Molecular Structures and High Resolution TEM

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When to use the projection assumption and the weak-phase object approximation in phase contrast cryo-EM

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An accurate description of the interaction between incident electrons and the specimen is an essential step in both forward modeling, contrast transfer function (CTF) correction and 3D reconstruction in cryo electron microscopy (cryo-EM). In cryo-EM, incident electrons with typical energies of 80-300 keV interact with the electrostatic interaction potential (IP) of the specimen, e.g. macromolecules that are similar in density to the surrounding vitreous ice. In order to describe the electron-specimen interaction (analytically) two approximations are often made: the weak-phase object approximation (WPOA) and the projection assumption (PA). The WPOA holds for weakly scattering objects and the PA assumes that the exit wave from the specimen can be computed via the projected IP of the whole specimen. These approximations have, of course, limitations as they cannot account for e.g. the curvature of the Ewald sphere or multiple scattering events; effects which become more critical for high resolution imaging. We show that to make the next step in resolution improvement in cryo-EM, it is important to revisit these two approximations as well as their limitations.

We present quantitative criteria for the applicability of the PA (via the Fresnel number) and WPOA (via the probability of multiple interactions) in phase contrast cryo-EM. Using these approximations, we derive four models that describe the electron wave propagation through the specimen (WPOA, PA, PA+WPOA and SWPOA). By combining the two approximations in a new way, we introduce an analytic image formation model that we call semi weak-phase object approximation (SWPOA). This model imposes less strict conditions on the interaction potential than PA or WPOA and gives comparable exit waves as a multislice calculation which is commonly used as a reference.

Figure 1A shows the computed exit waves for a tubulin tetramer (TT) (constructed from PDBid-1SA0) using the four models discussed above, i.e. PA, PA+WPOA, WPOA and SWPOA. In order to better visualize the effect of the approximations, we show in Figure 1B the differences of the four exit waves with a reference. This reference is computed by a multislice approach inspired by [1]. In the difference images we observe that the SWPOA is nearly identical to the multislice reference, whereas the WPOA shows deviations mostly in the stronger phase parts. For the PA we see deviations over the whole extent of TT and, of course, for the combined PA+WPOA the deviations are the largest.

In Figure 2 we present a practical reference to facilitate the model choice for electron wave propagation through an actual macromolecule such as hemoglobin, ribosome, or tubulin. To test the applicability of the four image formation models we compare the simulated exit waves again against a multislice reference. To quantify the difference we use the normalized mean squared error (MSE). Figure 2A shows the result of thresholding the MSE at 10%. We find a horizontal boundary for the WPOA and a vertical boundary for the PA. The boundaries of the combined models asymptotically approach the individual (WPOA and PA) approximations. In Figure 2B a sketched version qualitatively depicts the regions where the different approximations hold.

As practical conclusions we find that, when simulating images at resolutions of 5 Å, the applicability of the PA and WPOA need to be re-considered. Here, the SWPOA offers an excellent solution, as a fast but equally accurate alternative to the multislice approach. For tomograms with typical resolutions >30 Å, the PA and WPOA are generally applicable. In single particle analysis, structures can be obtained up to approximately 3.3 Å resolution [2] at which the PA and WPOA may be violated depending on the size of the macromolecule, while the SWPOA again offers a solid solution.

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2. X. Zhang, L. Jin, W. H. Fang, Q. Hui, Z. H. Zhou, Cell 141 (2010) p. 472 – 482.
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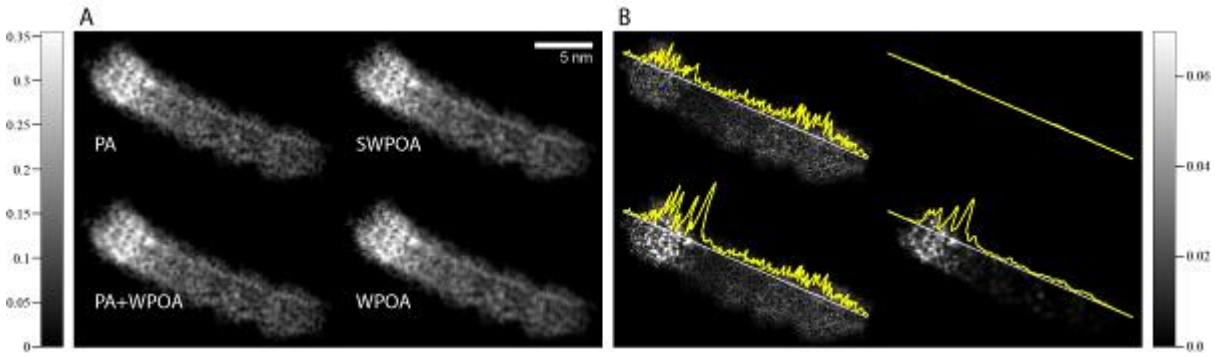

Figure 1. a.) Simulated exit waves of a tubulin tetramer (HT = 80 kV) using the PA, WPOA, PA+WPOA and SWPOA. b.) Difference image of the exit waves in A) and the exit wave computed with a multislice approach. The intensity scale bar indicates the phase of the exit wave subtracted by the mean.

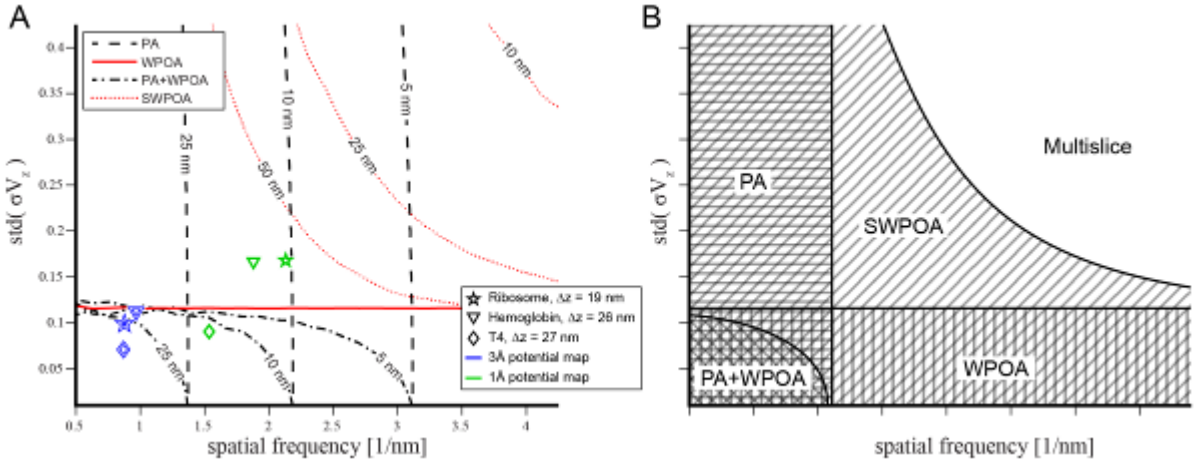

Figure 2. The applicability (at HT = 80 kV) of the PA, WPOA, PA+WPOA and SWPOA. A) Boundaries for each approximation where different lines represent different specimen thickness. Lines indicate 10% normalized MSE error of the respective approximation with a multislice reference. Left/below the boundary the approximation holds for a particular thickness. For three protein-complexes (Ribosome, Hemoglobin, TT) sampled at 1 Å and 3 Å, the potentials map properties are shown. B) A sketched diagram showing the qualitative results of A). The various striped regions depict region where each approximation holds.