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Description and comparison of algorithms for correcting anisotropic magnification in cryo-EM images

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ABSTRACT

Single particle electron cryomicroscopy (cryo-EM) allows for structures of proteins and protein complexes to be determined from images of non-crystalline specimens. Cryo-EM data analysis requires electron microscope images of randomly oriented ice-embedded protein particles to be rotated and translated to allow for coherent averaging when calculating three-dimensional (3D) structures. Rotation of 2D images is usually done with the assumption that the magnification of the electron microscope is the same in all directions. However, due to electron optical aberrations, this condition is not met with some electron microscopes when used with the settings necessary for cryo-EM with a direct detector device (DDD) camera. Correction of images by linear interpolation in real space has allowed high-resolution structures to be calculated from cryo-EM images for symmetric particles. Here we describe and compare a simple real space method, a simple Fourier space method, and a somewhat more sophisticated Fourier space method to correct images for a measured anisotropy in magnification. Further, anisotropic magnification causes contrast transfer function (CTF) parameters estimated from image power spectra to have an apparent systematic astigmatism. To address this problem we develop an approach to adjust CTF parameters measured from distorted images so that they can be used with corrected images. The effect of anisotropic magnification on CTF parameters provides a simple way of detecting magnification anisotropy in cryo-EM datasets.

1. Introduction

Anisotropic magnification in electron microscope images of 2D crystals was first described more than 30 years ago (Baldwin and Henderson, 1984) but more recently has not been detected with most modern electron microscopes used under conditions typical for cryo-EM with film or charge-coupled device (CCD) cameras. Direct detector device (DDDs) cameras have revolutionized single particle electron cryomicroscopy (cryo-EM) (Kühlbrandt, 2014; Smith and Rubinstein, 2014). Two DDD manufacturers, Gatan and Direct Electron, have produced cameras with pixel sizes between 5.0 and 6.5 μm, which is significantly smaller than is typical with CCD cameras. Because DDD cameras are placed below the projection chamber of the microscope, images formed on the DDD have an additional magnification relative to photographic film used with the same microscope. Consequently, electron microscopes used for high-resolution studies with a DDD may need to be set to a lower nominal magnification than was previously typical (Li et al., 2013). At these conditions, anisotropic magnification has been detected in several microscopes, representing a large fraction of the instruments where this issue has been investigated. The phenomenon was seen first in a modern electron microscope with a 300 kV FEI Titan Krios microscope used with a Gatan K2 Summit DDD (Grant and Grigorieff, 2015) and has subsequently been detected on other FEI microscopes, including at least a 300 kV Tecnai Polara and a 200 kV Tecnai TF20. With a 14 μm pixel size, the FEI Falcon series of DDDs does not require a low magnification setting and anisotropic magnification has not been detected with any microscope used in combination with this camera.

The consequence of anisotropic magnification in single particle cryo-EM is that images of molecules lying in different orientations on the specimen grid cannot be averaged coherently, limiting the resolution that can be obtained in 3D maps calculated from these data. For example, with 2% magnification anisotropy, a particle that is 300 Å long would appear to be 300 Å long when lying in one

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orientation and 306 Å long when lying in another orientation. This effect will have more severe consequences for larger particles than for smaller particles. Anisotropic magnification can be detected by an elliptical appearance of the powder diffraction patterns calculated from images of a variety of specimens including polycrystalline gold, graphite, or thallous chloride. The observed anisotropy in magnification exists at the low magnification settings used to acquire images, but not at the higher magnifications used for correcting objective lens astigmatism. The cause of this effect has been proposed to be dirt in the microscope column that becomes charged and acts as an additional lens; in one 120 kV microscope the anisotropy was found to change slowly from 1.4% to 2.5% over the 7 years between 1983 and 1990 (Richard Henderson, personal communication). This observation, as well as our own measurements described below, suggest that anisotropic magnification is stable over the course of weeks or months, but should likely be measured periodically with any microscope. The dependence of magnification anisotropy on the nominal magnification of the microscope explains why the issue was not detected in current microscopes when higher nominal magnifications were typical.

In order to calculate high-resolution maps from cryo-EM images the contrast transfer function (CTF) of the microscope must be corrected. Most algorithms currently in use correct for the CTF during calculation of the 3D map. Introduction of anisotropic magnification after correction of objective lens astigmatism causes Thon rings from images to be elliptical, even when there is no objective lens astigmatism present. CTF parameters determined from elliptical Thon rings will therefore suggest the presence of astigmatism. A consistent ellipticity for Thon rings from images despite several attempts at correcting astigmatism at high magnification could indicate anisotropic microscope magnification. Similarly, the problem may be detected by the presence of systematic astigmatism in CTF parameters for datasets obtained over several EM sessions where different amounts of astigmatism are expected. An electron optical method for removing the effect has not yet been described. Here we describe and compare algorithms for computationally correcting the effects of anisotropic magnification on EM images. We also develop a method for recovering true CTF parameters from CTF parameters calculated from images with anisotropic magnification. The purpose of this manuscript is to increase awareness that the potential for anisotropic magnification exists, illustrate how this distortion can be detected and quantified, and demonstrate how it can be corrected computationally.

2. Methods and results

2.1. Measurement of magnification anisotropy

To determine precisely the magnification of a FEI TF20 microscope operating at 200 kV and equipped with a Gatan K2 Summit direct detector, we acquired 99 movies containing crystalline thallous chloride particles. Anisotropic magnification parameters measured from these thallous chloride images can subsequently be used to correct cryo-EM images of biological specimens. The movies consisted of 30 frames collected at a rate of 2 frames s^{-1}, 5 e^- $pixel^{-1}$ s^{-1}, and 1 e^- $Å^{-2}$ $frame^{-1}$. From these movies, 558 thallous chloride particles that showed clear interatomic planes were selected (Fig. 1A). The thallous chloride lattice has a spacing of 3.842 Å. Consequently, the power spectrum from each crystal is expected to show a spot at distance ($p \cdot N/3.842$ Å) pixels from the origin, where p is the pixel size in Angstroms, and N is length in pixels along each edge of the image (Fig. 1B). The average of power spectra from images of particles is expected to produce a ring with this distance as the radius (Fig. 1C). However, the resulting average of power spectra had a slightly elliptical appearance. From 4096×4096 averaged power spectra of images, each containing several thallous chloride particles, we carefully recorded the lengths and angles of 209 vectors, $\mathbf{d}$, from the origin of the pattern to the diffraction ring. Fig. 1D shows a plot of the lengths of the vectors as a function of the angles they make with the positive k_x-axis of the power spectrum. The plot has a sinusoidal appearance, indicating that the diffraction ring from thallous chloride was indeed elliptical rather than round. Consequently, we fit the data to the equation

$$|\mathbf{d}| = \frac{|\mathbf{r_1}| + |\mathbf{r_2}| + \cos(2 \cdot [\theta_d - \theta_{r_1}])(|\mathbf{r_1}| - |\mathbf{r_2}|)}{2}, \quad (1)$$

where $|\mathbf{d}|$ is the distance from the origin of the diffraction pattern to a point on the elliptical diffraction pattern, $\mathbf{r_1}$ and $\mathbf{r_2}$ are the axes of the ellipse, θ_{r_1} is the angle between the positive k_x-axis and $\mathbf{r_1}$, and θ_d is the angle between $\mathbf{d}$ and the k_x-axis. The fit revealed that $\mathbf{r_1}$ had a length that was 1.02 times the length of $\mathbf{r_2}$ and is 1.3° from the k_x-axis of the pattern.

2.2. Correction of images for anisotropic magnification

Anisotropic magnification can be corrected in images by appropriate stretching or contracting along $\mathbf{r_1}$ or $\mathbf{r_2}$. Stretching an image along one direction is equivalent to contracting its Fourier transform in the corresponding direction and vice versa. This equivalence is due to the similarity theorem (Bracewell and Bracewell, 1986), which states that if $F(k_x)$ is the Fourier transform of $f(x)$, then $\frac{1}{a}F(\frac{k_x}{a})$ is the Fourier transform of $f(ax)$, where a is a constant and $a \neq 0$. Consequently, four possible approaches were explored to correct images for anisotropic magnification: (1) A real space stretch along $\mathbf{r_2}$ to make it equal to $\mathbf{r_1}$, (2) a real space contraction along $\mathbf{r_1}$ to make it equal to $\mathbf{r_2}$, (3) a Fourier space contraction along $\mathbf{r_2}$ to make it equal to $\mathbf{r_1}$, and (4) a Fourier space stretch along $\mathbf{r_1}$ to make it equal to $\mathbf{r_2}$. The effect of stretching or contracting on the position of a point in an image or Fourier transform can be expressed in matrices as a transformation $\mathbf{E}_{ani} = \mathbf{R}_{ani}\mathbf{S}_{ani}\mathbf{R}^{\mathrm{T}}_{ani}$, where $\mathbf{R}^{\mathrm{T}}_{\mathbf{ani}}$ and $\mathbf{R}_{\mathbf{ani}}$ rotate points about the origin by the angles $-\theta_{ani}$ and θ_{ani}, respectively, and $\mathbf{S}_{\mathbf{ani}}$ applies a stretch or contraction along the new x-axis. These matrices are defined by:

$$\mathbf{R}_{ani} = \begin{bmatrix} \cos\theta_{ani} & -\sin\theta_{ani} \\ \sin\theta_{ani} & \cos\theta_{ani} \end{bmatrix} \text{ and } \mathbf{S}_{ani} = \begin{bmatrix} a & 0 \\ 0 & 1 \end{bmatrix}. \quad (2)$$

With these operations, the position of any point in the image $\mathbf{p} = [x, y]$ or Fourier transform $\mathbf{p} = [k_x, k_y]$ is transformed as $\mathbf{p}' = \mathbf{E}_{\mathbf{ani}}\mathbf{p}$. Correcting anisotropic magnification requires applying the same transformation, but using a constant $1/a$ where the microscope caused a distortion with magnitude a. In each case, correcting anisotropic magnification requires interpolation because the positions of the transformed points of the image will in general not fall on previously sampled image points. Real space linear interpolation is a simple interpolation scheme (Fig. 2A). In linear interpolation in real space in one dimension, the value of the function $f(x)$ is estimated from the nearest known values of the function, $f(x_0)$ and $f(x_1)$, as $f(x) \approx \frac{x-x_0}{x_1-x_0}f(x_0) + \frac{x_1-x}{x_1-x_0}f(x_1)$. In other words, the unknown value of the function at the point x is treated as a weighted average of the known values of the function at the points x_0 and x_1. This approach can be extended two dimensions as bilinear interpolation, where $f(x,y)$ is estimated from $f(x_0,y_0), f(x_1,y_0), f(x_0,y_1), f(x_1,y_1)$ as

$$f(x,y) \approx \frac{x-x_0}{x_1-x_0} \cdot \frac{y-y_0}{y_1-y_0} f(x_0,y_0) + \frac{x_1-x}{x_1-x_0} \cdot \frac{y-y_0}{y_1-y_0} f(x_1,y_0) + \frac{x-x_0}{x_1-x_0} \cdot \frac{y_1-y}{y_1-y_0} f(x_0,y_1) + \frac{x_1-x}{x_1-x_0} \cdot \frac{y_1-y}{y_1-y_0} f(x_1,y_1). \quad (3)$$