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Simulating realistic imaging conditions for *in situ* liquid microscopy

David A. Welch [a,*], Roland Faller [a], James E. Evans [b], Nigel D. Browning [c]

[a] Department of Chemical Engineering and Materials Science, University of California, Davis, CA, USA
[b] Pacific Northwest National Laboratory, Environmental Molecular Sciences Laboratory, Richland, WA, USA
[c] Pacific Northwest National Laboratory, Fundamental Computational Sciences Directorate, Richland, WA, USA

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ABSTRACT

In situ transmission electron microscopy enables the imaging of biological cells, macromolecular protein complexes, nanoparticles, and other systems in a near-native environment. In order to improve interpretation of image contrast features and also predict ideal imaging conditions ahead of time, new virtual electron microscopic techniques are needed. A technique for virtual fluid-stage high-angle annular dark-field scanning transmission electron microscopy with the multislice method is presented that enables the virtual imaging of model fluid-stage systems composed of millions of atoms. The virtual technique is exemplified by simulating images of PbS nanoparticles under different imaging conditions and the results agree with previous experimental findings. General insight is obtained on the influence of the effects of fluid path length, membrane thickness, nanoparticle position, defocus and other microscope parameters on attainable image quality.

1. Introduction

Environmental cell transmission electron microscopy is an imaging technique that enables the observation of *in situ* chemical and biological processes in real-time with nanometer or better spatial resolution [1,2]. This technique utilizes two thin membrane windows to create an environmental chamber that can be inserted into the high vacuum of a transmission electron microscope while maintaining a sample within a liquid or gas environment at ambient pressure. The original designs for *in situ* environmental cells used two aluminum windows each approximately 500 nm thick [3] whereas silicon nitride, silicon oxide, amorphous silicon and graphene windows are now routinely used at thicknesses less than 50 nm. In addition to permitting both atomic resolution imaging and *in situ* spectroscopy [4], the thinner membranes have given improved contrast and expanded the range of samples amenable to *in situ* microscopy with applications to the study of macromolecular protein complexes, whole cells, nanoparticles in aqueous and organic solutions, catalysis, and electrochemistry [5–10]. Nonetheless, challenges still remain today in acquiring atomic resolution imaging conditions for every sample imaged using *in situ* electron microscopy.

Virtual transmission electron microscopy (VTEM) [11,12] (aka image simulation) enhances interpretation of experimental electron microscope images through comparison with accurate multislice image simulations [11]. VTEM techniques for high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) have been shown to be so accurate as to replace the need for experimental calibration standards in compositional analysis [13]. Through VTEM, contrast features due to the environment, detector angles, specimen thickness, specimen orientation, defocus, aberrations, structural strain, interfaces, and composition have been determined [14–25]. As recent studies have demonstrated lattice fringe observations during *in situ* liquid experiments performed under BF-TEM or HAADF-STEM imaging modes [1,26,27], VTEM can provide insight into such high resolution contrast features observed *in situ*.

Unfortunately, simulations based on complete fluid-stage models are missing. To ensure the accuracy of image simulation for *in situ* experiments, a correct structural description of the specimen, surrounding fluid and membrane enclosure is required, and this complexity can lead to large computational times. Furthermore, simple convolution models are not sufficient for handling such large systems as the evolution of the fluid-stage beam probe must be fully treated [28,29]. Detailed, multislice simulations are therefore needed to accurately determine the image that results from the interaction of the electron beam with the entire fluid-stage system. In this paper, the first multislice simulations of explicit, million-atom models of an *in situ* fluid-stage are presented.

The virtual imaging technique described here is demonstrated by reproducing observations of galena (*i.e.* PbS) nanoparticles in the fluid-stage. A recent *in situ* liquid electron microscopy study following the aqueous growth of galena nanoparticles shows the

* Corresponding author. Tel.: +1 207 907 9883.
E-mail address: dawelch@ucdavis.edu (D.A. Welch).

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observation of (220) lattice fringes of small galena nanoparticles, indicating excellent fluid-stage resolution [1]. This finding is reproduced here, and a quantitative analysis reveals the influence of the fluid-stage holder and imaging conditions on image resolution. Window thickness, fluid path length, the position of the particle within the holder, defocus, and particle features are all shown to affect atomic resolution capabilities.

2. Methods

2.1. Atomistic model construction

Virtual fluid-stage electron microscopy requires a structural breakdown, at the atomic level, of the entire fluid-stage system in terms of both static (*i.e.* equilibrium positions) and dynamic (*i.e.* Debye–Waller factors [12]) components. Representative amorphous and liquid structural descriptions are needed for the silicon nitride windows and fluid path, respectively. These two structures contribute background signal and also influence the evolution of the beam probe. When a complete fluid-stage model is built from these three structures (see Fig. 1c of section 3.1), image simulation can be performed.

Amorphous silicon nitride windows (~60,000–600,000 atoms in size representing membrane thicknesses of 10–100 nm) are created by first generating a representative 224 atom unit cell of the amorphous material. Generation of that cell is done with a cool-from-the-melt simulation based on procedures [30] and potentials [31] described in the literature, using the GULP simulation software [32]. Cool-from-the-melt parameters include 100 ps of melting at 4000 K followed by 100 ps of cooling to 298 K at a constant cooling rate, using a constant timestep of 0.2 fs and an NVT ensemble at a density of 3.14 g/cm^3. Buckingham-ionic potentials coupled with QTaper and damped dispersion potentials are used [31]; no two-body taper or three-body effects are included in simulation. The generated amorphous Si_3N_4 unit cell can then be repeated through space as needed in order to create a box-shaped window membrane of realistic thickness. In order to remove residual translational periodicity created by this process, all atoms are given position shifts by calculating and applying, per Cartesian axis, a random distance between −0.1 and 0.1 nm. This distance range may seem large, but it is required in order to remove contrast features in the image due to translational periodicity. Also, it is important to note that multislice simulations, which use 2D atomic potential projections, are not as sensitive as may be expected to the fine details of the amorphous structure. Fine structural inaccuracies in this model for amorphous silicon

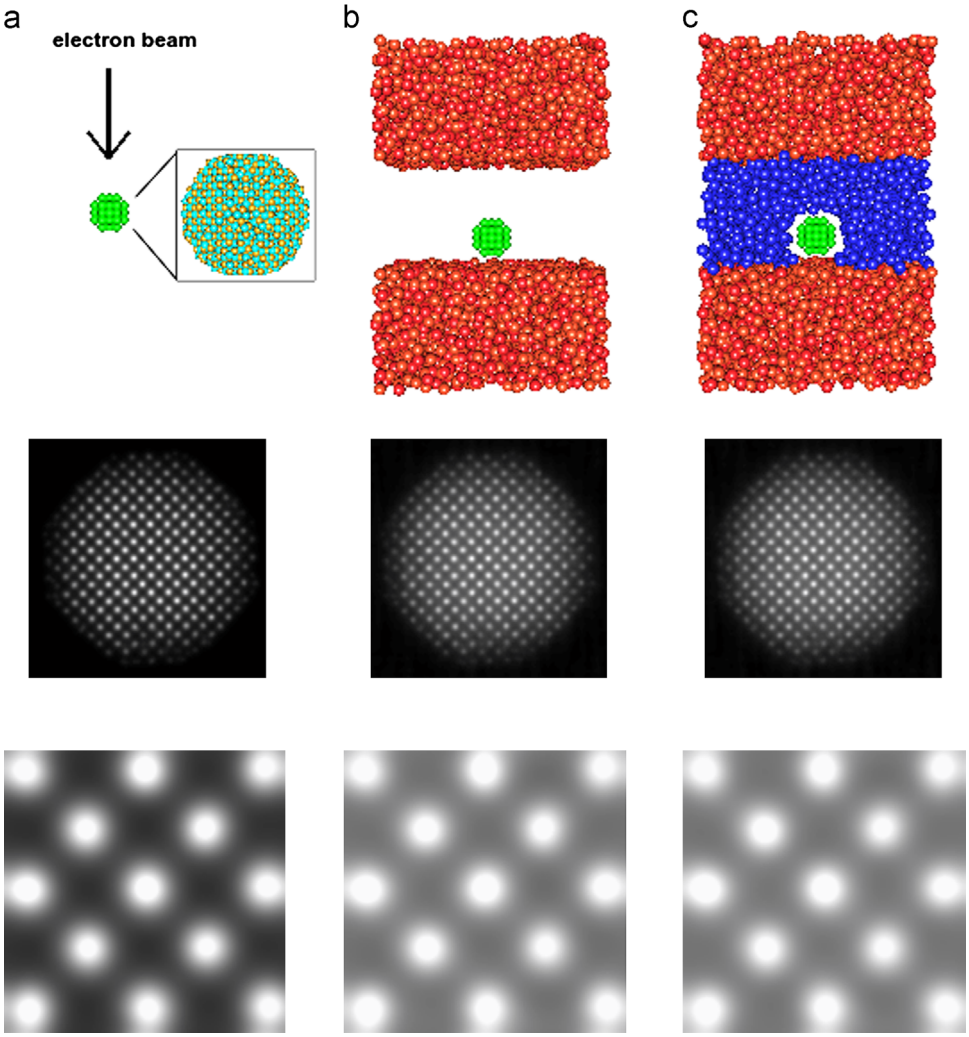

Fig. 1. Calculated HAADF-STEM images for a galena nanoparticle (a) in vacuum, (b) deposited at the bottom window of a gas-stage holder, and (c) deposited at the bottom window of a fluid-stage holder filled with water. For each system, a model of the system, the calculated nanoparticle image, and the calculated central 1×1 nm^2 area of the image (determined in a separate calculation) are shown. Atomic resolution is achieved in all cases. Window thickness and fluid/gas path length are 50 nm. Noise is not included.