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ScienceDirect
Journal of Structural Biology 161 (2008) 211–218
Journal of Structural Biology
www.elsevier.com/locate/yjsbi

Single particle analysis based on Zernike phase contrast transmission electron microscopy

Radostin Danev *, Kuniaki Nagayama

Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences, 5-1 Higashiyama, Myodaiji-cho, Okazaki, Aichi 444-8787, Japan

Received 3 October 2007; received in revised form 19 October 2007; accepted 21 October 2007
Available online 6 November 2007

Abstract

We present the first application of Zernike phase-contrast transmission electron microscopy to single-particle 3D reconstruction of a protein, using GroEL chaperonin as the test specimen. We evaluated the performance of the technique by comparing 3D models derived from Zernike phase contrast imaging, with models from conventional underfocus phase contrast imaging. The same resolution, about 12 Å, was achieved by both imaging methods. The reconstruction based on Zernike phase contrast data required about 30% fewer particles. The advantages and prospects of each technique are discussed.

Keywords: Zernike phase plate; Single particle reconstruction; Phase contrast; GroEL; Cryomicroscopy

1. Introduction

In the recent years, cryo-EM has become the main experimental approach for high-resolution structural studies of biological systems. The availability of high-performance cryo-microscopes, and developments in sample preparation and data processing, has transformed cryo-EM from an exotic technique to common practice. Energy filters, field emission guns and CCD cameras greatly improved experimental performance. The image quality is no longer limited by stability of the electron optics or the specimen stage. The experimental approach has reached a point where the results are limited mainly by the fundamental properties of the sample and the optical system: the low electron density of ice-embedded biological specimens, their radiation sensitivity, and the contrast transfer function (CTF) of the modulated phase contrast of the EM (Glaeser, 1999). The combination of these three factors produces weak contrast and a low signal-to-noise ratio in the images. A solution to one of these limitations will lead directly to higher-quality data.

There are three main EM-based techniques for 3D structure analysis of biological samples: electron crystallography (Glaeser et al., 2007), single particle analysis (SPA) (Frank, 2006a), and electron tomography (Frank, 2006b). The former two rely on averaging multiple objects to improve the signal-to-noise ratio, while tomography reconstructs a single instance of an object. In electron crystallography, success depends mostly on the availability and quality of crystals. The limiting factor in the case of SPA and tomography is the low signal-to-noise ratio in the cryo-EM images. SPA can partially compensate for this by increasing the amount of input data, followed by averaging. However, small or flexible molecules, as well as heterogeneous samples, can still be challenging. Used by itself, tomography does not employ averaging, so the quality of the result is directly bound to the signal-to-noise ratio of the data.

In the past, there were few attempts at implementing various types of phase-contrast enhancement devices (Kanaya et al., 1958; Badde and Reimer, 1970; Johnson and Parsons, 1973; Unwin, 1973; Krakow and Siegel,

* Corresponding author. Fax: +81 564 59 5564.
E-mail address: rado@nips.ac.jp (R. Danev).

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doi:10.1016/j.jsb.2007.10.015

1975; Willasch, 1975; Balossier and Bonnet, 1981). Most of them showed promising "proof of concept" results, but were eventually abandoned because of various practical problems. Recently, interest in such devices has been reborn and is growing rapidly (Nagayama, 1999; Danev and Nagayama, 2001, 2006; Nagayama and Danev, 2003; Lentzen, 2004; Hosokawa et al., 2005; Huang et al., 2006; Majorovits et al., 2007; Cambie et al., 2007; Malac et al., 2007). These devices work by modulating the phase of the electron wave at a diffraction plane. This alters the phase-contrast properties of the microscope. The most obvious effect is a boost in overall image contrast as a result of the enhanced transfer of low spatial frequencies (Tosaka et al., 2005; Kaneko et al., 2006; Ohta et al., 2006). The improved phase contrast characteristics of the microscope are especially beneficial for imaging of the low-Z specimens common in cryo-EM (Danev and Nagayama, 2006; Malac et al., 2007).

In this report, we present the first results on the application of Zernike phase contrast transmission electron microscopy (ZPC-TEM) to single-particle 3D structural analysis of proteins. We selected the chaperonin GroEL as a test specimen. The structure of the native GroEL has been thoroughly studied by several techniques including cryo-EM (Roseman et al., 2001; Ludtke et al., 2004; Stagg et al., 2006) and X-ray crystallography (Bartolucci et al., 2005). The protein is stable, easy to obtain, and its structure is well-known, making it an ideal benchmark sample.

The purpose of this work is not to compare the ultimate resolution attainable by conventional TEM (CTEM) versus ZPC-TEM, but to illustrate the differences in data collection efficiency. For this reason, the experimental conditions that were selected are a practical compromise between the number of particles per image and the pixel size, which limited the maximum resolution to about the same value for both techniques.

2. Theoretical considerations

The design and performance of the Zernike phase plate have been discussed in detail elsewhere (Danev and Nagayama, 2001, 2006; Malac et al., 2007). It consists of a thin material film with a small hole in the center. The film is positioned at a diffraction plane inside the TEM, so that the central beam of unscattered electrons passes through the hole. The film thickness is chosen so that the primary electrons experience a phase shift of $-\pi/2$. The main effect of the phase plate is a modification of the phase-contrast characteristics of the TEM. The CTF changes from a sine function to a cosine function. This has a drastic effect on the information content of the image; low spatial frequencies which are severely attenuated in the CTEM are preserved well by the ZPC-TEM. Furthermore, when the objective lens is close to focus ZPC-TEM produces a uniform spectral transfer of phase information without the characteristic zeros of the CTEM phase CTF.

Fig. 1 illustrates the differences between the phase CTFs of CTEM and ZPC-TEM. With CTEM, it is a common practice to collect data with a range of underfocus settings. This is done in order to compensate for the loss of information at the CTF zeros, as well as to aid particle selection by the increased contrast resulting from higher underfocus settings. Two of the curves in Fig. 1 represent CTEM images taken at different underfocus settings. The sum of the two

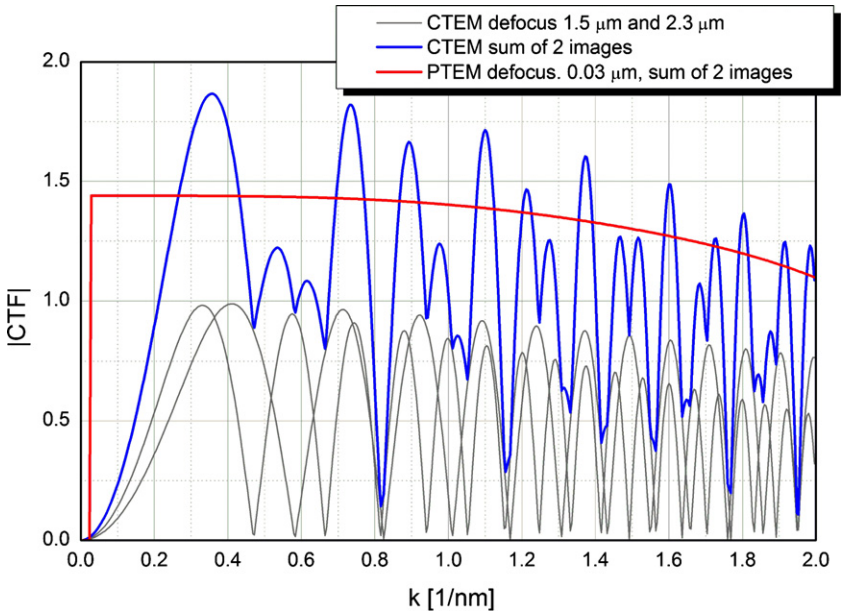

Fig. 1. Simulated weak-object phase-CTFs of conventional and Zernike phase contrast TEMs. The blue curve represents the sum of two images taken by varying the defocus of a conventional TEM. The red curve represents the sum of two images taken at a small defocus by Zernike phase contrast TEM. The simulation parameters were: acceleration voltage = 300 kV, spherical aberration Cs = 3.7 mm, chromatic aberration Cc = 4.0 mm, energy spread = 1.0 V, beam convergence = 0.04 mrad.