situation where the following problem occurs: when the beam is centered on the
viewing screen and aligned with the axis of the lenses, the beam cannot be centered on the lenses. However, the lenses are reasonably well aligned mechanically so the error introduced by the simplification is negligible.

Also, it may seem that correcting the translational error with the condenser shift (using coil sets 3 and 4) would cause the beam to be misaligned with the objective lens, but the shift introduced at coil sets 3 and 4 is magnified greatly by the lenses below. Thus, only a very small deflection is needed, and the shifting at coil sets 3 and 4 introduces negligible translational misalignment with the objective lens.

The initial column alignment procedure described above sets the alignment constants $C_{\text{tilt}}$, $C_{\text{shift}}$, $C_{5x}$, $C_{5y}$, $d_{7x}$, and $d_{7y}$. (Sections 2.5-2.6 will describe how beam precession is created by varying the parameters $p_t$, $d_5$, and $d_6$.) When these alignment constants are set properly, the beam passes through the lenses approximately on their objective axis when the zenith angle $\theta_0$ is 0, i.e. there is no beam precession. In the analysis that follows, all of the alignment constants are assumed to be zero as if the microscope lenses are perfectly aligned mechanically. This simplification is valid because (assuming small angle deflections and small lens alignment imperfections) the precession control parameters $p_t$, $d_5$, and $d_6$ would be set to the same values in either of the following cases: (1) the lens are aligned exactly and the alignment constants are zero or (2) the lenses are not exactly aligned and the nonzero alignment constants are used to return the beam to the optical axis of the lenses.
2.4 Beam trajectory model

2.4.1 Representation of beam deflection angle

The deflector coils create magnetic fields that deflect the beam. The direction of the magnetic field is indicated with $b_i$, where $i$ is the number of the coil set that produced the field. The effect of the field is to change the direction of the beam. We
model the change in direction as occurring at a single point in space. (In reality the
direction changes more gradually, so the path of the electron is curved where
deflection occurs.) The changes in direction can be represented with rotation
operations on the direction vector of the beam. These rotations are around the
direction vector of the magnetic field that causes them, according to the right hand rule
(Figure 21). The direction of the beam and the rotating effect of deflection may be
represented in the following ways:

1. **Three dimensional direction vectors and 3X3 rotation matrices**

   Suppose the beam is traveling in the direction $v_0$ and is changed to $v_1$. ($v_0$
   and $v_1$ are 3D direction vectors parallel to the beam direction.) A 3X3
   rotation matrix $R_{rotation}$ can be used to represent the change in direction:
   
   $v_1 = R_{rotation} v_0$

2. **Two dimensional rotation vectors**

   Alternatively, "rotation vectors" can be used to represent a rotation operation, or
   the direction of the beam. The operation of the rotation vector $r$ is defined as a
   rotation of $|r|$ radians around the vector's axis according to the right hand rule. The
   3X3 rotation matrix that performs the rotation operation associated with a rotation
   vector $r$ is $R(r)$. We use a convention that the 3D direction $v_0$ specified by a
rotation vector \( \mathbf{a} \) is $\begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$ rotated according to the rotation operation of \( \mathbf{a} \), so

\[
v_0 \equiv R(\mathbf{a}) \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}.
\] (17)

Rotation vectors have only x and y components, so they are restricted to the x-y plane. With this restriction it is still possible to represent any direction in 3-space. This restriction also means that all of the rotation operations must be around vectors in the x-y plane; this is acceptable because the direction of magnetic fields the deflector coils generate are confined to x-y planes. In this document the variable \( \mathbf{r} \) is used for rotation vectors representing rotation operations and the variable \( \mathbf{a} \) is used for rotation vectors that specify a direction.

As an example of using rotation vectors, suppose the beam direction is specified with a 2D rotation vector \( \mathbf{a}_0 \) and deflected according to the rotation vector \( \mathbf{r} \). The modified direction \( \mathbf{a}_1 \) is given by:

1. For any 3D vector \( \mathbf{v}_0 \), the crossproduct \( \mathbf{w} = \mathbf{v}_0 \times \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \) is confined to the x-y plane. Rotating \( \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \) around the axis of vector \( \mathbf{w} \) the proper amount, will yield a vector in the direction of \( \mathbf{v}_0 \). This shows the any direction \( \mathbf{v}_0 \) can be represented with a 2D rotation vector that has the x and y components of \( \mathbf{w} \).
As shown in equation 18, representation of direction and deflection in this way is convenient because rotation can be treated as addition of a vector rather than a matrix multiplication. Treatment of rotations as vector addition depends on a small angle assumption (see Section 2.4.2).

3. Two dimensional slope vectors

The beam can direction can be represented with the 2D "slope vector" \( a^* \).

With this representation the corresponding 3D direction vector is

\[
\begin{pmatrix}
  a^*_x \\
  a^*_y \\
  1
\end{pmatrix}
\]

The components of \( a^* \) are \( a^*_x = \frac{dx}{dz} \) and \( a^*_y = \frac{dy}{dz} \), slopes of the beam trajectory's x and y components. When using 2D slope vectors the vectors used to represent rotations are also slope vectors, \( r^* \), with \( r^*_x = \frac{dx}{dz} \) and \( r^*_y = \frac{dy}{dz} \). Using slope vectors, just as with rotation vectors, rotations operations can be represented as vector addition. If a beam moving in a direction indicated by \( a^*_0 \) is changed by the amount indicated by \( r^* \), the resulting direction \( a^*_1 \) is given by:

\[
a^*_1 = r^* + a^*_0
\]
beam, as it moves in the \( \hat{z} \) direction a distance of \( L \) is given by \( L \mathbf{a}^* \).

Notation: in this document for any rotation vector \( \mathbf{r} \), the vector \( \mathbf{r}^* \) is the corresponding slope vector which represents the same rotation operation as \( \mathbf{r} \). To convert between the rotation vector representation and the slope vector representation the following equations are used:

\[
\mathbf{r}^* \equiv \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix} \mathbf{r}
\]

(19)

\[
\mathbf{a}^* \equiv \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix} \mathbf{a}
\]

(20)

Section 2.4.3 will show that Equations 19 and 20 are valid.

2.4.2 Rotation operations as vector addition

When multiple rotations are performed on a 3D vector \( \mathbf{v} \), for example a rotation \( R(\mathbf{r}_1) \) followed by a rotation \( R(\mathbf{r}_2) \), the resulting rotation is

\[ R(\mathbf{r}_2)R(\mathbf{r}_1)\mathbf{v} \]; the effect of a two rotations is represented by matrix multiplication.

For small angle rotations, a simplification can be made: \( R(\mathbf{r}_2)R(\mathbf{r}_1) = R(\mathbf{r}_1 + \mathbf{r}_2) \).

With this simplification, the rotations are represented as vectors and combining rotations is vector addition, rather than matrix multiplication.

To show that a sequence of rotations can be represented as a sum of rotation vectors, we start with the rotation matrix corresponding to a rotation around the vector
Using the following rotation matrices around the x, y, and z axes,

\[
R_x = \begin{pmatrix}
1 & 0 & 0 \\
0 & \cos(\alpha) & -\sin(\alpha) \\
0 & \sin(\alpha) & \cos(\alpha)
\end{pmatrix},
\]

\[
R_y = \begin{pmatrix}
\cos(\beta) & 0 & \sin(\beta) \\
0 & 1 & 0 \\
-\sin(\beta) & 0 & \cos(\beta)
\end{pmatrix},
\]

\[
R_z = \begin{pmatrix}
\cos(\gamma) & -\sin(\gamma) & 0 \\
\sin(\gamma) & \cos(\gamma) & 0 \\
0 & 0 & 1
\end{pmatrix},
\]

the rotation matrix around the vector \( \begin{pmatrix} u \\ v \\ w \end{pmatrix} \) is given by:

\[
R(r) = \begin{pmatrix}
\frac{u^2 + (v^2 + w^2) \cos \theta}{u^2 + v^2} & \frac{uv(1 - \cos \theta) - w \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} & \frac{uv(1 - \cos \theta) + v \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} \\
\frac{uv(1 - \cos \theta) - w \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} & \frac{v^2 + (u^2 + w^2) \cos \theta}{u^2 + v^2} & \frac{vw(1 - \cos \theta) - u \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} \\
\frac{uv(1 - \cos \theta) + v \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} & \frac{vw(1 - \cos \theta) - u \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} & \frac{w^2 + (u^2 + v^2) \cos \theta}{u^2 + v^2}
\end{pmatrix}
\]

(The derivation of 21 is described in [12] and [17].)

Since we are considering rotations vectors confined to x-y plane, we set \( w \) to zero which gives

\[
R(r) = \begin{pmatrix}
\frac{u^2 + (v^2 + w^2) \cos \theta}{u^2 + v^2} & \frac{uv(1 - \cos \theta)}{u^2 + v^2} & \frac{uv(1 - \cos \theta)}{u^2 + v^2} \\
\frac{uv(1 - \cos \theta)}{u^2 + v^2} & \frac{v^2 + (u^2 + w^2) \cos \theta}{u^2 + v^2} & \frac{-uv \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} \\
\frac{uv(1 - \cos \theta)}{u^2 + v^2} & \frac{-uv \sqrt{u^2 + v^2 + w^2} \sin \theta}{u^2 + v^2} & \frac{(u^2 + v^2) \cos \theta}{u^2 + v^2}
\end{pmatrix}
\]

Where \( r = \begin{pmatrix} u \\ v \end{pmatrix} \) is a rotation vector.
Using the length of the vector \( r \) to represent the angle

\[
\theta = \sqrt{u^2 + v^2} = |r|
\]  

(23)

and small angle (\( \theta < 10^\circ \)) approximations

\[
sin(\theta) = \theta
\]

(24)

\[
\cos(\theta) = 1
\]

(25)

Equation 22 simplifies to the following approximation

\[
R(r) = \begin{pmatrix}
1 & 0 & v \\
0 & 1 & -u \\
-v & u & 1
\end{pmatrix}
\]

(26)

\[
R(r_1)R(r_2) = \begin{pmatrix}
1-v_1v_2 & u_2v_1 & v_1 + v_2 \\
u_1v_2 & 1-u_1u_2 & -u_1 - u_2 \\
-v_1-v_2 & u_1 + u_2 & 1 - u_1u_2 - v_1v_2
\end{pmatrix}
\]

Since the angle \( \theta \) is small, the components \( u_i \) and \( v_i \) are small, the terms with two components multiplied together are small enough to be ignored, which gives

\[
R(r_1)R(r_2) = \begin{pmatrix}
1 & 0 & v_1 + v_2 \\
0 & 1 & -u_1 - u_2 \\
-v_1 - v_2 & u_1 + u_2 & 1
\end{pmatrix} = R(r_1 + r_2)
\]

(27)
So, the matrix multiplication is approximately equal to the effect of adding the rotation vectors.

2.4.3 Relationship between slope vectors and rotation vectors

Slope vectors and rotation vectors representing direction

In this section we will verify Equation 20. As explained earlier, the rotation vector \( \mathbf{a} \) indicates the direction \( \mathbf{v}_0 \) of \( \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} \) rotated around the axis of \( \mathbf{a} \).

\[
\mathbf{v}_0 = R(\mathbf{a}) \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} = \begin{pmatrix} 1 & 0 & a_y \\ 0 & 1 & -a_x \\ -a_y & a_x & 1 \end{pmatrix} \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix} = \begin{pmatrix} a_y \\ -a_x \end{pmatrix}
\]

Also explained earlier, the slope vector \( \mathbf{a}^* \) indicates the amount the position changes in x and y with respect to z as one moves along the direction of the beam. Based on this definition, a 3D vector pointing in the direction of the beam is

\[
\mathbf{v}_0 = \begin{pmatrix} a_x^* \\ a_y^* \\ 1 \end{pmatrix}
\]

The slope vector \( \mathbf{a}^* \) and the corresponding rotation vector \( \mathbf{a} \) must indicate
the same direction \( v_0 \) so

\[
\begin{pmatrix}
    a_x^* \\
    a_y^* \\
    1
\end{pmatrix}
= \begin{pmatrix}
    a_y \\
    -a_x \\
    1
\end{pmatrix}
\]

so

\[ a_x^* = a_y, \]

\[ a_y^* = -a_x. \]

This verifies Equation 20.

\textbf{Slope vectors and direction vectors representing rotation operations}

In this section we will show that

\[ a_1 = r + a_0, \tag{28} \]

where \( a_0 \) is a rotation vector indicating a direction, \( r \) is a rotation vector indication a rotation operation, and \( a_1 \) is the resulting rotation vector indicating the new direction. We will also show that the similar statement

\[ a_1^* = r^* + a_0^* \tag{29} \]
where $a_0^*$ is a slope vector indicating a direction, $r^*$ is a rotation vector indication a rotation operation, and $a_1^*$ is the resulting rotation vector indicating the new direction. (The equations were stated but not proved in section 2.4.1.)

A vector $v_1$ represents the direction specified by $a_0$ rotated according to $r$:

$$v_1 = R(r) R(a_0) \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$$ \hfill (30)

Using Equation 27, Equation 30 can be rewritten as

$$v_1 = R(r + a_0) \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}.$$ \hfill (31)

By the definition of a rotation vector, the vector in the direction specified by $a_1$ is

$$v_1 = R(a_1) \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}.$$ \hfill (32)

Setting equation 31 and 32 equal gives Equation 33, which shows the validity of Equation 28.
Multiplying both sides of the equation by \[
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}
\] gives:

\[
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}
a = \begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}r + \begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}a_0
\]  \hspace{1cm} (34)

Using Equation 20 we rewrite Equation 34 as

\[
a^* = \begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}r + a^*_0
\]  \hspace{1cm} (35)

Using Equation 19 define we have \[
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}r = r^*
\] so we can rewrite Equation 35 as

\[
a^* = r^* + a^*_0
\] , which validates Equation 29.
2.4.4 Beam trajectory in the JEOL JEM-3200

Figure 22: Model of beam trajectory. Deflector coils are labeled from 3 to 6. The +z direction is downward along the optical axis. The vectors $c_i$ specify a point in x-y plane where the beam crosses the plane of a deflector, the specimen, or the objective lens, which is specified with the subscript i. The vectors $a_i$ describe the direction of each line segment.

As shown in Figure 22, the path of the electron beam is modeled as a sequence of
line segments. The direction of each line segment is represented with a rotation vector \( \mathbf{a} \) or a slope vector \( \mathbf{a}^* \) with a subscript identifying the segment. Deflection of the beam (at a deflector coil plane or at a lens plane) is represented as a rotation vector \( \mathbf{r} \) or a slope vector \( \mathbf{r}^* \) with a subscript identifying the deflector set or lens as shown in Figure 22. The x-y position of the beam at the horizontal plane of a deflector set or lens is written as \( \mathbf{c} \) with a subscript identifying the plane.

Each deflection adds to the angle of the beam, so the direction of each segment is given by:

\[
\begin{align*}
\mathbf{a}_b^* &= \mathbf{r}_3^* \\
\mathbf{a}_c^* &= \mathbf{a}_b^* + \mathbf{r}_4^* \\
\mathbf{a}_d^* &= \mathbf{a}_e^* \\
\mathbf{a}_e^* &= \mathbf{a}_d^* + \mathbf{r}_{obj}^* \\
\mathbf{a}_f^* &= \mathbf{a}_e^* + \mathbf{r}_5^* \\
\mathbf{a}_g^* &= \mathbf{a}_f^* + \mathbf{r}_6^*
\end{align*}
\]

Separation distance in the z direction between planes of lenses or deflectors are written as \( L \) with a subscript, as shown in Figure 22. The positions of the beam at each horizontal plane are:

\[
\begin{align*}
\mathbf{c}_3 &= 0 \\
\mathbf{c}_4 &= L_b \mathbf{a}_b^* \\
\mathbf{c}_{spec} &= \mathbf{c}_4 + L_e \mathbf{a}_e^* \\
\mathbf{c}_{obj} &= \mathbf{c}_{spec} + L_d \mathbf{a}_d^* \\
\mathbf{c}_5 &= \mathbf{c}_{obj} + L_e \mathbf{a}_e^*
\end{align*}
\]
\[ c_6 = c_5 + L_f a_f^* \] (47)

In this document, tilting the beam "in the \( \hat{x} \) direction" means increasing the \( x \) component of \( a^* \) which describes the beam direction. Likewise, the tilting the beam in the \( \hat{y} \) direction corresponds to increasing the \( y \) components of \( a^* \). The coils labeled "x" tilt the beam approximately in the \( \hat{x} \) direction the coils labeled "y" tilt the beam approximately in the \( \hat{y} \) direction. The deflection directions are approximate because the deflector coils do not create ideal magnetic fields that are perfectly aligned with the \( x \) or \( y \) axis.

2.4.5 Modeling deflector coil effects

The scan and de-scan systems sets \( d_i \) to achieve the proper beam deflections. This section describes how the currents represented by \( d_i \) for each coil set map to the deflection that coils produce, which can be represented with a rotation vector \( r_i \).
According to [22] the angle (in radians) at which a beam is deflected for a magnetic field of magnitude $B$ is given by:

$$\theta = \frac{e v B}{m v}$$  \hspace{1cm} (48)

(Note that Equation this is an approximation valid for small angle deflections less than $10^\circ$.) Adapt ing 48 to model a deflection caused by both $x$ and $y$ deflector coils gives:

$$r = \mu b$$  \hspace{1cm} (49)

where  $\mu = \frac{e A}{m v}$,  $e$ is the the charge of an electron;  $A$ is the length along the $z$ axis that the magnetic field acts;  $|b|$ is the magnetic field magnitude;  $m$ is the mass of the electron. When subscripts are used to indicate the coil set we write:
\[ r_i = \mu b_i \] (50)

For ideal x and y deflector coil set (Figure 23a) that generates orthogonal magnetic fields aligned with the x and y directions, the following equation is valid,

\[ r^* = \xi d \] (51)

where \( \xi \) is a positive constant that defines the ratio of deflection angle to current for a particular coil. With ideal coils, as \( d_{ix} \) is increased, the beam would tilt toward the \( \hat{x} \) direction, and as \( d_{iy} \) increased, the beam would tilt toward the \( \hat{y} \) direction. Using equations 19 and 49 to replace \( r^* \) in equation 51 gives:

\[
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix} \mu b = \xi d
\] (52)

Solving for \( b \) gives the following equation, which is the relationship between the magnetic field direction and the current assuming coils are ideal:

\[
b = \begin{pmatrix}
0 & -\xi \\
\xi & \mu \\
\xi & 0
\end{pmatrix} d
\] (53)

Equations 51-53 describe the effect of ideal coils. However, the coils are not ideal and the magnetic fields they produce are not exactly orthogonal and not exactly aligned with the x and y axis (see Figure 23b). To correct for the imperfection of the coils use
to represent the relationship between the coil currents and the magnetic field direction.

The components of matrix $S$ are not restricted to the form
$$\begin{pmatrix} 0 & -\frac{\xi}{\mu} \\ \frac{\xi}{\mu} & 0 \end{pmatrix}$$

nonzero values for any of its components. It can represent errors such as misalignment of the coil with an axis (rotation in the x-y plane) or the effect of one coil being stronger than another (different $\xi$ values for x and y coils). Because $S$ is roughly equal to
$$\begin{pmatrix} 0 & -\frac{\xi}{\mu} \\ \frac{\xi}{\mu} & 0 \end{pmatrix},$$
it is invertible, and this property will be used throughout this document. For convenience, we define $M$ matrices as
$$M = \mu S .$$

So, the mapping from coil current to rotation vector representing the deflection is given by
$$r = M d .$$
2.5 Scan control system

The scan control system uses coil sets 3 and 4 to produce a conical precession of the beam with the cone apex (pivot point) centered on the object being imaged, as shown in Figure 22. Conical precession is created using the shift and tilt compensation system as a basis. The shift and tilt compensation system is useful as a basis because it produces tilt of the beam without movement of the beam relative to the specimen. In the following sections, the build-in tilt and shift compensation system is modeled mathematically, and then the scanning system that produces the conical precession is modeled. Finally the scan calibration procedure (used to ensure a pivot point is formed at the specimen) is described.
2.5.1 Tilt and shift purity

![Diagram showing pure tilt, pure shift, and mixed shift and tilt]

**Figure 24:** (a) Pure tilt: The angle at which the beam enters the specimen changes, and the position of the beam at the specimen is unchanged. (b) Pure shift: The position of the beam at the specimen is changed, and the angle at which the beam enters the specimen is unchanged. (c) Both the angle and the position of the beam change at the specimen, so this is not pure shift or pure tilt.

Conventional transmission electron microscopy often requires adjustments in the angle and position of the beam as it enters the specimen. For example, the angle is adjusted to ensure that the beam will pass through the objective lens on the optical axis, and beam position is adjusted to ensure the area of interest on the specimen is illuminated. These changes in position and angle of the beam before it passes through the specimen are performed with coil sets 3 and 4. The control system for these coils, expressed in equations 7-10, is designed to allow the user to introduce a "pure tilt" and a "pure shift" of the beam by adjusting the parameters $p_t$ and $p_s$, respectively. Pure tilt refers to a change in angle of the beam with no movement of the beam on the specimen (see Figure 24a). Pure shift refers to movement of the beam on the specimen with no change in beam angle (see Figure 24b).

Equation 57 is derived from Equation 36 and Equation 37 and states that the
rotation vector \( \mathbf{a}_c \) describing angle of the beam at the specimen is the sum of the rotation vectors from deflector coil set 3 and 4. (A necessary assumption for this equation to be valid is that deflection angles are small.) The location that the beam intersects the specimen plane, \( \mathbf{c}_{\text{spec}} \), is given in equation 58, which is derived from Equations 36, 37, 43, and 44.

\[
\mathbf{a}_c = \mathbf{r}_3 + \mathbf{r}_4
\]

\[
\mathbf{c}_{\text{spec}} = L_b \mathbf{r}_3^* + L_c (\mathbf{r}_3^* + \mathbf{r}_4^*)
\]

A pure tilt will result in a change in the rotation vector \( \mathbf{a}_c \) with no change in the position \( \mathbf{c}_{\text{spec}} \). A pure shift will result in a change in the position \( \mathbf{c}_{\text{spec}} \) with no change in the rotation vector \( \mathbf{a}_c \). Equations 7-10 are built into the microscope and designed to produce pure shift when \( p_s \) is adjusted and pure tilt when \( p_t \) is adjusted.

### 2.5.2 Pure tilt

To simplify the discussion here we assume the user has specified there should be no beam shift, so \( p_{sx} \) and \( p_{sy} \) are zero; in section 2.5.4 this restriction will be removed. We also assume that there are no dependences on \( \phi \); physically this means that we are only considering a single beam trajectory rather than the set of beam trajectories that form the cone; the same reasoning will apply when all beam trajectories are considered. With these assumptions, equations 7-10 reduce to
equations 59-62.

\[ d_{3x} = C_{tx,3x}p_{tx} \quad \text{(59)} \]
\[ d_{3y} = C_{ty,3y}p_{ty} \quad \text{(60)} \]
\[ d_{4x} = p_{tx} + C_{ty,4x}p_{ty} \quad \text{(61)} \]
\[ d_{4y} = p_{ty} + C_{tx,4y}p_{tx} \quad \text{(62)} \]

Figure 25: (a) Deflection with deflector coil set 4 alone does not produce pure tilt. (b) Pure tilt is produced using deflector coil set 3 and 4.

As an example of how equations 59-62 ensure pure tilt, suppose the user increases \( p_{ty} \) by \( \Delta p \), with \( p_{tx} \) set to zero. In this example, the adjustment to \( d_{4y} \) causes a deflection in the \( +\hat{y} \) direction. If only \( d_{4y} \) were used without balance from \( d_{3x} \) (as shown in Figure 25a) the beam would no longer intersect the specimen plane at the correct point. To compensate the deflection from \( d_{4y} \), an opposite deflection is introduced by the term \( C_{ty,3y}p_{ty} \) in equation 60, which deflects the beam approximately in the \( -\hat{y} \) direction (as shown in Figure 25b). If
the coils deflected the beam precisely in the $\hat{y}$ and $-\hat{y}$ direction, this is all that would be necessary. But the direction of the deflection is imprecise, another correction is needed, represented as the term $C_{ty,4x} p_{ty}$ in equation 61, and may be in the positive or negative $\hat{x}$ direction depending on the particular misalignment of the coils.

To formally show that equations 59-62 ensure pure tilt, consider the condition that the beam must pass through the center of the specimen:

$$c_{spec} = 0$$

With this constraint, Equation 58 gives:

$$-(\frac{L_b + L_c}{L_c}) r_3 = r_4$$

This negative scalar $-(\frac{L_b + L_c}{L_c})$ in Equation 64 indicates, as expected, that the deflection represented by $r_4$ will be opposite to the deflection represented by $r_3$ so that the beam is brought back to the correct position on the specimen.

Using Equations 56, and adding subscripts to designate the coil set, we have the following relationships for coil sets 3 and 4:

$$r_3 = M_3 d_4$$

$$r_4 = M_4 d_4$$

Equation 64 is the condition that must be satisfied for tilt to occur without shift.
Since the control system sets currents, we rewrite Equation 64 in terms of currents by substituting in Equations 65 and 66:

\[- \left( \frac{L_b + L_c}{L_c} \right) M_3 d_3 = M_4 d_4 \quad (67)\]

So

\[ d_4 = Q_t d_3 \quad (68)\]

where

\[ Q_t \equiv - \left( \frac{L_b + L_c}{L_c} \right) M_4^{-1} M_3 \quad (69)\]

gives the relationship between \( d_3 \) and \( d_4 \) that must be maintained for tilt to occur without shift.

If the compensation system expressed in Equations 59-62 is capable of representing the relationship in Equation 68, it can create pure tilt. To show that it can, we rewrite Equations 59-62 in matrix form:

\[
\begin{align*}
    d_3 &= \begin{pmatrix} C_{tx,3x} & 0 \\ 0 & C_{ty,3y} \end{pmatrix} \begin{pmatrix} p_{tx} \\ p_{ty} \end{pmatrix} \quad (70) \\
    d_4 &= \begin{pmatrix} 1 & C_{ty,4x} \\ C_{tx,4y} & 1 \end{pmatrix} \begin{pmatrix} p_{tx} \\ p_{ty} \end{pmatrix} \quad (71)
\end{align*}
\]

From Equation 70,

\[
\begin{pmatrix} p_{tx} \\ p_{ty} \end{pmatrix} = \begin{pmatrix} C_{tx,3x} & 0 \\ 0 & C_{ty,3y} \end{pmatrix}^{-1} d_3 \quad (72)
\]
Substituting Equation 72 into Equation 71:

\[
d_4 = \begin{pmatrix}
1 & C_{ty,4x} \\
C_{tx,4y} & 1
\end{pmatrix}
\begin{pmatrix}
C_{tx,3x} & 0 \\
0 & C_{ty,3y}
\end{pmatrix}^{-1}
d_3
\]  

(73)

With \( Q_t \) set to the following,

\[
Q_t = \begin{pmatrix}
1 & C_{ty,4x} \\
C_{tx,4y} & 1
\end{pmatrix}
\begin{pmatrix}
C_{tx,3x} & 0 \\
0 & C_{ty,3y}
\end{pmatrix}^{-1}
\begin{pmatrix}
C_{ty,4x} & \\
C_{tx,3x} & C_{ty,3y}
\end{pmatrix}
\]  

(74)

Equation 73 becomes the same as Equation 68, so the compensation system expressed in Equations 59-62 is capable of producing pure tilt.

Calibrating the compensation system to ensure pure tilt involves adjusting the subscripted \( C \) parameters in Equation 74. Since the variables on the right hand side of Equation 69 are not known, the components of \( Q_t \) are determined experimentally during a calibration procedure described in section 2.5.6.

### 2.5.3 Pure shift

To simplify the discussion here we assume the user has specified there should be no beam shift, so \( p_{tx} \) and \( p_{ty} \) are zero; in section 2.5.4 this restriction will be removed. As in the last section, we also assume that there are no dependences on \( \Phi \). With these assumptions, equations 7-10 reduce to equations 75-78.

\[
d_{3x} = p_{sx}
\]

(75)

\[
d_{3y} = p_{sy}
\]

(76)

\[
d_{4x} = C_{sx,4x} p_{sx} + C_{sy,4x} p_{sy}
\]

(77)
As an example of how Equations 75-78 ensure pure shift, suppose the user increases $p_{sy}$ by $\Delta p$, with $p_{sx}$ set to zero. According to 76, $d_{3y}$ will increase by $p_{sy}$, so coil set 3 will deflect the beam approximately in the $\hat{y}$ direction. If only $d_{3y}$ were used the beam would no longer intersect the specimen plane with the same angle (Figure 12a). To correct this, an opposite deflection is introduced by the term $C_{sy,4y} p_{sy}$ in Equation 78, which deflects the beam approximately in the $-\hat{y}$ direction. If the coils deflected the beam precisely in the $\hat{y}$ and $-\hat{y}$ direction, this is all that would be necessary. However, as with tilt compensation, the direction of the deflection is imprecise, so another correction is needed, represented as the term $C_{sx,4y} p_{sx}$ in Equation 78, which may be positive or negative depending on the particular misalignment of the coils.

\[
d_{4y} = C_{sy,4y} p_{sy} + C_{sx,4y} p_{sx}
\]  

(78)

Figure 26: (a) Deflection with deflector coil set 3 alone does not produce pure shift. (b) Pure shift is produced using deflector coil set 3 and 4.
To formally show that equations 75-78 ensure pure shift, consider the condition that the beam angle must be aligned with the z axis:

$$a_c = 0$$ \hfill (79)

With this constraint, Equation 57 gives:

$$r_3 = -r_4 \hfill (80)$$

Equation 80 is intuitively reasonable because the rotation for \( r_4 \) will be in the opposite direction as for \( r_3 \), so that the tilt created by coil set 3 is canceled by coil set 4.

Rewriting 80 using Equations 65 and 66 gives

$$M_3 d_3 = -M_4 d_4 \hfill (81)$$

so

$$d_4 = -M_4^{-1}M_3 d_3 \hfill (82)$$

Rewriting Equation 82 with the definition in Equation 84,

$$d_4 = Q_s d_3 \hfill (83)$$

$$Q_s \equiv -M_4^{-1}M_3 \hfill (84)$$

Equation 83 gives the relationship between \( d_3 \) and \( d_4 \) that must be maintained for shift to occur without tilt. If the compensation system expressed in
Equations 75-78 is capable of representing the relationship in Equation 83, it can create pure shift. Next we show that it is capable. Rewriting Equations 75-78 in matrix form:

\[
\begin{align*}
\mathbf{d}_3 &= \begin{pmatrix} p_{sx} \\ p_{sy} \end{pmatrix} \\
\mathbf{d}_4 &= \begin{pmatrix} C_{sx, 4x} & C_{sy, 4x} \\ C_{sx, 4y} & C_{sy, 4y} \end{pmatrix} \begin{pmatrix} p_{sx} \\ p_{sy} \end{pmatrix} \\
\mathbf{d}_4 &= \begin{pmatrix} C_{sx, 4x} & C_{sy, 4x} \\ C_{sx, 4y} & C_{sy, 4y} \end{pmatrix} \mathbf{d}_3 \\
\mathbf{Q}_s &= \begin{pmatrix} C_{sx, 4x} & C_{sy, 4x} \\ C_{sx, 4y} & C_{sy, 4y} \end{pmatrix}
\end{align*}
\]

Equation 84 describes the values for the components of \( \mathbf{Q}_s \), but since \( M_3 \) and \( M_4 \) are not known, \( \mathbf{Q}_s \) is calibrated according to the microscope manufacturer's procedure.

### 2.5.4 Tilt and shift combined

This sections shows how adding the shift and tilt coil currents from the previous sections gives a full compensation system. In previous sections, when adjusting \( \mathbf{p}_t \) we assume \( \mathbf{c} \) is zero and \( \mathbf{a}_c \) varies, and when adjusting \( \mathbf{p}_s \) we assumed \( \mathbf{a}_c \) is zero and \( \mathbf{c} \) varies. In this section both \( \mathbf{a}_c \) and \( \mathbf{c}_{\text{spec}} \) are allowed to vary. From section 2.5.2 we have following coil currents for pure tilt:
From section 2.5.3 we have the following coil currents for pure shift:

\[ d_{3x}^{\text{tilt}} = C_{tx,3x} p_{tx} \] (85)
\[ d_{3y}^{\text{tilt}} = C_{ty,3y} p_{ty} \] (86)
\[ d_{4x}^{\text{tilt}} = p_{tx} + C_{ty,4x} p_{ty} \] (87)
\[ d_{4y}^{\text{tilt}} = p_{ty} + C_{tx,4y} p_{tx} \] (88)

The JEM-3200 compensation system uses the sum of the tilt and shift currents to control coil sets 3 and 4:

\[ d_{3} = d_{3}^{\text{tilt}} + d_{3}^{\text{shift}} \] (89)
\[ d_{4} = d_{4}^{\text{tilt}} + d_{4}^{\text{shift}} \] (90)

Applying Equations 65-66 gives:

\[ M_{3}^{-1} r_{3} = M_{3}^{-1} r_{3}^{\text{tilt}} M_{3}^{-1} r_{3}^{\text{shift}} \] (95)
\[ M_{4}^{-1} r_{4} = M_{4}^{-1} r_{4}^{\text{tilt}} M_{4}^{-1} r_{4}^{\text{shift}} \] (96)

Equations 95 and 96 imply Equations 97 and 98:

\[ r_{3} = r_{3}^{\text{tilt}} + r_{3}^{\text{shift}} \] (97)
\[ r_{4} = r_{4}^{\text{tilt}} + r_{4}^{\text{shift}} \] (98)

Using the deflections described in Equation 97 and Equation 98, \( a_{c} \) and \( c_{spec} \)
are:

$$a_c = r_3^{\text{tilt}} + r_4^{\text{shift}}$$

$$c_{\text{spec}} = L_b(r_3^{*\text{shift}} + r_4^{*\text{shift}})$$

(99) (100)

Applying the conditions of Equation 58 and Equation 80 gives:

$$a_c = r_3^{\text{tilt}} + r_4^{\text{tilt}}$$

$$c_{\text{spec}} = L_b r_3^{*\text{shift}} + L_c (r_3^{*\text{shift}} + r_4^{*\text{shift}})$$

(101) (102)

So, as desired, the tilt controls affect only the angle, specified with \( a_c \), and the shift controls affect only the position, specified by \( c_{\text{spec}} \).

### 2.5.5 Formation of conical beam precession

The scan system uses coil sets 3 and 4 to ensure the beam trajectories for all azimuthal angles form a cone. Ideally the angle of the beam at the specimen, described with the rotation vector \( a_c \) is

$$a_c = \tan(\theta_0) \left( \begin{array}{c} -\sin(\phi) \\ \cos(\phi) \end{array} \right).$$

(103)

In this section we will show that the Equations 15 and 16 (which determine the tilt control parameter \( p_t \)), do produce conical beam precession as described ideally with 103. First, using the beam trajectory model and Equations 15 and 16, we determine and expression for \( a_c \) in terms of \( p_t \). For convenience we define
Using $H_3$ and $H_4$, we rewrite equation 70 and 71 as:

$$d_3 = H_3 p_t$$

$$d_4 = H_4 p_t$$

From Equation 65 and 66:

$$r_3 = M_3^{-1} H_3 p_t$$

$$r_4 = M_4^{-1} H_4 p_t$$

Substituting Equation 106 and 107 into Equation 57 gives:

$$a_c = (M_3^{-1} H_3 + M_4^{-1} H_4) p_t$$

Using Equations Equations 15 and 16 we have

$$a_c = (M_3^{-1} H_3 + M_4^{-1} H_4) \begin{pmatrix} A_{tilt} & 0 \\ 0 & A_{tilt} \end{pmatrix} \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix}$$

Defining the matrix $T$ as

$$T \equiv (M_3^{-1} H_3 + M_4^{-1} H_4) \begin{pmatrix} A_{tilt} & 0 \\ 0 & A_{tilt} \end{pmatrix}$$
we write Equation 108 as

\[ \mathbf{a}_c = T \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix} \]  

(109)

The matrix \( T \) is set to scale the x and y sinusoidal signals to produce conical precession. Ideally \( T \) should be set to \( T_{cone} \) to produce precession with a zenith angle of \( \theta_0 \):

\[ T_{cone} \equiv \begin{pmatrix} 0 & -\tan(\theta_0) \\ \tan(\theta_0) & 0 \end{pmatrix} \]

(Substituting \( T_{cone} \) into \( T \) in equation 109 gives ideal conical precession as in equation 103.) However \( T \) cannot be set directly; \( T \) depends on \( H_3 \), \( H_4 \), \( M_3 \), and \( M_4 \). The values \( H_3 \) and \( H_4 \) are fixed to values that ensure pure tilt and shift. The values \( M_3 \) and \( M_4 \) are determined by the design and performance of the deflector coil sets 3 and 4. The only variable in the definition of \( T \) (Equation 108) that can be set arbitrarily is \( A_{tilt} \). We will show that assuming ideal deflector coils that Equations 15 and 16 will produce a conical precession of the beam. We will also show how \( A_{tilt} \) is used to set the zenith angle \( \theta_0 \). The following Equations (110-114) are valid for ideal deflector coils:

\[ M_3 = \begin{pmatrix} 0 & -\nu_3 \\ \nu_3 & 0 \end{pmatrix} \]  

(110)
\[ M_4 = \begin{pmatrix} 0 & -\nu_4 \\ \nu_4 & 0 \end{pmatrix} \]  
(111)

\[ C_{\nu,4x} = 0 \]  
(112)

\[ C_{tx,4y} = 0 \]  
(113)

\[ C_{tx,3x} = C_{ty,3y} \]  
(114)

where

\[ \nu \equiv \frac{\xi}{\mu} \]  
(115)

Equations 110 and 111 give ideal matrices for \( M_3 \) and \( M_4 \) as described in section 2.4.5. Equations 112 and 113 are based on the assumption that the x and y coils for coil sets 3 and 4 produce fields ideally aligned with the x and y axis. That is, because of the assumed precise orientation, tilting in the y direction with set 3 does not require correction with x coil of set of 4 so \( C_{\nu,4x} = 0 \). Likewise, tilting in the x direction with coil set 3 does not require correction with the y coil of set 4, so \( C_{tx,4y} = 0 \). Equation 114 states an assumption that the tilt balance ratio, which ensures tilt purity, is the same for the x coils as it is for the y coils. For convenience, the variable \( k \) is defined to represent \( C_{tx,3x} \) and \( C_{ty,3y} \).

\[ k \equiv C_{tx,3x} = C_{ty,3y} \]

Substituting Equations 110-114 into Equation 108 and multiplying matrices gives \( T \) for an ideal microscope:
To form a cone we want to set $T$ to the ideal value $T_{\text{cone}}$

$$T = T_{\text{cone}}.$$

Writing out the matrices gives

$$\begin{pmatrix}
0 & A_{\text{tilt}} \left( -\frac{k}{v_3} - \frac{1}{v_4} \right) \\
A_{\text{tilt}} \left( \frac{k}{v_3} + \frac{1}{v_4} \right) & 0
\end{pmatrix} = \begin{pmatrix}
0 & -\tan(\theta_0) \\
\tan(\theta_0) & 0
\end{pmatrix},$$

which implies that

$$A_{\text{tilt}} = \frac{1}{\left( -\frac{k}{v_3} - \frac{1}{v_4} \right)} \tan(\theta_0).$$

This gives an expression for the value $A_{\text{tilt}}$ should be set to to produce a conical precession with zenith angle $\theta_0$.

So, assuming ideal coil performance, the control signal in Equations 15 and 16 will guarantee the formation of a conical precession, and $A_{\text{tilt}}$ can be adjusted to produce the desired zenith angle $\theta_0$. In reality the coil performance is not ideal, Equations 110-114 are not exactly true, but because they are approximately true, the
beam does precess around a cone approximately. The data in section 2.7 shows experimentally how the angle of the beam changes as it precesses around the cone.

Note that the assumptions expressed in Equations 110-114 are used in this section only to show that the beam precesses in an approximately conical manner. These assumptions will not be used in the rest of this document: the calibration to form of a proper apex and the de-scan system described in sections that follow will not assume that the coils behave in the ideal way.

2.5.6 Scan calibration

The purpose of calibrating the scan system is to balance the deflections from coil set 3 and coil set 4 so that a pivot point exists at the specimen plane, i.e. \( c_{\text{spec}} \) remains 0 as the beam precesses around its conical trajectory. The tilt control parameter \( p_t \) is varied to precess the beam. As long as the the tilt control system (described by Equations 7-10) is calibrated, the tilt will be pure and a pivot point will exist at the specimen plane.

Note that during the scan calibration procedure, the SA aperture is removed so that the beam will not be blocked. Once the scan calibration and de-scan calibration is complete, the transmission spot remains centered at the SA aperture plane (which is the back focal plane), so the aperture can then be put into place without blocking the beam.

During scan calibration the zenith angle is set to the value that will be used for imaging. The zenith angle is typically set near or at the maximum possible value to
increase z resolution of volumetric reconstructions (see Appendix B). For the
JEM-3200 microscope used in this work, the maximum zenith angle is 1.7 degrees,
and it is limited by the strength of the de-scan coils. (When the beam is tilted beyond
1.7 degrees with the scan system the de-scan coils do not produce a strong enough
magnetic field to bring the beam back onto the optical axis.)

Conventionally the tilt compensation system, which ensures tilt purity, is
calibrated by continuously varying the tilt parameter $p_t$, and adjusting constants
$C_{tx,3x}$, $C_{ty,3y}$, $C_{tx,4x}$, and $C_{tx,4y}$ so that the illumination spot remains
stationary relative to the image of the specimen. At small zenith angles, less than 1
degrees, the image remains stationary on the viewing screen while tilting. Once the
calibration is complete the tilt of the beam can be varied and the illumination spot
remains stationary relative to the features of the specimen. This indicates that beam is
forming a pivot point at the specimen plane during tilting. However, the standard tilt
compensation calibration procedure is not useful when the beam tilt has a zenith angle
greater than approximately 1 degree because the image of the specimen moves
significantly on the viewing screen when $p_t$ varies; this movement makes the
visual task of insuring that the illumination spot is stationary with respect to the
features in the image virtually impossible.

The reason for image motion when tilting beyond 1 degree is imperfection of the
objective lens; when the periphery of the lens is used the image forms in a different
place on the viewing screen than when the center of the lens is used. As the beam
precesses around a cone with zenith angle 1.7 degrees, the image moves in a circular
path on the viewing screen. (If the objective lens was ideal, the angle at which the sample is illuminated would not effect the location of objects in the final image on the viewing screen, but it is not.)

Because of the image motion mentioned above, calibration of the tilt compensation system at a conical precession zenith angle of 1.7 degrees required a custom calibration procedure which corrects the motion of the image. As shown in Figure 27, using coil set 5, a compensating deflection was introduced (calibrated with parameters $A_{sx}$, $A_{sy}$, $\sigma_{sx}$, and $\sigma_{sy}$) that keeps the image centered on the viewing screen.
screen as the beam processes. Parameters $C_{tx,3x}$, $C_{ty,3x}$, $C_{ty,4x}$, and $C_{tx,4y}$ (which define the matrix $Q_t$) are then adjusted so that the illumination spot remains stationary on the viewing screen. (Coil set 6 is unused during scan calibration.)

Once scan calibration is complete, the $Q_t$ matrix is set correctly, so a pivot point is formed at the specimen plane. During the de-scan calibration, which is performed after scan calibration, the beam configuration is changed from that shown in Figure 27 to that shown in Figure 1. Regarding control of coil set 5, the de-scan procedure will re-configure $A_{5x}$, $A_{5y}$, $\sigma_{5x}$, and $\sigma_{5y}$, and deflections with coil set 6 will also be introduced as described in section 2.6.4.

## 2.6 De-scan control system

The purpose of the de-scan system is to ensure that the beam is on the optical axis before it enters the mini-objective lens. As described earlier, coil sets 3 and 4 produce a tilt of the beam. As shown in Figure 22, coil set 5 deflects the beam so it reaches the position of the optical axis at plane of deflector coil set 6. Coil set 6 then deflects the beam so that its direction is aligned with the optical axis. In this section we compute what deflections are needed to bring the beam on axis, i.e. we derive expressions for $r^*_5$ and $r^*_6$ in terms of $r^*_3$ and $r^*_4$. In following sections these results will be used to justify Equations 11-14 which define the de-scan control system.

To ensure that the beam is aligned with the optical axis, the de-scan coils must deflect in the beam in such a way that the following equations become true:
When analyzing the de-scan system we assume that the scan system is functional and creates a pivot point at the specimen, so \( c_{\text{spec}} = 0 \). The following equations describe the angle and position of the beam at various locations in the column (see Figure 19); these equations will be used in the sections that follow:

\[
a^*_d = r^*_3 + r^*_4 \quad (118)
\]

\[
a^*_e = a^*_d + r^*_\text{obj} \quad (119)
\]

\[
a^*_f = a^*_d + r^*_\text{obj} + r^*_5 \quad (120)
\]

\[
a^*_g = a^*_d + r^*_\text{obj} + r^*_5 + r^*_6 \quad (121)
\]

\[
c_{\text{spec}} = 0 \quad (122)
\]

\[
c_{\text{obj}} = L_d a^*_d \quad (123)
\]

\[
c_5 = L_d a^*_d + L_e a^*_e \quad (124)
\]

\[
c_6 = L_d a^*_d + L_e a^*_e + L_f a^*_f \quad (125)
\]

### 2.6.1 Finding the deflection angle for coil set 5

In this section we find \( r^*_5 \) in terms of \( r^*_3 \) and \( r^*_4 \). A key requirement is that the beam remain centered at the plane of deflector coil set 6 (as expressed in Equation 116) as the beam precesses. Substituting 116, 119, and 120 into 47:

\[
0 = L_d a^*_d + L_e (a^*_d + r^*_\text{obj}) + L_f (a^*_d + r^*_\text{obj} + r^*_5)
\]
Moving $r^*_5$ to the left hand side gives:

$$r^*_5 = \frac{L_d(a_d^* + L_e(a_d^* + r^*_{obj}) + L_f(a_d^* + r^*_{obj})}{-L_f}$$  \hspace{1cm} (126)$$

Rearranging 126 gives:

$$r^*_5 = \frac{(L_d + L_e + L_f) a_d^* + (L_e + L_f) r^*_{obj}}{-L_f}$$  \hspace{1cm} (127)$$

Using the paraxial approximation for a thin lens [16] gives:

$$r^*_{obj} = -\frac{1}{f_{obj}} c_{obj}$$  \hspace{1cm} (128)$$

Substituting Equation 45 into Equation 128 gives:

$$r^*_{obj} = -\frac{1}{f_{obj}} L_d a_d^*$$  \hspace{1cm} (129)$$

Substituting 129 into 127 gives:

$$r^*_5 = \frac{(L_d + L_e + L_f) a_d^* + (L_e + L_f) (-\frac{1}{f_{obj}} L_d a_d^*)}{-L_f}$$  \hspace{1cm} (130)$$

$$r^*_5 = \frac{(L_d + L_e + L_f) + (L_e + L_f) (-\frac{1}{f_{obj}} L_d)}{-L_f} a_d^*$$  \hspace{1cm} (131)$$

For convenience, we introduce a scalar $C_0$ defined as
Using $C_0$, we rewrite Equation 131 as:

\[ r_5^* = C_0 \alpha_d \]  (133)

Substituting Equation 118 into Equation 133:

\[ r_5^* = C_0 (r_3^* + r_4^*) \]  (134)

### 2.6.2 Finding the deflection angle for coil set 6

In this section we find $r_6^*$ in terms of $r_3^*$ and $r_4^*$. A key requirement is that the beam angle be aligned with the optical axis after being deflected by coil set 6 (as expressed in Equation 117).

Substituting 117 into Equation 121:

\[ r_6^* = - (\alpha_d^* + r_{obj}^* + r_5^*) \]  (135)

Substituting 129 and 133 into 135:

\[ r_6^* = - (\alpha_d^* + \frac{-1}{f_{obj}} L_d \alpha_d^* + C_0 \alpha_d^*) \]  (136)

\[ r_6^* = (-1 + \frac{1}{f_{obj}} L_d - C_0) \alpha_d^* \]  (137)
Substituting Equation 118 into Equation 137:

\[ r_6^* = (-1 + \frac{1}{f_{\text{obj}}} L_d - C_0) (r_3^* + r_4^*) \]  

(138)

### 2.6.3 Single azimuthal angle de-scan calibration

De-scan calibration is the process of determining the necessary currents for \( d_5 \) and \( d_6 \), which set the deflector coils sets 5 and 6 to return the beam to the optical axis. Writing 134 and 138 in terms of \( d_i \) rather than \( r_i \) we have:

\[ d_5 = M_5^{-1} C_0 (M_3 d_3 + M_4 d_4) \]  

(139)

\[ d_6 = M_6^{-1} (-1 + \frac{1}{f_{\text{obj}}} L_d - C_0) (M_3 d_3 + M_4 d_4) \]  

(140)

To use these equations in a control system to set \( d_5 \) and \( d_6 \) it would be necessary to know the terms on the right hand side, \( M_3 \), \( M_4 \), \( M_5 \), \( M_6 \), \( L_d \), \( L_e \), \( L_f \), \( f_{\text{obj}} \), \( d_3 \), \( d_4 \). (Note the definition of \( C_0 \) given in Equation 132.) The separation, \( L_d + L_e \), between coil set 4 and the objective lens and the separation \( L_f \) between coil sets 5 and 6, are known by the microscope manufacture but undocumented. The distances \( L_d \) and \( L_e \) depend on the height of the specimen, which is reported by the JEM-3200 microscope software. The focal length of the objective, \( f_{\text{obj}} \), is documented as 3.9mm. The currents \( d_3 \) and
$d_4$ are known, just as all the coil currents are known because they are set with the microscope hardware. However, $M_3$, $M_4$, $M_5$, $M_6$ are not known. Because not all of these terms are known and because the necessary corrections can change due to unpredictable effects (such as magnetization of soft iron components of the system), Equations 139 and 140 are not currently used to set $d_3$ and $d_4$. However estimating the value of the unknown parameters and using the beam trajectory model to speed up the process of calibration may be a subject of future work.
Overview of de-scan calibration procedure

Figure 28: Beam trajectory illustration showing width of the beam. The beam is concentrated most at the diffraction plane. The darkened path is a ray at the center of the beam. (a) Untilted beam (b) Tilted beam

The following text describes a procedure that can be used to calibrate the de-scan of the beam at each azimuthal angle. (A more streamlined procedure that calibrates the full set of azimuthal angles simultaneously is given in section 2.6.4.) During calibration, the trajectory of the beam is observed visually and adjustments are made manually to \( d_4 \) and \( d_5 \) iteratively until the beam is brought onto the optical
axis. Although the complete trajectory of the beam, as shown in Figure 22, is not directly observable, the microscope can show an image in imaging mode or an image in diffraction mode. Imaging mode produces an image of the specimen on the viewing screen, and diffraction mode produces an image of the back focal plane on the viewing screen. These two modes can be used to verify that the beam is aligned with the optical axis. The calibration is done in two parts, an initialization procedure followed by an iterative centering procedure.

**Initialization procedure:**

- Column alignment is performed as described in section 2.3. During this alignment the scan and de-scan systems are disabled so that the beam is not tilted.

- Initializing diffraction mode: For an ideal microscope, the diffraction spot of the un-tilted beam would be at the center of the viewing screen, but in reality it typically is not. The projector lens deflectors (coil set 11) are used to position the transmission spot on the center of the viewing screen, which is marked with a dot that is physically written on the screen. This adjustment makes the calibration process easier because the diffraction spot has to be repetitively returned to the un-tilted diffraction spot location. (If the projector lens deflectors are not used to center the transmission spot, the location of the un-tilted transmission spot on the viewing screen has to be recorded in some way so that the beam can be returned to that location during the centering procedure.)
Initializing imaging mode: The specimen stage is used to move some easily
identifiable "reference feature" to the center of the viewing screen. (The
projector lens deflectors are unused in imaging mode.)

**Centering procedure:**

At each azimuthal angle this centering process is performed. The tilt control
\( p_t \) sets the beam direction so it enters the specimen at the desired zenith and
azimuthal angle. Coil sets 5 and 6 are adjusted manually to return the beam to the
optical axis with steps (a) and (b) described below. When the following two
observable conditions are met the beam is returned to the optical axis:

- Condition 1. In diffraction mode, the transmission spot is centered on the
  viewing screen.
- Condition 2. In imaging mode, the reference feature is at center of the viewing
  screen.

**Manual calibration procedure:**

- Step (a) In diffraction mode, use deflector coil set 5 to move the transmission
  spot to the center of the viewing screen.
- Step (b) In image mode, use deflector coil set 6 to move the reference object to
  the center of the viewing screen.

This processes is repeated, alternating between steps (a) and (b). After about 10
iterations, both conditions are met: the transmission spot is centered in diffraction
mode and the reference feature is centered in imaging mode.
Explanation of the calibration process

Figure 29: Iterative procedure used to ensure that the beam is centered on the back focal plane and the image plane. Angles are labeled $\alpha$ and $\beta$ with subscripts that indicate the current iteration. Initially the beam is tilted and off center in the back focal plane (shown in diffraction mode) and approximately centered in the image plane (shown in imaging mode). In iteration 1, step (a), the deflector coil set 5 is used to center the beam on the back focal plane. In iteration 1, step (b), the deflector coil 6 is used to center the beam on the image plane. In the second iteration steps (a) and (b) are repeated. Multiple iterations are performed until the beam trajectory is sufficiently similar to the ideal final state, where the beam is aligned with the optical axis.
The reasoning behind this calibration process is illustrated in Figure 29. The figures shown the beam at one azimuthal angle. (In Figure 29 subscripts on $\alpha$ and $\beta$ specify the current iteration.) Each time step (a) is performed, the $\alpha$ angle is increased. It is not possible to increase $\alpha$ by too much in step (a) because as $\beta$ decrease in subsequent steps, $\alpha$ will only need to be increased more. Each time step (b) is performed, the $\beta$ angle is decreased. It is not possible to decrease $\beta$ by too much because as $\alpha$ is increased in subsequent steps, $\beta$ will need to be decreased more. So, the angles $\alpha$ and $\beta$ tend to move to correct values without overshooting. After an infinite number of iterations they converge on a final state where the beam is returned to the optical axis. In practice, about 10 iterations is enough to ensure that the calibration is accurate enough.

2.6.4 Sinusoidal de-scan calibration

The process described in the previous section describes how to calibrate the beam at one particular azimuthal angle $\phi$. Since we used one degree increments to approximate a continuous precession around the cone, the process would have to be performed 360 times, for azimuthal angle 0 up to 359. Each azimuthal angle requires about 5 minutes of manual work, so the manual alignment procedure would require 30 hours of labor. This method may have been feasible if the deflection system behavior was highly stable over time, but the adjustments must be changed periodically for
reasons described earlier. Also, changes in specimen height and focus must be adjusted for, so it would be impractical to perform a 30-hour calibration procedure each time an adjustment to specimen height or focus was required.

To increase calibration efficiency, a calibration procedure based on adjusting sinusoidal signals was implemented, which requires about 30 minutes. This procedure, described in section 2.6.4, is based on the fact that the electrical current signal to the correction coils can be assumed to be sinusoidal with a period of 360°. With this restriction, the number of parameters is 8: the amplitudes of 4 sinusoidal waves \( A_{5x}, A_{5y}, A_{6x}, A_{6y} \) and the phase of 4 sinusoidal waves \( \sigma_{5x}, \sigma_{5y}, \sigma_{6x}, \sigma_{6y} \) according to Equations 11-14. Since only 8 parameters must be manually adjusted rather than 1440 (d5x, d5y, d6x, d6y for 360 different angles), the process requires significantly less time to perform.

**Mathematical basis for sinusoidal de-scan signal**

Sinusoidal currents produce the scanning conical precession, so it is plausible that sinusoidal waves could also be used to "de-scan" and bring the beam back onto the optical axis. The following discussion will verify this claim mathematically; we will show that the sinusoidal waves described by Equations 11-14 are sufficient to bring the beam back on axis.

Note that although the dependence is not written explicitly, the variables \( a, r, a^*, r^*, d \) in this section are all a function of \( \phi \). As described earlier,
$M$ matrices map coil current to deflection. Applying subscripts to indicate the matrix for the coil set 5 and the matrix for the coil set 6 we have:

$$
\begin{align*}
\mathbf{r}_5 &= M_5 \mathbf{d}_5 \\
\mathbf{r}_6 &= M_6 \mathbf{d}_6
\end{align*}
$$

(141)  
(142)

**De-scan current signal for coil set 5:**

Equation 109 describes the conical precession of the beam. Using Equation 109 and multiplying by

$$
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix}
$$

we have:

$$
\begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix} \mathbf{a}_d = \begin{pmatrix}
0 & 1 \\
-1 & 0
\end{pmatrix} T \begin{pmatrix}
\cos(\phi) \\
\sin(\phi)
\end{pmatrix}
$$

Using Equation 20 gives:

$$
\mathbf{a}_d^* = T^* \begin{pmatrix}
\cos(\phi) \\
\sin(\phi)
\end{pmatrix}
$$

(143)

where $T^* \equiv \begin{pmatrix} 0 & 1 \\ -1 & 0 \end{pmatrix} T$, and $T$ is defined in Equation 108.

The compensation that $\mathbf{r}_5$ must provide is given in Equation 133. Substituting

Equation 143 into Equation 133:

$$
\mathbf{r}_5^* = C_0 T^* \begin{pmatrix}
\cos(\phi) \\
\sin(\phi)
\end{pmatrix}
$$

(144)

Using Equation 141 and Equation 19 we can rewrite Equation 144 as
$d_5 = M_5^{-1} C_0 T \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix}$ \hfill (145)

For convenience, we define the matrix $Q$ as

$$Q \equiv M_5^{-1} C_0 T \hfill (146)$$

Substituting Equation 146 into Equation 145 gives:

$$d_5 = Q \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix} = \begin{pmatrix} Q_{11} & Q_{12} \\ Q_{21} & Q_{22} \end{pmatrix} \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix} = \begin{pmatrix} Q_{11} \cos(\phi) + Q_{12} \sin(\phi) \\ Q_{21} \cos(\phi) + Q_{22} \sin(\phi) \end{pmatrix}$$

To simplify the linear combination of sines and cosines, the following trigonometric identity will be used:

$$a \sin(x) + b \cos(x) = \sqrt{a^2 + b^2} \sin(x + g(b, a)) \hfill (147)$$

$$g(b, a) = \arctan \left( \frac{b}{a} \right) + \begin{cases} 0 & \text{if } a \geq 0 \\ \pi & \text{if } a < 0 \end{cases} \hfill (148)$$

Applying the trigonometric identities described in Equations 147-148:

$$d_5 = \begin{pmatrix} \sqrt{Q_{12}^2 + Q_{11}^2} \sin(\phi + g(Q_{11}, Q_{12})) \\ \sqrt{Q_{22}^2 + Q_{21}^2} \sin(\phi + g(Q_{21}, Q_{22})) \end{pmatrix} \hfill (149)$$

To show that Equation 149 agrees with Equations 11 and 12, parameters are set as follows:

$$A_{5x} = \sqrt{Q_{12}^2 + Q_{11}^2} \hfill (150)$$

$$A_{5y} = \sqrt{Q_{22}^2 + Q_{21}^2} \hfill (151)$$
\[ \sigma_{sx} = g(Q_{11}, Q_{12}) \]  
\[ \sigma_{sy} = g(Q_{21}, Q_{22}) \]  

Substituting Equations 150-153 into Equation 149 gives:

\[ d_5 = \begin{pmatrix} A_{sx} \sin(\phi + \sigma_{sx}) \\ A_{sy} \sin(\phi + \sigma_{sy}) \end{pmatrix} \]

This verifies Equations 11 and 12 as it shows that sinusoidal signals produce the desired effect for deflector coil set 5.

**De-scan current signal for coil set 6:**

Rewriting Equation 137 using Equations 19 and 20:

\[ r_6 = (-1 + \frac{1}{f_{obj}} L_d - C_0) a_d \]  

(154)

Using Equation 142, we rewrite Equation 154 as:

\[ d_6 = M_6^{-1}(-1 + \frac{1}{f_{obj}} L_d - C_0) a_d \]  

(155)

Substituting Equation 103 into Equation 155 (noting that \( a_c = a_d \)) we have:

\[ d_6 = M_6^{-1}(-1 + \frac{1}{f_{obj}} L_d - C_0) T \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix} \]

For convenience, we define the matrix \( P \) as:

\[ P \equiv M_6^{-1}(-1 + \frac{1}{f_{obj}} L_d - C_0) T \]
\[ d_6 = P \begin{pmatrix} \cos(\phi) \\ \sin(\phi) \end{pmatrix} \]

Using the trigonometric identities of Equations 147-148:

\[
d_6 = \begin{pmatrix} \sqrt{P_{12}^2 + P_{11}^2} \sin(\phi + g(P_{11}, P_{12})) \\ \sqrt{P_{22}^2 + P_{21}^2} \sin(\phi + g(P_{21}, P_{22})) \end{pmatrix}
\]

\[
A_{6x} = \sqrt{P_{12}^2 + P_{11}^2} \tag{156}
\]

\[
A_{6y} = \sqrt{P_{22}^2 + P_{21}^2} \tag{157}
\]

\[
\sigma_{6x} = g(P_{11}, P_{12}) \tag{158}
\]

\[
\sigma_{6y} = g(P_{21}, P_{22}) \tag{159}
\]

\[
d_6 = \begin{pmatrix} A_{6x} \sin(\phi + \sigma_{6x}) \\ A_{6y} \sin(\phi + \sigma_{6y}) \end{pmatrix}
\]

This verifies Equations 13 and 14 as it shows that sinusoidal signals produce the desired effect for deflector coil set 6.
De-scan calibration: Determining the amplitude and phase parameters

Figure 30: Calibration of de-scan system at all azimuthal angles. This procedure is similar to that shown in Figure 29 although in this case the beam is precessing at 2Hz during the procedure. (For clarity, the objective lens and specimen, which are shown in Figure 29, are omitted from this Figure.) Illustrations show the circular paths that the beam traces in each or four planes as it precesses conically. In the initial state, deflectors 5 and 6 are not energized: since all of the beam trajectories at each azimuthal angle originate from approximately the same point in the specimen, they all converge approximately at the imaging plane, but the diffraction image moves significantly in the back focal plane. At iteration 1, step (a), the sinusoidal signal driving coil set 5 is adjusted until the circle that the transmission spot traces at the back focal plane converges into one spot. At iteration 1, step (b), the sinusoidal signal driving coil set 6 is adjusted until the circular motion of the image in the image plane converges, i.e. the image stops moving. Iteration 2 repeats steps (a) and (b). Multiple iterations are performed until the ideal final state is reached (approximately). The criteria for the ideal final state are that during beam precession, (1) the image remains stationary on the imaging plane and (2) the transmission spot remains stationary in the back focal plane.
Equations 150-153 and 156-159 describe expressions for amplitude and phase of electric current signals needed to de-scan the beam, but the expressions involve unknown terms such as the $M$ matrices (as discussed in section 2.6.3). So, the parameters $A_{5x}$, $A_{5y}$, $A_{6x}$, $A_{6y}$, $\sigma_{5x}$, $\sigma_{5y}$, $\sigma_{6x}$, and $\sigma_{6y}$ are determined experimentally during the calibration process described below. As with single azimuthal angle de-scan calibration, there is an initialization procedure and a centering procedure.

**Initialization procedure:**

Initialization is the same as with single azimuthal angle, except that there is no need to adjust the projector deflectors to center the transmission spot. Since a stationary transmission spot is the desired condition rather than centering, the spot does not need to be centered on the viewing screen.

**Centering procedure:**

To perform calibration, the beam is precessed continuously around the cone at a rate of about three cycles per second. The user alternates between steps (a) and (b) until both conditions are met: the transmission spot is stationary in diffraction mode and the image is stationary in imaging mode. Figure 30 illustrates this procedure.

- Step (a). In diffraction mode, adjust parameters for deflector coil set 5 ($A_{5x}$,
\( A_{5y}, \sigma_{5x}, \sigma_{5y} \) to make the transmission spot stationary.

- Step (b). In image mode, adjust parameters for deflector coil set 6 (\( A_{6x}, A_{6y}, \sigma_{6x}, \sigma_{6y} \)) to make the reference feature stationary.

The reasoning behind this process (see Figure 30) is analogous to single azimuthal angle de-scan calibration.

### 2.7 Beam Control Results

Three criteria were used to characterize the performance of the conical beam precession system:

1. The angle at which the beam enters the specimen should indicate that the beam is undergoing conical precession.

2. Although some parallax is expected, the image of the specimen should remained stationary on the CCD camera during precession (see section 2.7.2). This is especially essential for electron optical sectioning because instability in position of the image tends to blur the results.

3. The transmission spot in diffraction mode at the back focal plane should stay within the selective area aperture. (In B-mode the selective area aperture serves the function that the objective aperture does in conventional transmission
electron microscopy.) The beam remained inside of a 50 micron diameter aperture during imaging. Therefore, the error of the control system in the back focal plane is less than 50 microns, with $\theta_0$ of 1.7 degrees.

### 2.7.1 Angular performance

Appendix C characterizes the angle of the beam produced by the beam scan system for zenith angles from 0 to 4.4 degrees. An ancillary measurement of conical beam trajectory presented in this section was performed at a zenith angle of 1.4 degrees using bundle adjustment. As explained earlier, $G_j$ defines the orientation of the specimen relative to the beam direction. Bundle adjustment was performed to experimentally determine $G_j$ for each view and model of the 3D bead configuration (see Equation 6). Figure 31 shows elements of the transformation matrix $G_j$ for each view $j$, and Figure 7A shows a plot of lines in directions specified by each $G_j$.

$$
G_j = 
\begin{pmatrix}
\begin{array}{ccc}
b_{11} & b_{12} & b_{13} \\
b_{21} & b_{22} & b_{23} \\
b_{31} & b_{32} & b_{33}
\end{array}
\end{pmatrix}
$$

(The elements $b_{31}$, $b_{32}$, and $b_{33}$ are not used because, considering the value for $P$ given earlier, they do not play a part in Equation 6.)
Figure 31: Comparison of experimental (top) and theoretical (bottom) components of matrix representing the rotation of the specimen relative to the beam.

Top: the view number $j$ is on the horizontal axis. Views were acquired at azimuthal angles $\phi$ from 0 to 350 degrees in 10 degree (.174 radian) increments; Bottom: Theoretical plot of components assuming precession; The vertical axis in all plots is unitless (see Equation 160).
A plot of a theoretically ideal precession is shown in Figure 31(bottom). This plot was generated according to:

\[ G(\phi) = R_z^{-1}(\phi) \, R_x(\theta_0) \, R_z(\phi) \]  \hspace{1cm} (160)

Where:

\[ R_x(a) = \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos(a) & \sin(a) \\ 0 & -\sin(a) & \cos(a) \end{pmatrix} \]

\[ R_z(a) = \begin{pmatrix} \cos(a) & \sin(a) & 0 \\ -\sin(a) & \cos(a) & 0 \\ 0 & 0 & 1 \end{pmatrix} \]

\[ \theta_0 = .03 \text{ radians (1.7 degrees)} \]

Figure 31 shows that the experimentally measured beam trajectories have the
same sinusoidal form as the theoretical. The most noticeable difference between theoretical and actual plots of $G$ components is a phase offset and a factor of 5 difference in amplitude. The phase offset is unimportant as it only indicates that the precession of the beam starts at a different azimuthal angle in the theoretical model than in the actual data. To explain the difference in amplitude, note that bundle adjustment describes the shape of the trajectory cone but does not give information about the absolute trajectory angles. The measure is not absolute because bundle adjustment finds a solution (a set of projections and a 3D model of bead locations) that is consistent but not necessarily unique: the trajectory cone may be scaled in the X, Y, or Z direction for instance. (The absolute angle is measured in Appendix C.)

2.7.2 Image centering performance

Apparent motion of beads during precession of the beam was used to characterize the centering performance of the coil control system. As described earlier, the de-scan system balances signals to coil sets 5 and 6 to ensure that the image remains stable as the beam precesses. Spherical gold beads were used as they have a well defined point-like positions and high contrast. To generate this dataset, calibration was performed as described in Chapter 2. As an enhancement to the standard calibration procedure, the last step of the de-scan calibration procedure was performed with the crosscorrelation method described in Appendix H.

As illustrated in Figure 4 the image of a bead remains stable on the CCD camera as the beam precesses if the bead is at the plane of the cone apex. Figure 33B shows
position of beads in images as the beam precesses. The z position in the plot corresponds to the z position of the stage during acquisition. At each z-stage position the beam was precesses from $\phi = 0$ to $\phi = 330$ degrees in 30 degree increments. (The zenith angle $\theta_0$ was fixed at 0.8 degrees.) The apparent motion of each bead traces out a ring in each X-Y plane of Figure 33B.

Thirty-one gold beads were tracked. An example view is shown in Figure 33A with the beads circled in green. Beads were at various z positions within the specimen. The radius of the ring traced by a bead is approximately zero when the z stage moves the bead to the plane of the beam cone apex, so different beads show a "waist" at different z locations (see Figure 33B). An example of the varying radius of the rings traced by a single example bead as the z-stage moves is shown in Figure 31.

In Figure 33B, each bead shows an "hour-glass" shaped plot. The radius of the hourglass is smallest at the "waist" of the hour-glass and increases as the z-stage moves away from the waist. To characterize the centering error, radius at the waist for each bead was used. Determination of the z level that is at the waist of each bead and measurement of the approximate radius at this level for each bead is described in the following text.
Figure 33: Plot of apparent motion of bead as the beam precesses at various z-stage positions; zenith angle 0.8 degrees. At each z level, 12 views were acquired, from azimuthal angles of 0 degrees to 330 degrees in 30 degree increments. (This data set is also shown in Figure 6.) Pixel size in X and Y directions is 1.7 nm.
Mathematical characterization of waist size

The 2D position of bead $i$ in view $j$ with the z-stage at level $z$ is given by vector $u_{ijz}$. The ring that each bead traces at each z-stage level is plotted in Figure 33B. The middle point of a ring for a particular bead at a particular z-stage level is given by

$$m_{iz} = \frac{\sum_j u_{ijz}}{N_{\text{views}}}$$

where $N_{\text{views}}$ is the number of views.

Throughout this section, $j$ is the index of the view and $i$ is the index of the bead, $z$ is the index of the z-stage level. The "radius" for the ring that bead $i$ traces at z-stage value $z$ is approximated by:

$$r_{iz} = \frac{\sum_j |m_{iz} - u_{ijz}|}{N_{\text{views}}}$$
Figure 34: Plot of the radius $r_{iz}$ as a function of z-stage position for a single example bead. The minimum, which represents the "waist" of an hour-glass shaped figure (see Figure 33B) is near z-stage position 17.

The waist of the hour-glass traced by a bead is at the z level where radius $r_{iz}$ is minimum. This is illustrated in Figure 34. So, for a given bead, the waist of the minimum radius considering every z level:

$$w_i = \min_{z}(r_{iz})$$

The waist-size $w_i$ for each tracked bead is shown in Figure 35.
Figure 35: $w_i$ for each bead in Figure 33

The value $w_i$ is useful as a measure of the stability of the image on the CCD camera. If the de-scan system does not work properly, the beads will tend to move in paths of larger radius. Ideally the waist $w_i$ of the cone should be zero. This precision is especially important for EOS as inaccuracies lead to unwanted blurring and reduction in resolution.

Considering all beads, the average waist size is given by

$$a = \frac{\sum w_i}{N_{beads}}$$

where $N_{beads}$ is the number of beads.

The value $a$, which was calculated and found to be 1.02, gives an average measure of image centering error considering all beads that were tracked.
Because of geometric image distortion that depends on azimuthal angle, the image stability (measured by the "waist size") during precession is not uniform across the entire image. The crosscorrelation alignment described in Appendix H was performed on features in the bottom right quadrant of the image, so alignment is more optimal in this region. The color coding in Figure 33B shows that the waist of the hourglass traces associated with each bead are smaller (as low as 0.7 pixels) in the lower right quadrant of the image and larger (as high as 1.5 pixels) in the opposite upper left quadrant.
3. Discussion

In this work, a conical beam precession system is designed and tested on multiple 3D imaging applications. This is the first report of beam precession applied to electron optical sectioning (EOS) and conical backprojection, hybrid backprojection, and object precession. Beam precession facilitates high throughput tomography by replacing or augmenting tedious physical specimen rotation with high speed and high accuracy beam deflection. A novel aspect of this conical beam precession system is ability to accurately maintain the position of the beam in both the back focal plane and the image plane at the CCD camera during beam precession. The speed of conical precession based methods of reconstruction is generally higher than mechanical tilt based methods because there is no need for physical rotation or tracking.

Volumetric reconstruction:

Results from volumetric reconstruction methods of electron optical sectioning (EOS) and conical backprojection produce 3D reconstructions of a spherical gold bead with the expected elongating effects of the conical point-spread function. EOS and conical backprojection were applied to a spiny dendrite specimen, and while EOS does produce some depth discrimination, the conical backprojection reconstructions show more easily perceivable results. While both methods have a theoretical point-spread function of a hollow cone, conical backprojection is different in that it uses precise alignment of images based on bundle adjustment and filtering that can be applied before backprojection. It is likely that the alignment and filtering techniques of backprojection are responsible for the clearer results (see Figure 8).
Results from hybrid acquisition show that conical beam precession in combination with physical specimen tilt does produce a reduction in the backprojection artifact, although the resolution is still limited by the missing wedge in Fourier space which remains approximately the same size. This hybrid approach serves to enhance reconstruction quality adding little to the total effort required to acquire data. With a high speed CCD camera the hybrid acquisition would take only slightly longer than the single axis tilt acquisition.

**Realtime imaging applications:**

Two applications presented are capable of realtime or nearly realtime operation: single slice electron optical sections and object precession. The first technique, single slice EOS, can be performed in approximately two seconds (with a single CCD acquisition) and gives some sense of the 3D nature of the sample with depth discrimination. The second technique, object precession, is a simple but effective means of observing some 3D features of the specimen with parallax effects. Acquisition of a set of 12 images for object precession requires approximately 24 seconds. If a faster CCD camera was used (12 frames per second) for object precession, it would be a realtime method, which could be especially useful for searching the specimen for areas of interest.

**Acquisition speed enhancement:**

The focus of this work was on development of a beam control system and creation of preliminary data, so beam deflection speed was not fully optimized. But,
even without optimization, performance of beam deflection (10 ms per change in angle) is orders of magnitude faster than mechanical tilting of the specimen (1 s per change in angle). When beam deflection is used, the dominant time requirement is CCD camera acquisition. A high speed CCD camera was not used in this work but commercially available cameras, which are capable of 12 frames per second at 1024X1024 resolution, would deliver a significant speed-up (see Table 1). Another potential optimization not explored in this work is adjusting the number of views used in conical backprojection (see Equation 161) so that only the minimum number needed is acquired.

**Future work, improving Z resolution:**

The most important improvements to the applications of EOS and conical backprojection in the future would be increase of the zenith angle, since it has a dramatic effect on Z resolution (see Figure 2). (Larger zenith angle would allow for a more complete sampling of Fourier space that would ultimately improve Z resolution.) Increasing this angle would require the design of a new microscope with a deflector and lens system capable of producing the larger deflection and maintaining image quality.

Although the Z resolution from convolving with a cone is characterized with equation 162, this theory does not characterize effects of deconvolution in the presence of imperfect representations of point spread function and incomplete sampling the full blurred volume. To address this, simulation was performed to show
the effects of zenith angle on deconvolution quality (see Appendix G). Simulations showed that deleterious effects are generally reduced with increases in zenith angle. These results re-iterate the point stated previously, that increase in zenith angle is beneficial, and imply that deconvolution in EOS will deliver better results with larger zenith angle. (Increase of the zenith angle would require a modified deflection system physically capable of producing larger deflections and a lens system capable of maintaining image quality at larger angles.) Results from imaging simulation also show that deconvolution tends to perform better when the point spread function is a solid rather than a hollow cone. So, as a future improvement, the beam could be precessed at a set of zenith angles rather than only one, approximating a solid cone PSF rather than a hollow cone.

**Future work, reducing calibration time:**

While previous work has published the use of sinusoidal waves to produce beam precession [9][8][18][21], they have not shown in mathematical detail why sinusoidal signals are sufficient. The mathematical model of the beam developed in this work validates use of sinusoidal signals to produce stability in both the back focal plane and the imaging plane. A way of building on this work in the future would be to more fully characterize the microscope, recording parameters such as the precise angle tilt expected from changes in current on each coil. An advantage of having a fully calibrated mathematical model of the precession system would be that it could be used to design a more efficient calibration procedure. For example, adjustments to the system such as changes in objective focus of zenith angle would not require
recalibration because the change in scan and de-scan coil current parameters could be
determined with the model.
4. Appendices

Appendix A. Time Required for Tissue Reconstruction

Reconstruction of a complete mammalian neural circuit would be valuable and arguably necessary to ultimately understand human brain function. As a model circuit, the rodent whisker barrel is appropriate as it is widely recognized as a distinct module with many internal connections but few connections to the outside. The project of reconstructing the circuitry of the whisker barrel demonstrates that increases in electron microscopy throughput would greatly facilitate circuit reconstruction efforts. Reconstructing the whisker barrel circuitry at approximately 10 nm resolution is described in [20]; however the description in [20] addresses serial electron microscopy rather than serial electron tomography. The following text adapts the argument given in [20] to determine the approximate time required for acquisition if a whisker barrel reconstruction were performed using serial electron tomography [14].

A block of tissue 0.66 X 0.66 X 1.55 mm would be large enough to contain an entire whisker barrel. This block would be physically sliced in to 1550 sections, each with dimensions of 660 X 660 X 1 \( \mu \)m. (The one-micron sections would be thin enough to image with energy filtered TEM.) A mechanical stage would be tilted around a single axis to 120 positions for each tomographic reconstruction, requiring one 1 sec for each position. Each section would be imaged in tiles, each 40 X 40 \( \mu \)m, generating 289 tiles per section. (This is assuming a 4K by 4K pixel CCD camera with 10 X 10 nm pixel size.) Considering all 1550 the sections, this is a total of 447950
tomographic reconstructions. Therefore, with current methods of automatic
tomography this project would require approximately 15,000 hours (625 days) of
mechanical stage movement time.

Appendix B. Resolution of Conical Tomography

Conical backprojection and volumetric electron optical sectioning both give a
volumetric image of the original object convolved with a conical point-spread
function, so resolution for each can be addressed with the same theory, which is
presented in [19] and [7]. Analysis in this section will assume finite steps in azimuthal
angle as the beam precesses around the cone. (The mode of optical sectioning where
the beam is precessed continuously can be approximated as taking very small steps in
azimuthal angle.)

The "central section theorem" states that each projection defines a plane in
Fourier space orthogonal to the projection direction [10]. The quality of the resulting
3D volumetric image dependent on the completeness of the sampling of Fourier space.
With conical tomography, the resolution in the X-Y plane of the reconstruction is the
same as the resolution in the individual projection views, \( d \), as long as a sufficient
number of images are acquired (i.e. the step in azimuthal angle is sufficiently small).
The required number of images \( N_c \) is given by:

\[
N_c = \frac{2 \pi D \sin(\theta_0)}{d}
\]  (161)
where \( D = \frac{T}{\cos(\theta_0)} \) and \( T \) is the specimen thickness, and \( \theta_0 \) is the zenith angle of the conical precession (see Figure 1).

The missing cones illustrated in Figure C tend to reduce the Z resolution of reconstructions based on conical tomography. This reduction in Z resolution can be represented with a theoretical elongation factor that gives the ratio of Z of resolution to X-Y resolution. (For the data acquired at zenith angle of \( \theta_0 = 1.7 \) degrees, this theoretical elongation factor \( e_c \) is 41 as shown in Figure 2.)

\[
e_c = \sqrt{\frac{3 - \sin^2(\theta_0)}{2 \sin^2 \theta_0}}
\]  

(162)

An important difference between conical backprojection and volumetric electron optical sectioning is a 3D "clipping" effect. The conical point-spread function (PSF) that image is convolved with (using both modes) theoretically extends to infinity in the \( +z \) and \( -z \) directions. Therefore the object \( o(x, y, z) \) that is convolved with the PSF during imaging gives a volumetric image, \( i(x, y, z) \), that extends to infinity in \( +z \) and \( -z \). During optical sectioning this blurred object \( i \) is effectively sampled one layer at a time with each step of the \( z \) stage. Therefore the sampling of \( i \) is truncated with optical sectioning because the stage is moved a limited amount in the \( z \) direction, typically a few microns. In contrast, with conical backprojection, the filtering was performed before backprojection, so the aforementioned 3D clipping problem does not arise. (Simulated effects of 3D clipping are shown in Appendix G.)
For information on filtering as a 2D operation, see [19].

Appendix C. Beam deflection characterization

This appendix reports on measurement of the mapping between numeric digital value for tilt parameter \( p_t \) in Chapter 2 and the actual zenith angle on our JEM-3200 microscope. (The parameter \( p_t \) was controlled with custom software through an application programming interface that gives access to microscope settings.) Also, the performance of the tilt compensation system for coil sets 3 and 4 at large zenith angle was measured. To generate data in this appendix, two cameras were used: the viewing screen camera and the internal camera. The viewing screen camera is mounted outside of the TEM and shows the viewing screen, and the internal camera is inside of the microscope column positioned at the bottom of the column. A summary of measurement results is given below followed by a detailed description of the procedures used.

Summary of Results

1. **Measuring camera length**: A prerequisite to measuring the beam tilt angle was measurement of the camera length. The camera length relates the diffraction spot displacement (which can be measured directly) to the angle of beam tilt (Equation 164). Diffraction rings of a powder of thallous chloride were used for calibration of camera length, and the camera length was found to
be $L = 88$ cm. This camera length value was then re-checked by tilting a single molybdenum trioxide crystal by 5 degrees (according to the goniometer) and ensuring that the displacement of the Kikuchi pattern was the expected amount assuming an 88 cm camera length.

2. **Measuring zenith angle of conical precession:**

The zenith angle during conical precession was measured and results showed that the digital numerical value for magnitude of tilt, $A_{\text{tilt}}$, corresponds to the zenith angle according to

$$K \theta_0 = A_{\text{tilt}}$$

(163)

where $K$ was measured and found to be 5700. This measurement was based on displacement of the diffraction pattern and consideration of the camera length described above. For an alternative verification of the value $K$, the angle $\theta_0$ computed by measuring the cone angle of a conical bead position plot, similar to that shown in Figure 6A, and the results agreed.

3. **Measuring unwanted beam shift during precession:** Ideally the center of the beam should remain stationary relative to the specimen as the beam precesses around the cone. The amount of displacement in the beam relative to the specimen was measured by observing the movement of the illumination spot
on the specimen. At a zenith angle of 1.4 degrees, approximately 20 microns of shift was measured. It should be noted that larger zenith angles (above 1°) tend to produce more shift error and smaller zenith angles (below 1°) produce negligible shift error.

Measurement Procedures

1. Measuring Camera Length

Figure 36: Camera length L

Figure 36 shows a simplified diagram of the beam in diffraction mode. The lenses are not shown in the figure because they only act to change the effective camera length L [22]. From trigonometry, \[ \sin(\theta_0) = \frac{R}{L} \], and using the small angle approximation,
\[ \theta_0 = \frac{R}{L} \quad (164) \]

So, if we know the camera length \( L \) and the displacement from the center \( R \) we can compute the angle \( \theta_0 \) of the beam.

To calculate \( L \), a specimen of powdered thallous chloride with diffraction rings of known radii \( R_i \) was used. (Here \( i \) is used as an index to specify a particular ring.) The following two equations [22] characterize \( L \) based on the radius of diffraction rings:

\[ L = \frac{R_i d_i}{\lambda} \quad (165) \]
\[ \lambda = \frac{1.22}{E^{1/2}} \quad (166) \]

where \( d_i \) is the principle lattice spacing that corresponds to ring \( i \), \( E \) is the electric potential in electron volts, and \( \lambda \) is the wavelength of an electron in nm. The \( d_i \) principle lattice spacing for thallous chloride is 0.384 nm. For the JEM-3200, \( E = 300 \text{kV} \), so \( \lambda = .0022 \text{nm} \).

Based on the size of \( R_i \) (4.6mm), the TEM camera length given by Equation 165 was \( L = 88 \text{cm} \). (The viewing screen camera was used to measure \( R_i \); each millimeter on the viewing screen corresponds to 3.125 pixels on the viewing screen camera, ...
measured horizontally.)
Verification of camera length with Kikuchi pattern movement

Figure 37: Marks are drawn where Kikuchi lines cross. The position of the goniometer was moved from -5° to 5° in 5° increments. Top: -5°. Middle: 0°. Bottom: 5°.
Unlike the diffraction rings, Kikuchi lines are effectively rigidly fixed to the specimen [22], so they move on the viewing screen as the specimen is tilted. Equation 164 gives the relationship between displacement of the pattern $R$ and the angle $\theta_0$. The specimen was tilted from $-5^\circ$ to $5^\circ$ in $5^\circ$ increments, and the diffraction images during tilting are shown in Figure 37. (The specimen used was a single molybdenum trioxide crystal.) The Kikuchi pattern moved by approximately $v=158$ viewing screen camera pixels per $5$ degrees of tilt. Using the camera length computed above, the expected angle based on the movement of the Kikuchi pattern was calculated as follows:

- $C=1.5$ represents the horizontal stretch of the viewing screen camera image. The viewing screen is circular in reality so it was used to determine this factor. $C$ is the ratio of the width of the viewing screen to the height of the screen in the viewing screen camera image.
- $v$ is the displacement of the Kikuchi pattern in a vertical direction, measured in viewing screen camera pixels.
- $p=3.125$ represents the number of viewing screen camera pixels per millimeter, measured horizontally.
- $v=158$ is the approximate vertical displacement of Kikuchi lines in pixels when the goniometer is moved $5$ degrees.
The angle based on camera length is given by

\[ \theta_0 = \frac{(vC)/p}{L}, \]

which is Equation 164 with a conversion from pixels to millimeters and a correction for distortion of the image in the viewing screen camera. This gives an expected angle of 4.9 degrees, which matches the goniometer angle of 5 degrees reasonably well.

2. Measuring zenith angle of conical precession

The amount of tilt in the x and y direction (\( P_{tx} \) and \( P_{ty} \) in Chapter 2) are digital values measured in "ticks." The purpose of this experiment is to measure the number of ticks per change in the angle \( \theta_0 \). As shown in Figure 38, the calibration result is that \( K = 5700 \) ticks per degree.
Verification of zenith angle with tracked bead data

In addition to checking the displacement of the diffraction spot, the beam tilt angle was computed from a conical precession and Z stepping dataset similar to that shown in Figure 6A (expect that motor driven Z stage was used rather than the piezo driven stage). The numerical value of the zenith angle tilt was $A_{\text{tilt}} = 8000$ ticks (see Equations 15 and 16). The zenith angle was measured as the angle of the cone off...
the Z axis. At the magnification used (5K), one pixel of the CCD camera corresponded to 8.5nm on the sample. Mechanical Z stage movement was read from the microscope's built in gauge (measured in microns). For a tilt amplitude $A_{\text{tilt}}$ of 8000 ticks, the cone had a 119nm radius when checked 4800nm away from the apex along Z. So, trigonometry gives $\theta_0 = 1.4$ degrees of tilt off the axis, which agrees with the expected value of 1.4 degrees based on the assumption that $K$, the ratio of "ticks" to degrees, is 5700 (see Equation 163).

3. Measuring unwanted beam shift during precession

At zenith angles less than 1 degree the tilt compensation system that the JEM-3200 uses to control coil sets 3 and 4 works properly and maintains the beam on one point at the specimen plane. As the zenith angle increases above 1 degree, the beam begins to shift on the sample. Although unwanted shift is reduced at small zenith angles, the resolution of reconstruction is improved by larger zenith angles (as described earlier), so it is useful to increase the tilt as much as possible, as long as error remains manageable. The following experiment quantifies the amount of unwanted shift occurring when the beam is at $\theta_0 = 1.4$ degrees.
Figure 39: Illustration of shift error caused by coil sets 3 and 4 during precession; (a) Proper formation of a pivot point at the specimen plane. (b) Lack of a pivot point at the specimen plane. (In both (a) and (b) the figures shown coil sets 5 and 6 returning the beam to the optical axis)
Figure 40: At tilt amplitude 1.4° (8000 ticks), five different images of illumination spots are superimposed on a background image to illustrate shift. From 90 to 270 degrees there is a shift of 118 pixels on the viewing screen camera.

Figure 41: A full CCD camera image digitally superimposed on the viewing screen camera image to show field of view of the CCD camera. The darkened rectangular region shows the size of the full field of view of the CCD camera. The width of the CCD camera corresponds to 102 pixels on the viewing screen camera.
The tilt compensation system was first calibrated according to the manufacturer's instructions. After this calibration was complete, the following procedure was used to measure shift error. One "background" image was taken with the illumination spot spread enough to fill the entire viewing screen. The illumination spot was then condensed so that its center could be located. The tilt amplitude was set to 1.4° (8000 ticks) and the magnification was set to 2.5K. A total of five images were recorded: one "background" image was recorded with no tilt and then an image at each of the azimuthal angles $\varphi = 0°, 90°, 180°, \text{and } 270°$. For the background image, the beam was spread to fill the screen, but with the four images at various azimuthal angles, the beam was condensed to a visible spot. Each of the four images with the condensed beam were pasted onto the background image with the spread beam, according where features of the image aligned. The result is shown in Figure 40. Ideally, if there was no shift error, the five circular illuminated disks in Figure 40 would appear at the same position; the difference in their position describes the shift error of the tilt compensation. The amount of shift error (which is large as the beam moves from 90 degrees to 270 degrees) is about 118 viewing screen camera pixels as shown in Figure 40.

So, at 2.5K magnification and $\theta_0 = 1.4$ degrees the illumination spot center moves by 118 viewing screen camera pixels (see Figure 40) as the beam precesses around the cone; this displacement is approximately 1.1 times the width the internal camera field of view. At a typical magnification of 10K, the beam would move by $4 \times 118 = 472$ viewing screen camera pixels, approximately 4.7 times the width of the
internal CCD camera. At this magnification, the width of the internal camera corresponds to 4.4 microns at the specimen, so the shift error is approximately 20 microns at the specimen during precession. (The scan system calibration procedure described in Chapter 2 tends to deliver better results, so the results presented here using the manufacturer's alignment procedure give a conservative estimate of performance.)

In conclusion, with \( \theta_0 = 1.4 \) degrees, the shift error is manageable, but not desirable. Accepting the shift error and spreading the beam over a large area, it is possible to keep a particular region illuminated during conical precession, although this is not ideal. The beam has to be spread more that it typically would be, so the amount of intensity on the region being imaged is decreased. When imaging thick specimens, most of the intensity was blocked by the selective area aperture, so loosing more intensity by spreading the beam is not desirable. Reduction of the unwanted shifting may require modification to the microscope control system and or physical modification of coil sets 3 and 4.

Appendix D. Power spectrums of reconstructions

This appendix shows resolution performance for the techniques described previously in Chapter 1. Figures 42-47 show 2D slices through 3D reconstructions and corresponding power spectrums of the 2D slices, which are plotted using a logarithmic scale.
Figure 42: Left: cross-sections through a volumetric reconstruction based on electron optical sectioning; Right: power spectrums corresponding to images on the left; This reconstruction is also shown in Figure 8A-B.

Figure 43: Left: cross-sections through a volumetric reconstruction based on electron optical sectioning with deconvolution; Right: power spectrums corresponding to images on the left; This reconstruction is also shown in Figure 8C-D.
Figure 44: Left: cross-sections through a volumetric reconstruction based on conical backprojection; Right: power spectrums corresponding to images on the left; This reconstruction is also shown in Figure 8E-F.

Figure 45: Left: cross-sections through a volumetric reconstruction based on conical backprojection; Right: power spectrums corresponding to images on the left
Figure 46: Left: cross-sections through a volumetric reconstruction based on mechanical tilt around a single axis; Right: power spectrums corresponding to images on the left; This reconstruction is also shown in Figure 12A-B and 13A.

Figure 47: Left: cross-sections through a hybrid volumetric reconstruction based on mechanical tilt around a single axis combined with beam precession (left); Right: power spectrums corresponding to images on the left; This reconstruction is also shown in Figure 12C-D and 13B.
Appendix E. Energy Filtering

Since the beam is tilted off the optical axis of the objective lens during beam precession, more chromatic aberration is formed [6]. Energy filtering was used, which reduced chromatic aberration and thereby helped to enhance image contrast. Filtering was performed with an Omega energy filter with slit width ranging from 30 to 80 eV depending on the specimen. This filtering was especially important for datasets where beads were tracked automatically (such as shown in Figure 6A) because high contrast images of beads were needed for IMOD to automatically locate the bead centers.

Figure 48: Images at zenith angle 0.8°, without energy filtering and with energy filtering. Insets show a enlarged view of two gold beads. (a) A smearing effect obfuscates the location of the two spherical gold beads. (2) Energy filtering reduces chromatic aberrations; bead locations are well defined and can be tracked automatically using IMOD.
Appendix F. Effect of Aperture Size on Image Quality

Because the size of the selective area aperture affects image quality (especially with thicker samples [6]), an aperture of 50 micron diameter or smaller was used for all data collected. To produce scattering contrast, this transmission spot must remain inside of the selective area aperture (which is at the back focal plane in B-mode) as the beam precesses. Ideally the beam would not move at the back focal plane at all during precession, although some motion is inevitable due to imperfection in the control of the beam. It was necessary to ensure the beam moved less than 50 microns in the back focal plane so it would stay within the selective area aperture. (As described in Chapter 2, motion of the beam relative to the aperture can be viewed in diffraction mode.)

To measure the effect of aperture size on image quality, three images were acquired. The specimen was 2 microns thick and energy filtering with a slit width of 50eV was used to reduce chromatic aberration. The beam was tilted to zenith angle of 1.7 degrees and set to an arbitrary azimuthal angle of 90 degrees. Images show the effect of a completely open aperture, a 100 micron diameter aperture, and a 50 micron aperture. The 100 micron and 50 micron apertures accept electrons that are .44 and .22 degrees off of the unscattered beam axis, respectively.
Figure 49: Effect of selective area aperture size on resolution. Images are shown on the left and corresponding power spectrums are shown on the right (logarithmic scale). (A and B) Completely open aperture; (C and D) 100 micron diameter aperture; (E and F) 50 micron diameter aperture
**Appendix G. Deconvolution study**

These simulations show how imperfections in data affect the final optical sectioning output. Three sources of error were simulated: (1) inaccuracies in point spread function (PSF), (2) clipping, and (3) thresholding of the de-blurring filter. Each is simulated assuming various cone angles, 5, 10, 20, and 40 degrees off axis. Each simulation was done assuming a hollow cone, hollow cone with two mechanical tilts, and a solid cone PSF. The 3D test image is a cube taken from a double tilt tomographic reconstruction. The views shown are a Y-Z cross-section (vertical plane cut through center of cube) and a X-Y cross-section (horizontal plane cut through center of cube).

**Three types of PSF’s were simulated:**

- Hollow cone
- Hollow cone rotated -20 degrees superimposed with another hollow cone rotated 20 degrees (This was done to simulate two mechanical tilt angles, -20 degrees and +20 degrees.)
- Solid cone

**The following types of error were simulated:**

1. **Sheared PSF:** The point spread function used for de-blurring is slightly different than the actual point spread. It is sheared to an angle of 0.4 degrees off the Z axis.
2. **Clipped:** The blurred image is clipped before de-blurring. Thirty pixels were cut from the top and bottom of the blurred image. This is a conservative introduction of error. The Z stage is typically moved from the bottom of the physical specimen to the top so the imaging process clips off more than this much in reality.

3. **Thresholded:** The filter used for de-blurring (deconvolution) is thresholded to reduce noise in the output. This threshold (0.25) was chosen because it was high enough to remove excess noise in data in one of our previous reconstructions with real data. The thresholding of the filter is set to match the thresholding that was needed with the dataset shown in Figure 8 to mitigate excessive noise from deconvolution.

4. All three errors combined

**Details of simulation:**

- \(i(x,y,z)\) is the input image (phantom):
- \(p(x,y,z)\) is the exact point spread function \(p\):
- For hollow cone, value of point spread is inversely proportional to the circumference of the circles it forms in each X-Y plane.
- For solid cone, value of point spread is inversely proportion to the area of the circular region it forms in each X-Y plane.
- The approximate PSF, \(p_a\), is a the actual PSF, \(p\), sheared like so:
  \[
p_a(x,y,z) = p(x, y + (z \tan(\theta)), z)
  \]
• $b(x,y,z)$ is the convolved or "blurred" image.

\[ b = i*p \]

• The filter, $f$, is given by the Fourier transform of $p_a$:

\[ f = \text{threshold} (\text{normalize} (F(p_a)), \text{minValue}) \]

• Definition of "threshold(v)" function:

\[
\begin{align*}
\text{if} & \quad v < \text{minVal} \quad \text{threshold}(v, \text{minValue}) = \text{minValue} \\
\text{if} & \quad v \geq \text{minVal} \quad \text{threshold}(v, \text{minValue}) = v
\end{align*}
\]

• Definition of "normalize" function: Scale the data so that the average value of a voxel is 1.

• The deconvolved image $i_d$ is given by

\[ i_d = F^{-1}(F(b) / f) \]

where $F$ is a Fourier transform and $F^{-1}$ is an inverse Fourier transform

• Plots show the absolute value of $i_d$
Table 2: Effect of sheared PSF; hollow cone PSF

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Table 3: Effect of sheared PSF; double hollow cone PSF

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Table 4: Effect of sheared PSF; solid cone PSF

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Table 5: Effect of clipping; hollow cone PSF

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Table 6: Effect of clipping; double hollow cone PSF

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Table 7: Effect of clipping: solid cone

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<th>XY (original)</th>
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<th>XY (deconvolved)</th>
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Table 8: Effect of thresholding: hollow cone PSF

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<th>XY (original)</th>
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Table 9: Effect of thresholding; double hollow cone PSF

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Table 10: Effect of thresholding; solid cone PSF

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<th>XY (original)</th>
<th>XY (convolved)</th>
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Table 11: Effect of all errors; hollow cone PSF

<table>
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**Table 12: Effect of all errors; double hollow cone PSF**

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Table 13: Effect of all errors; solid cone PSF

<table>
<thead>
<tr>
<th>Actual PSF</th>
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<th>XY (convolved)</th>
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clipError: 30.00  
coneError: 0.40  
minValue: 0.25 | ![Image] | ![Image] | ![Image] | ![Image] |
| zenithAngle: 10.00  
clipError: 30.00  
coneError: 0.40  
minValue: 0.25 | ![Image] | ![Image] | ![Image] | ![Image] |
| zenithAngle: 20.00  
clipError: 30.00  
coneError: 0.40  
minValue: 0.25 | ![Image] | ![Image] | ![Image] | ![Image] |
| zenithAngle: 40.00  
clipError: 30.00  
coneError: 0.40  
minValue: 0.25 | ![Image] | ![Image] | ![Image] | ![Image] |
Appendix H. Calibration with cross-correlation

When calibrating the beam for optical sectioning, image stability is essential, so an automatic image centering software module written by Dr. James Bouwer was used to enhance the accuracy of the de-scan calibration. When calibrating for optical sectioning, the de-scan calibration was performed in two parts. In the first part, the sinusoidal signals (see Equations 11-14) were adjusted manually as described in section 2.6.4. The sinusoidal calibration procedure reduces the shift error in the imaging plane from many thousands of pixels to approximately 20 pixels, and the procedure reduces the shift error in the diffraction plane so that unscattered beam remains inside of the selective area aperture. (The magnification was 35000X at the plane of the CCD camera, and the size of the a pixel on the CCD camera was 60 microns.) After the last iteration of this sinusoidal procedure, automatic image centering was used to re-perform step "b" of single azimuthal angle alignment described in section 2.6.3. This automatic centering is performed at each azimuthal angle \( \phi \).

To perform image centering automatically, a technique involving cross-correlation is used. It is assumed that the relationship between image movement on the screen and deflection is linear. That is, if the displacement of the image in x and y is given by the 2D vector \( \mathbf{v} \) and the corresponding adjustment to the x and y deflectors is given by the 2D vector \( \mathbf{k} \), then

\[
\mathbf{v} = A \mathbf{k}
\]  

(167)

where \( A \) is an invertible 2X2 matrix.
The following matrix calibration procedure is used to determine $A$:

1. The beam is set to no tilt (i.e. $A_{5x}$, $A_{5y}$, $A_{6x}$, $A_{6y}$, $A_{\text{tilt}}$ are set to zero).

2. A reference image is acquired.

3. $d_6$ is set to $e_1 = \begin{pmatrix} 1 \\ 0 \end{pmatrix}$

4. An image is acquired and compared to the reference image with cross-correlation. The maximum peak of the cross-correlation gives the image shift, $v_1$, that the deflection $e_1$ produced.

5. $d_6$ is set to $e_2 = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$

6. An image is acquired and compared to the reference image with cross-correlation. The maximum peak of the cross-correlation gives the image shift, $v_2$, that the deflection $e_2$ produced.

Using Equation 167 gives:

$$v_1 = Ar_1$$

(168)

$$v_2 = Ar_2$$

(169)

The two vector Equations 168 and 169 represent four equations, and they are used to solve for four unknowns, the components of the matrix $A$. The matrix $A$ is used on the following automatic centering procedure.

When performing optical sectioning, Equations 170 and 171 are used in place of
Equations 13 and 14 so that a correction term $j(\phi)$ is used. The correction term is a vector valued function defined at each azimuthal angle $\phi$.

\[
\begin{align*}
    d_{6x}(\phi) &= A_{6x} \sin(\phi + \sigma_{6x}) + j_x(\phi) \\
    d_{6y}(\phi) &= A_{6y} \sin(\phi + \sigma_{6y}) + j_y(\phi)
\end{align*}
\]  

The following procedure is performed to determine corrections $j(\phi)$ which are small adjustments to beam position that improved the centering of the image during beam precession.

1. Acquire a reference image with the beam untilted.
2. For each azimuthal angle $\phi$:
   a) Set the beam to the azimuthal angle $\phi$ according to Equations 11-14.
   b) Compute the cross-correlation between the current image and the reference image and find the maximum peak to determine the shift error $\mathbf{v}$ (measured in pixels).
   c) Set $j(\phi)$ to $-A^{-1}\mathbf{v}$, which is the deflection needed to return the image to the reference image position.
Appendix I. Names of JEOL JEM-3200 parameters

This appendix gives the mapping between names of parameters used in the JEOL JEM-3200 microscope software and symbols used in this thesis.

- **SHIFT Y**: $C_{sy,4y}$
- **SHIFT X**: $C_{sx,4x}$
- **TILT X**: $C_{tx,3x}$
- **TILT Y**: $C_{tx,3x}$
- **ANGLE X**: $C_{ty,4x}$ and $C_{sy,4x}$ (which are required to be equal)
- **ANGLE Y**: $C_{tx,4y}$ and $C_{sx,4y}$ (which are required to be equal)
Bibliography


