Journal of Structural Biology 192 (2015) 204–208

Contents lists available at ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Automatic estimation and correction of anisotropic magnification distortion in electron microscopes

Timothy Grant, Nikolaus Grigorieff *

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Drive, Ashburn, VA 20147, USA

ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form 11 August 2015
Accepted 12 August 2015
Available online 13 August 2015

Keywords:
Electron cryo-microscopy
Resolution
Magnification anisotropy
Image correction

ABSTRACT

We demonstrate a significant anisotropic magnification distortion, found on an FEI Titan Krios microscope and affecting magnifications commonly used for data acquisition on a Gatan K2 Summit detector. We describe a program (mag_distortion_estimate) to automatically estimate anisotropic magnification distortion from a set of images of a standard gold shadowed diffraction grating. We also describe a program (mag_distortion_correct) to correct for the estimated distortion in collected images. We demonstrate that the distortion present on the Titan Krios microscope limits the resolution of a set of rotavirus VP6 images to ~7 Å, which increases to ~3 Å following estimation and correction of the distortion. We also use a 70S ribosome sample to demonstrate that in addition to affecting resolution, magnification distortion can also interfere with the classification of heterogeneous data.

1. Introduction

Single particle electron cryo microscopy (cryo-EM) has undergone a recent surge in attainable resolutions (Bartesaghi et al., 2015; Campbell et al., 2015; Grant and Grigorieff, 2015). These gains are in large part due to the use of new direct electron detectors (Milazzo et al., 2005; Faruqi and Henderson, 2007; Li et al., 2013b), which have an improved detective quantum efficiency (DQE) (Ruskin et al., 2013; McMullan et al., 2014) and are capable of recording movies to reduce the blurring in images due to beam induced movement (Brilot et al., 2012; Campbell et al., 2012; Li et al., 2013a; Scheres, 2014; Rubinstein and Brubaker, 2014). A number of these direct detectors use relatively small physical pixel sizes, for example 5 μm (Gatan K2 summit, Gatan Inc., Pleasanton, CA) or 6.4 μm (DE-20, Direct Electron, San Diego, CA) and experience significant additional magnification due to their required positioning under the microscope column. This additional magnification means that the recorded images are taken at lower nominal magnification on the microscope than with traditional charge coupled device (CCD) cameras or the FEI Falcon detectors. It now appears that many microscopes may suffer from an anisotropic magnification distortion at these magnifications, which may have gone undetected until now, as these magnifications were not used for high-resolution work.

An anisotropic magnification distortion results in an image whose magnification varies with direction, and thus effectively leads to a directional scaling of the image. For example, a perfect circle, when imaged on a system with anisotropic magnification will appear as an ellipse. In single-particle cryo-EM, which relies on the averaging of many copies of a protein imaged with random orientations, anisotropic magnification will result in particles with different apparent dimensions that depend on their orientation in the image, and any subsequent averaging of these particles will not be fully coherent. The distortion will displace particle features from their undistorted locations and, for a centered particle, these displacements become larger with distance from the particle center. Therefore, larger particles will be affected more than small particles. For example, a 2% distortion will cause a location at the edge of a 700 Å diameter rotavirus DLP to differ by 7 Å in the most displaced directions, or 3.5 Å from the average location. Locations in other directions will also be displaced from the average position, by an amount that is dependent on the direction. Averaging many DLP images with different displacements into one 3D reconstruction will effectively apply a B-factor to the reconstruction of about 1000 Å^2 (Jensen, 2001), ultimately limiting its resolution. Correcting the distortion prior to averaging will reduce or remove this B-factor. In the case of a 100 Å diameter particle, locations at the edge will be displaced by only 1 Å from the average position and the effect of the magnification distortion on the 3D reconstruction will be much smaller with a B-factor of about 100 Å^2.

We have recently reported a 2.6 Å resolution reconstruction of the rotavirus VP6 trimer, which forms the outer shell of the

* Corresponding author.
E-mail address: niko@grigorieff.org (N. Grigorieff).

http://dx.doi.org/10.1016/j.jsb.2015.08.006
1047-8477/

rotavirus double-layer particle (DLP) (Grant and Grigorieff, 2015). The data for this reconstruction was taken on an FEI Titan Krios at a nominal magnification of 29,000x with images recorded on a K2 summit detector with a pixel size of ~1 Å per pixel. During analysis of this and other datasets, we became aware of anisotropic magnification distortion on the Titan Krios microscope that affects all magnifications typically used for data collection on a K2 summit detector. We believe the distortion arises from an issue in the projection system based on experimental observations that the distortion is magnification-dependent but not dependent on the objective lens setting. The projection system is composed of four lenses, which work in a pre-calibrated and fixed manner set by the manufacturer when changing magnification. Slight imperfections in the calibration/alignment between the four lenses such as astigmatism in one of the lenses could lead to the observed distortion.

We set out to characterize the distortion at each of the magnifications we normally use for data collection, and developed a program to automatically estimate the distortion from a set of images of a gold covered diffraction grating. The measured values can then be used to correct images for the distortion after they are taken and prior to processing.

2. Method

The conversion of a circle to an ellipse and vice versa can be described by two scaling values, describing the amount of stretching or shrinking along two orthogonal axes, and an angle which describes the orientation of these axes (see Fig 1A.). Our algorithm relies on first taking a number of images (~10) of a sample containing polycrystalline gold. A cross-grating diffraction standard, which can easily be purchased and is likely to be readily available in most EM labs, works well for this purpose. Amplitude spectra of images of polycrystalline gold will exhibit diffractions spots at ~2.4 Å and ~2 Å spacings. The number and directions of these spots will depend on the number and orientation of crystals in the image; however, averaging a sufficient number of amplitude spectra from different images of randomly orientated gold crystals will result in a spectrum containing rings at the 2.4 and 2 Å spacings (e.g. Fig 1B.). In an ideal microscope, these rings would be circular; however, in a microscope with anisotropic magnification they will form an ellipse, which describes the distortion. By estimating the parameters required to make the gold diffraction rings circular, we can estimate any magnification distortion present at a given magnification.

To facilitate this process, we developed a program to perform this analysis automatically (mag_distortion_estimate), and another to use the estimated values to correct images for any found distortion (mag_distortion_correct). The automatic estimation takes a set of images of polycrystalline gold and an estimate of the pixel size as input and prints the estimated anisotropic magnification parameters as output. The set of input images are Fourier transformed and converted to amplitude spectra. These amplitude spectra are averaged to provide an image which should contain rings at the

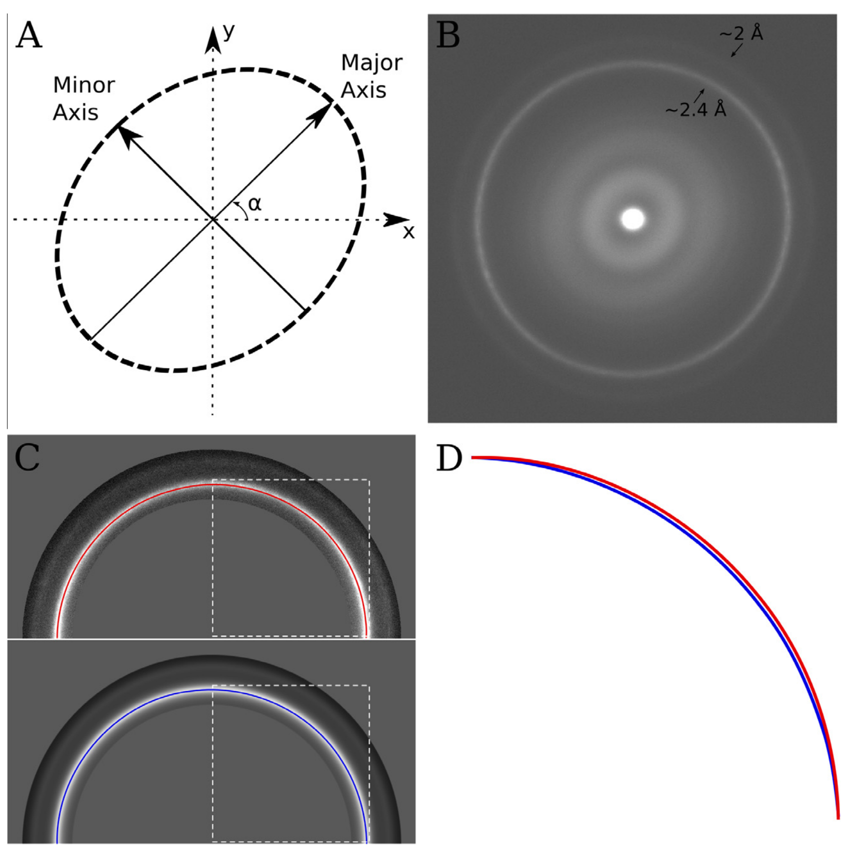

Fig. 1. (A) An anisotropic magnification distortion can be described with 3 parameters (in this case, two scale factors which describe the scaling along the major and minor axes and α, which describes the orientation of the major axis). (B) Sum of the amplitude spectra from 10 images of a polycrystalline gold covered diffraction grating. The images were taken at a nominal magnification of 22,500 on an FEI Titan Krios. The ~2.4 Å and ~2 Å gold rings are visible in the image and are slightly elliptical suggesting anisotropic magnification. (C) Top – half of the image shown in B, with the gold rings masked out and a path tracing the ~2.4 Å gold ring. Bottom – half of the rotational average of the image shown in B, also with the gold rings masked out and a path tracing the ~2.4 Å gold ring. The dashed white box illustrates the area, which is shown zoomed and overlaid in panel D. (D) Overlay of the section of the paths traced in C surrounded by the dashed white box. The path from the original image is different compared with the rotationally averaged version, indicating anisotropic magnification. In this instance the difference is ~1%, which combined with a ~1% difference in the orthogonal direction indicates a ~2% anisotropic magnification distortion at this magnification.