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Image formation modeling in cryo-electron microscopy

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ABSTRACT

Accurate modeling of image formation in cryo-electron microscopy is an important requirement for quantitative image interpretation and optimization of the data acquisition strategy. Here we present a forward model that accounts for the specimen's scattering properties, microscope optics, and detector response. The specimen interaction potential is calculated with the isolated atom superposition approximation (IASA) and extended with the influences of solvent's dielectric and ionic properties as well as the molecular electrostatic distribution. We account for an effective charge redistribution via the Poisson–Boltzmann approach and find that the IASA-based potential forms the dominant part of the interaction potential, as the contribution of the redistribution is less than 10%. The electron wave is propagated through the specimen by a multislice approach and the influence of the optics is included via the contrast transfer function. We incorporate the detective quantum efficiency of the camera due to the difference between signal and noise transfer characteristics, instead of using only the modulation transfer function. The full model was validated against experimental images of 20S proteasome, hemoglobin, and GroEL. The simulations adequately predict the effects of phase contrast, changes due to the integrated electron flux, thickness, inelastic scattering, detective quantum efficiency and acceleration voltage. We suggest that beam-induced specimen movements are relevant in the experiments whereas the influence of the solvent amorphousness can be neglected. All simulation parameters are based on physical principles and, when necessary, experimentally determined.

1. Introduction

The structures of macromolecules, macromolecular complexes and subcellular assemblies provide insight into their functions. Knowledge of the 3D structure of a macromolecule is also the cornerstone for rational drug design (Wang et al., 2005).

Cryo-electron microscopy (cryo-EM) of biological specimens in an unstained, frozen-hydrated state has become an indispensable tool for structural biology (Sali et al., 2003). Advances in cryo-EM single particle analysis (SPA) (Frank, 2006) and cryo-electron tomography (cryo-ET) (Lučić et al., 2005; McIntosh et al., 2005; Leis et al., 2009) provide opportunities to characterize the structures of macromolecular complexes that are either too flexible, heterogeneous or transient to be explored by crystallographic methods (Henderson, 2004; Glaeser et al., 2006). The level of structural detail that can be obtained by cryo-EM is largely limited by specimen heterogeneity, the effective contrast transfer function (CTF), the detector's detective quantum efficiency (DQE), and radiation damage which limits the integrated electron flux that can be used, resulting in a poor signal-to-noise ratio (SNR) in images.

In addition to hardware developments, computational methods will continue to improve, enabling more information to be extracted from inherently noisy cryo-EM images. Simulations of electron images will be increasingly important in order to optimize the data acquisition strategy, to improve image interpretation and resolution, and to provide insight on ways to improve instrumentation. An accurate forward model of image formation in cryo-EM should rely on all relevant physical properties such as the specimen's elastic and inelastic scattering properties and the effects of the CTF and the detector.

Simulation of transmission electron microscope (TEM) images of biological specimens is implemented in a number of software packages for SPA and ET such as Xmipp (Sorzano et al., 2004; Bilbao-Castro et al., 2004), IMAGIC (van Heel et al., 1996), SPIDER (Frank and Shimkin, 1978; Shaikh et al., 2008), EMAN2 (Tang et al., 2007), Bsoft (Heymann and Belnap, 2007), and TOMToolbox

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(Nickell et al., 2005). In most cases, these simulations are used to facilitate Euler angles determination in SPA and to evaluate reconstruction methods for SPA (Marabini et al., 1998; Sorzano et al., 2001) and ET (Marabini et al., 1997). Usually a virtual model of a biological specimen is created using 3D primitives (phantoms) such as spheres, ellipsoids, cubes, and cylinders (Bilbao-Castro et al., 2004). In some cases, the specimen volume is constructed based on information from the RCSB Protein Data Bank (PDB) and TEM images are computed by projecting the 3D specimen; the effects of the solvent and detector are rarely accounted for. In general, projecting the 3D electron density distribution into a 2D image is not correct, since it does not represent the actual physical electron–specimen scattering properties (interaction potential). In addition, the noise is often simplified as being additive Gaussian noise. Below, we discuss two related work that aim to provide more realistic simulations.

In Hall et al. (2011), image simulations were performed to assess the attainable benefits of phase plates. The solvent (water) was treated explicitly via molecular dynamics (MD) simulations generating a box of amorphous water and a multislice approach was used to account for the specimen thickness and multiple scattering. The generated noise was Poisson distributed, but the detector response was not included. Unfortunately, the methods were not validated experimentally.

TEM-simulator (Rullgård et al., 2011) aims to provide accurate simulations based on physical principles. It was the first simulator whose results were compared to experimental data, albeit not in depth. There, the specimen thickness has been neglected, and low-pass filtering to a certain resolution exceedingly damps the interaction potential (IP). Although most simulation parameters described there are based on physical principles, a calibration protocol needs to be employed for some parameters that are phenomenologically introduced, leading to a situation where nuisance parameter tuning is required. Examples of such phenomenological parameters are amorphousness (granularity), absorption potential, as well as camera parameters such as the modulation-transfer function (MTF), detective quantum efficiency (DQE), and conversion factor. Furthermore, none of the aforementioned approaches have considered chemical bonding and/or interaction of the sample with solvent and ions. For completeness, it should be mentioned that recently Shang and Sigworth (2012) parameterized a function that describes the distribution of water molecules around a protein. In previous work the solvent was assumed to be water, instead of less dense vitreous ice, leading to possible artificial damping of the contrast between the protein (which has a higher density than water) and solvent.

For material science applications, numerous TEM simulators have been developed (reviewed by Kirkland, 2010). Many assume that the atoms of a specimen are periodically ordered which is not fulfilled for non-crystalline biological specimens. Some of the simulators, such as YAMS (Dinges and Rose, 1995; Müller et al., 1998) and SimulaTEM (Gómez-Rodríguez et al., 2009), have been used for image simulations of biological specimens. They do not assume that the specimen is periodic and although YAMS propagates the mutual coherence function through the specimen, a method more appropriate for treating the partial incoherence, only elastic scattering was assumed for biological specimens (Sorzano et al., 2001). In both simulators the specimen thickness and multiple scattering events were treated via a multislice approach (Cowley and Moodie, 1957), but inelastic scattering, the detector response, and solvent were ignored. In high resolution electron microscopy (HREM) the contrast in experimental images has been frequently reported to be much less, typically about a factor of three, than predicted by image simulation (Hytch and Stobbs, 1994; Boothroyd et al., 1995). It was suggested in Thust (2009) that this discrepancy, often called the Stobbs-factor, originates from neglecting the detector's MTF in image simulations.

Here we present, analyze and validate an image formation model in TEM based on physical principles. In addition to computing the 3D potential distribution where atoms are treated in isolation, the interaction redistribution potential due to the solvent, ions and molecular interactions is computed. Beam-induced motion and amorphousness of the vitreous ice are also addressed. For validation, comparisons between experiments and simulations were performed on cryo-embedded specimens. Some of the parameters such as defocus, astigmatism and camera properties are accurately estimated from experiments via available toolboxes (Vulović et al., 2010, 2012), without introducing nuisance parameters. The simulator presented here, InSilicoTEM, has been implemented in DIPimage (www.DIPlib.org), a MATLAB toolbox for scientific image processing and analysis, and is freely available for non-commercial use upon request.

2. Theory

Forward modeling approaches in cryo-EM describe the complex image formation process. Below, we will shortly outline our image formation model whose main ingredients are: the interaction potential, electron wave propagation and intensity detection by the camera. The Supplementary material (S.M.) provides a detailed description of all steps and approximations.

2.1. Interaction potential (IP)

The interaction between the incident electron wave and a macromolecule embedded in the surrounding medium is modeled as a sum of two interaction potential components: (1) "atom" contributions, i.e. the superposition of atomic potentials as if each atom was in isolation; and (2) "bond" contributions, i.e. the influence of the charge redistribution due to the solvent, ions and molecular interactions

$$\mathcal{V}^{\text{int}}(\boldsymbol{r}) = \mathcal{V}_{\text{atom}}(\boldsymbol{r}) + \mathcal{V}_{\text{bond}}(\boldsymbol{r}), \tag{1}$$

where $\boldsymbol{r} = (x,y,z)$ is the position of the electron wave. Since $\mathcal{V}_{\text{atom}}$ considers the specimen as a set of isolated atoms, we get $\mathcal{V}_{\text{atom}}(\boldsymbol{r}) = \sum_{j=1}^{m} \mathcal{V}_{Z_j}(\boldsymbol{r} - \boldsymbol{R}_j)$, where $\mathcal{V}_{Z_j}$ is the electrostatic potential of an isolated neutral atom with atomic number Z_j centered at $\boldsymbol{R}_j$. With the first Born approximation, such a potential can be written as the inverse Fourier transform of the electron scattering factor of the atom (Peng et al., 2004; Rullgård et al., 2011) (see Section 1.2 in S.M.).

The isolated atom superposition approximation (IASA) ignores the potential due to the charge redistributions, $\mathcal{V}_{\text{bond}}$, which accounts for the interaction with neighboring atoms, solvent and ions. As $\mathcal{V}_{\text{atom}}$ provides the most significant contribution to the scattering of the incident electron, this computationally convenient approximation provides a good starting point for initial interpretation of high-energy electron diffraction and microscopy experiments (Kirkland, 2010; Peng et al., 1996). Biological specimens are embedded in an amorphous solvent and the potential distribution depends also on the dielectric and ionic properties of the solvent. It seems appropriate to include the contribution of the solvent and ions modeled by $\mathcal{V}_{\text{bond}}$. This potential due to the charge redistribution can be accounted for via a continuum electrostatics approach (see 1.3 in S.M.), described by the solution of the linearized Poisson–Boltzmann equation:

$$-\epsilon_0 \nabla(\epsilon_r(\boldsymbol{r}) \nabla \mathcal{V}_{\text{bond}}(\boldsymbol{r})) = \rho_{\text{mol}}^{\text{bond}}(\boldsymbol{r}) + \rho_{\text{sol}}^{\text{bond}}(\boldsymbol{r}) - \alpha(\boldsymbol{r}) \sum_i \frac{q_i^2 n_i^0 \mathcal{V}_{\text{bond}}(\boldsymbol{r})}{k_B T}, \tag{2}$$