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Computational Modeling of the Benefits Expected For Zernike Phase Contrast in Cryo-EM

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Large values of defocus must be used to image biological macromolecules that are prepared as unstained, cryo-EM specimens. One consequence is that high-resolution information becomes delocalized and can only be imperfectly recovered [1]. In addition, rapid oscillations in the CTF produce an envelope function that also limits information recovery. Use of a phase-contrast aperture rather than high values of defocus might thus be a better solution for electron microscopy of weak phase objects [2, 3]. Among the hypothesized benefits are: (1) image contrast may become sufficient to "box" particles as small as 100 kDa, (2) particle alignment may become more accurate, and (3) assignment of particles to distinct conformational states may become more accurate.

In order to test whether these hypothesized benefits have a sound mathematical basis, we used a multislice algorithm [4] to simulate images of biological macromolecules embedded in vitreous ice. Molecular dynamics calculations were used to simulate large volumes of vitreous ice [5]. Atomic coordinates, taken from the PDB, were then placed into the simulated vitreous ice, displacing water molecules based on Van der Waals distances. Images of TMV were simulated (Figure 1a) using two different defocus values (1200 nm and 2400 nm), while the phase-plate was modeled by a constant $\pi/2$ phase shift for spatial frequencies greater then 1/67nm.. Using average intensity profiles the contrast in the TMV images can be quantitatively compared (Figure 1b). The simulated images show a much greater increase in contrast between defocused images and images from the simulated phase plate than what current experimental images have shown [3], suggesting that current experimental phase-plate implementations are far from optimal. To further study how phase contrast microscopy may aid in the analysis of heterogeneous macromolecular assemblies, images of RNA polymerase II in three distinct assemblies were simulated. For each assembly 50 images with different Euler angles were generated. Images simulated using either defocus or the phase plate were aligned to projections from three models of RNA polymerase II and images were classified as being in the same conformation as the model to which they had maximal cross correlation (Figure 4).

These simulations support the hypothesis that in-focus phase contrast will improve the capabilities of cryo-EM of biological macromolecules. The results suggest that not only will the study of smaller macromolecules be possible, due to greater image contrast, but that the ability of current methods to separate heterogeneous data will be greatly improved.

[1] K.H. Downing and R.M. Glaeser, *Ultramicroscopy* 108 (2008) 921.
[2] H. Boersch, *Zeit. f. Naturforsch. A* 2 (1947) 615.
[3] R. Danev, R.M. Glaeser and K. Nagayama, *Ultramicroscopy* (2009) In Press.
[4] E.J. Kirkland, Advanced Computing in Electron Microscopy (1998) Springer.
[5] E. Lindahl and B. Hess and D. van der Spoel: Journal of Molecular Modeling 7 (2001) 306.

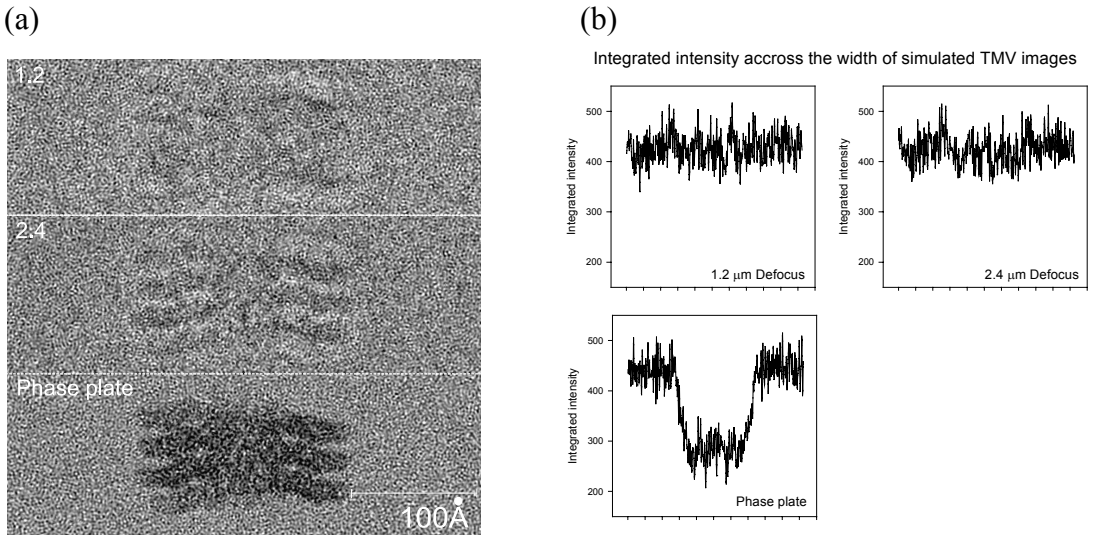

Figure 1. (a) Simulated images of ice-embedded TMV for a defocus of 1200 nm (top), 2400 nm (middle) and for in-focus Zernike phase contrast (bottom), with a simulated exposure of 10 $e^-/Å^2$. (b) Averaged intensity profiles embedded across the width of the respective, simulated images of TMV.

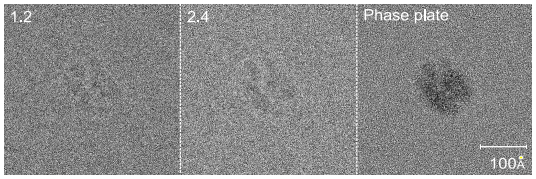

Figure 2. Simulation of images of ice-embedded RNAP II for a defocus of 1200 nm (left), 2400 nm (middle) and for Zernike in-focus phase contrast (right), with a simulated exposure of 10 $e^-/Å^2$.

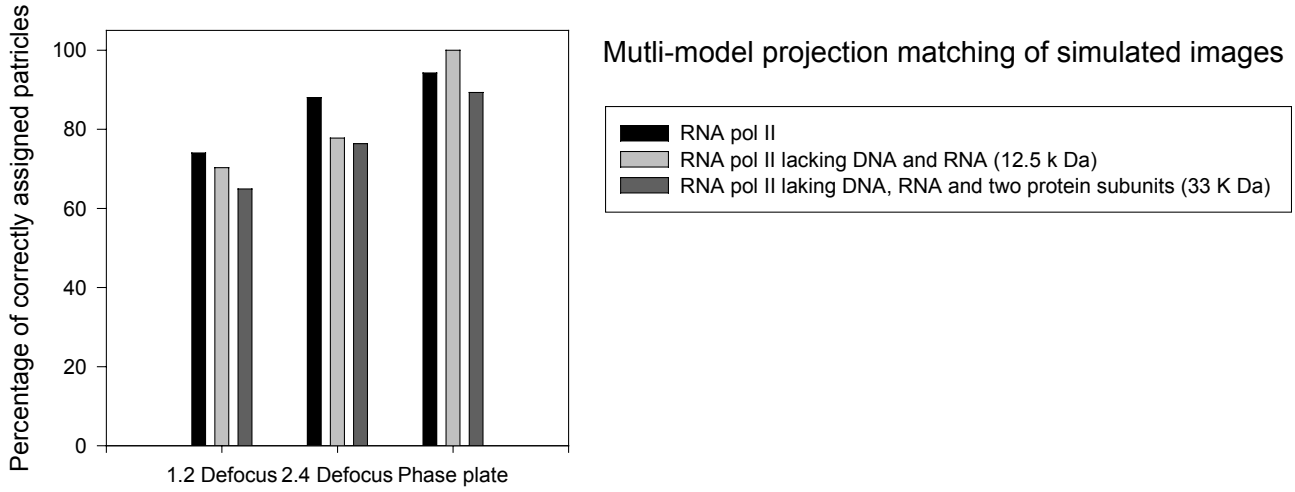

Figure 3 Comparison of the accuracy with which images of RNAP II particles in a structurally heterogeneous mixture are assigned to their correct sub-class. We assume that the small differences among the three structures, for any given imaging-condition, reflect statistical fluctuations in the results. The data indicate that assignments are about 70% correct for images defocused by 1200 nm; 80% correct for images defocused by 2400 nm; and 90% correct for in-focus Zernike phase-contrast images.

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