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Experimental evaluation of a forward model in cryo-electron microscopy

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Accurate modeling of image formation in cryo electron microscopy (cryo-EM) is an important requirement for optimizing data acquisition and ultimately, interpreting the data at the highest possible resolution. Such a forward model (InSilicoTEM) that accounts for the specimen's scattering properties, microscope optics, and detector response is presented in [1].

The model was validated by comparing simulated and experimental images of 20S proteasome, earthworm hemoglobin, and GroEL acquired under various microscope settings and experimental conditions. The effects and parameters that have been analyzed include: solvent constituents, defocus, integrated electron flux, inelastic scattering, detective quantum efficiency (DQE), acceleration voltage, and amorphousness ("structural noise") of the solvent. All simulation parameters are based on physical principles and, when necessary, experimentally determined. The detector characteristics and CTF parameters (defocus and astigmatism) were determined using toolboxes provided in [2] and [3], respectively.

The dominant part of the interaction potential is calculated via isolated atom superposition approximation (IASA) [4, 5]. The influence of the effective charge redistribution due to the solvent's dielectric and ionic properties and molecular electrostatic distribution is modeled via a Poisson-Boltzmann (PB) approach [6]. Various buffer compositions (50mM up to 3M ammonium acetate) have been used to evaluate our modeling of the influence of charge redistributions for the hemoglobin sample. The contribution of the PB-based potential to the interaction potential appears to be less than 10 % for all these cases. The influence of the PB-based potential inclusion is mostly recognized by slightly less contrast at protein-solvent interfaces compared to the images calculated using only the IASA-based potential.

Various defocus series were acquired for which the simulations correctly predicted changes in the experimental images. After each defocus series another region was imaged with a different integrated electron flux. At acceleration voltage of 80 kV, simulated images at higher integrated fluxes often gave stronger contrast compared to the experiments. This observation is consistent with the effect of random beam-induced movements which depend on the integrated flux and can significantly damp the contrast in cryo-EM [7]. The inclusion of a motion factor blurs the simulated images to become more similar to experimental. The required motion factor to match simulations with experiments is in the range between 0 and 10 Å. It appears to be stronger for higher integrated electron fluxes, thinner ice layers or lower acceleration voltages, which is in agreement with values experimentally measured [7].

Inelastic scattering of electrons is modeled as the imaginary part of the interaction potential. Figure 1. allows a comparison between (B) simulated images where the model of inelastics is incorporated and (C) experimental energy-filtered images of earthworm hemoglobin and proteasome. In addition, simulated images in which only pure phase contrast is considered (A) and unfiltered experimental images (D) are included.

We analyzed images both at 80kV and 300kV. The latter show less contrast compared to the lower voltage images. For both voltages, experimental data were in agreement with simulations.

For typical electron fluxes in cryo-EM we show that the influence of the solvent/specimen amorphousness (structural noise) can be neglected. Apparently Poisson noise is the dominant noise source in the image and the solvent can be modeled as a continuum medium.

InSilicoTEM could be used to predict the optimal experimental parameters and its modularity allows efficient and inexpensive investigation of the influence of new hardware components.

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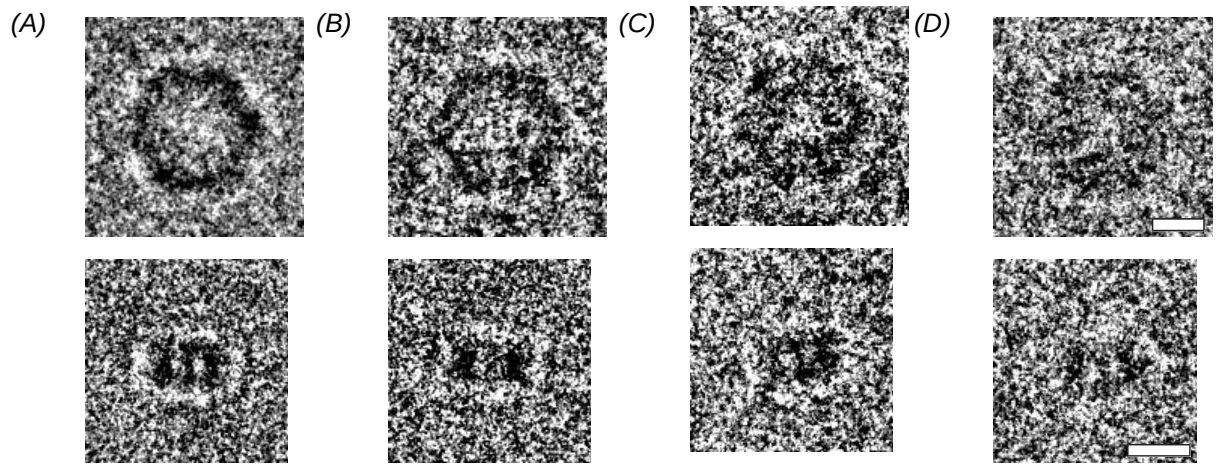

Figure 1. Influence of inelastic scattering. (A) simulations of pure phase contrast, (B) simulations with inelastic scattering, (C) experimental zero-loss filtered images, and (D) experimental unfiltered images. From top to bottom are presented earth worm hemoglobin (t_{exp}=1 s, Δf = 4918 nm, thickness d = 142 nm), and side view of 20S proteasome (t_{exp}=1 s, Δf = 6713 nm, d = 80 nm). Some acquisition and simulation parameters: Krios@80 kV, pixel size 0.135 nm, flux 2.5 $e^-/Å^2/s$, C_s 2 mm, C_c 2 mm, ΔE 0.7eV, illumination aperture 0.03 rad, energy slit 10 eV, conv. factor 20 ADU/pe^-, MTF@0.5Nq 0.223, DQE@0.5Nq 0.315, readout 3 ADU, dark current 0.11 ADU/s. The scale bars correspond to 10 nm.