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Simulating Realistic Imaging Conditions For In-Situ Liquid Microscopy

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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.

Keywords

Image simulation; atomistic model; in situ microscopy; nanoparticle growth

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],

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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 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 Figure 1c of Section 3.1), image simulation can be performed.

Amorphous silicon nitride windows (~60,000 to 600,000 atoms in size representing membrane thicknesses of 10 to 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 nitride are thus assumed to be insignificant to image simulation results, an assumption often used in image simulations involving amorphous material [21, 33]. A default Debye-Waller factor of $0.5 \text{ \AA}^2$ is assigned to all atoms in the window.

Creating the fluid path follows a very similar procedure. We assume that the entire fluid path can be represented with a large, amorphous structure. Additionally, Debye-Waller factors account for the mobility of water molecules, effectively creating the most fluid-like model as is possible with the current state of the image simulation code. Water-surface densification effects that occur at the window and particle surfaces, as discussed later, are assumed to be insignificant. To create the amorphous structure, room-temperature water simulation is performed with the SPC/Fw water model, an NVT box of 216 water molecules at a density of 1 g/cm^3 , an equilibration time of 100 ps, and a timestep of 0.5 fs [34]. The resulting water box is then repeated through space, in the same manner as with silicon nitride, as to create the full fluid path. Hydrogen atoms are not included in image simulation, as they are very weak scattering centers and can thus be ignored. To account for the high mobility of water on the timescale of STEM imaging, a large Debye-Waller factor of $10 \text{ \AA}^2$ is assigned to all oxygen atoms. This is an arbitrary value that is not physically intuitive; it is based on the nature of phonon sampling that is performed by the image simulation software [12]. The specific value of $10 \text{ \AA}^2$ is large enough as to effectively include water mobility effects during phonon sampling, and it is small enough as to not create peculiar structural results. It is worth noting that two additional simulations using Debye-Waller factors of $4 \text{ \AA}^2$ and $25 \text{ \AA}^2$ have been performed, and results do not significantly vary.

Modeling of the structure of a galena nanoparticle is based on experimental findings and is done by first constructing a spherical nanoparticle from an ideal, rock-salt lattice of galena [35]. A group of atoms are selected from the ideal lattice by taking all atoms within a maximum distance (i.e. radius) from an origin positioned at the center of a lead atom. Particle radii used are integer multiples of half the ideal near-neighbor distance. Next, the spherical particle is charge-balanced by random removal of ions from the particle surface. At this point in the particle construction process, atomic positions are still those of the ideal, bulk lattice. Molecular mechanics is now used to account for the galena particle's surface relaxation. An atomistic potential is thus derived for galena by fitting a Buckinghamionic potential [32] to the elastic constants of galena [36, 37] with GULP [32]. This potential (shown in supp. info.) is used with GULP to structurally relax the nanoparticle structure by means of energy optimization. Energy optimization techniques, which determine equilibrium structure, are used. For our image simulation, dynamic Debye-Waller factors specifically account for phonon effects, and the atomic structural input should therefore represent equilibrium geometry.

Accurate Debye-Waller factors (DWF) are required for quantitative description of the contributions of thermal diffuse scattering to HAADF-STEM images [12]. Molecular

dynamics simulation via GULP is used to calculate DWF as a function of ionic coordination number, which can then be used as input for image simulation. Determination of DWF (itself a function of mean square displacements of atoms from their respective equilibrium positions) is performed by first calculating the molecular dynamics trajectory of a structurally relaxed galena nanoparticle placed in vacuum, using room temperature conditions, a simulation time of 100 ps, and a timestep of 0.5 fs. DWF are then determined using 100 coordinate samples from that trajectory. The average DWF for a certain coordination number is determined by averaging the calculated DWF of all particle atoms having that coordination number. It should be noted that this coordination-based DWF analysis is an approximation that agrees with trends seen during trajectory analysis. It is meant to be a compromise between the two extremes of ignoring surface effects and defining a DWF for every atom in the specimen. To reduce simulation error, all calculated DWF are scaled by the same value such that the factor at full coordination (i.e. that of the bulk) matches the experimentally determined, dynamic DWF found in the literature for bulk PbS [38]. With our approach, the static DWF component should not be included in the DWF used in image simulation as the structural relaxation described above inherently includes static effects. DWF for lead ions and sulfur ions, in order of decreasing coordination number from 6 to 3, are 1.09, 1.63, 2.35, 3.71 and 0.60, 0.91, 1.19, 1.77 Å², respectively.

With the window model, fluid model, and specimen model defined, the complete fluid-stage model can be constructed. This step is a simple procedure in which these three components, either being box-like or sphere-like in shape, are positioned together in the manner expected for a fluid-stage system. The nanoparticle is positioned 0.5 nm from the top or bottom window membrane as to represent its deposition at these windows which is required in experiment in order to prevent particle flow/motion blur and achieve atomic resolution [1, 2]. Note that this requirement will likely be relaxed in future applications of dynamic transmission electron microscopy (DTEM) with the *in situ* fluid-stage [1, 39]. Nanoparticle orientation is set under zone axis conditions that result in the (220) lattice plane being observed (as in experiment [1]). No specimen tilt is included. In order to effectively make room in the fluid path for the particle, all oxygen atoms closer than a minimum distance to the PbS nanoparticle atoms are removed (this minimum distance is equal to the sum of the oxygen atom's ionic radius and the particle atom's ionic radius multiplied by 1.2 [32]). This overall approach leaves out component-component interaction effects such as water densification at the nanoparticle/window surfaces, but these effects are negligible when considering the relatively weak scattering properties of a very thin layer of water. More sophisticated models, however, may want to consider the change in particle surface structure due to hydration. Once the complete fluid-stage model is built, it ranges in atomic size from 200,000 to 1,600,000 atoms and consists of two $8 \times 8 \times A$ nm ($A = 5, 10, 30, 50$, or 100) silicon nitride membranes sandwiching a $8 \times 8 \times B$ nm ($B = 50, 100, 250$, or 500) fluid layer containing a structurally relaxed galena nanoparticle deposited at either the top or bottom window. This model can be used as input for image simulation software.

2.2. Image Simulation

HAADF-STEM image simulations are performed with the QSTEM image simulation software (V2.22), which was designed for obtaining quantitative HAADF-STEM images that include the effects of thermal diffuse scattering and inelastic scattering [12]. This software uses the multislice simulation technique which is required for the non-periodic system studied in this work [11, 28]. Microscope parameters for the 200 kV JEOL 2100F AC-STEM microscope used in the study of PbS nanoparticle growth are used in simulation. These are $C_s = 0.01$ mm, $C_c = 1.8$ mm, $dE = 1.5$ eV, $\theta = 30$ mrad, and HAADF inner detector angle = 100 mrad. Additional, approximate parameters used in simulation meant to include effects not well understood for this microscope are $C_5 = 3.2$ mm and source size =

0.1 nm. The outer detector angle, limited by the maximum scattering angle in simulation, is 167.2 mrad. Unless otherwise stated, the defocus plane is set to the optimal value of 4 nm below the center of the nanoparticle. Slice thickness is 0.14706 nm, which is about half the interplanar spacing of the nanoparticle's lattice planes along the beam axis. Image pixel size is 0.05×0.05 nm. Quantitative analysis is performed with an assumed brightness of 1.5×10^{10} A/cm²sr and pixel dwell time of 2 μ s. Beam energy spreading that takes place within the specimen has not been exactly accounted for in the HAADF-STEM image simulation technique, and the influence of energy filtering on image intensity has thus not been considered.

Slice positioning must be performed with care, especially with image simulation techniques that divide the projected potentials of single atoms between multiple slices. As the nanoparticle is not located at the same position in the fluid-stage holder over the various calculations we present, we have to ensure that the multislice simulation's 2D potential slices still divide the nanoparticle at the same positions relative to the particle's structure. This is crucial as changing the slice position (i.e. a potential z-offset) has been found to significantly influence the calculated contrast of the nanoparticle image (background signal, however, does not change). Hence, especially when performing comparative calculations, the slices must be consistently defined according to the specimen in terms of both slice thickness and slice position.

The simulation window size for almost all statistically analyzed images is 2.56×2.56 nm (i.e. 512×512 pixels with 0.005 nm point sampling, which is found to give sufficient convergence). This window size is not sufficient, however, for simulations involving large fluid path lengths (≥ 250 nm) where the nanoparticle is specifically deposited at the bottom window. With these tests, a great extent of beam spreading occurs before the beam interacts with the nanoparticle, and a larger simulation window size is required in order to describe the entire beam-particle interaction. A simulation window size of 5.12×5.12 nm (i.e. 1024×1024 pixels) is instead used for those tests. Note that image simulation, in general, is limited in its ability to accurately describe electron diffraction. One significant limitation is that the scattering of electrons out of (not into) the detector's annular range is not accounted for. Due to these ignored signal attenuation effects, the calculated effect of the *in situ* environment on image formation is only approximate. This simulation error can be expected to be much more significant as system thickness increases.

2.3. Analysis

The goal of this paper is to compare image quality under a variety of conditions, and the following statistical analysis is used to specifically give the most accurate image comparisons. All images are averaged over 20 phonon configurations [12, 24, 40], which gives sufficient convergence in contrast. Image comparisons are then performed by comparing calculated image patterns, particle signal-to-noise (SNR), and atomic SNR. Particle SNR is determined by averaging the the five image pixels with greatest intensity, and the Poisson noise characterizing the image is then determined by taking the square root of this average. Five pixels are used as it will be shown that there are five atomic columns at the center of the particle image, and therefore, there are at least five local contrast maxima in every calculated image. Image background intensity is determined with a separate calculation on a fluid-stage cell in which no specimen is present, taking the resulting minimum-intensity pixel as the background intensity value. Only one phonon configuration need be sampled to determine this background intensity. Atomic SNR is determined from the peak-valley contrast difference within a diagonal linescan across the image (note: this is also the linescan across near-neighbor atomic columns). It will be shown that there are three peak-valley contrast differences within a line-scan that are to be averaged. Presented linescans are not background subtracted. Any image pixels within two pixels from an edge

of a calculated image are not used in statistical analysis as to avoid errors from non-periodic image boundaries. Oversampling techniques are only used in the generation of images for this paper, not in statistical analysis. Given this statistical approach and the analysis of ten repeated simulation runs for the simulation showing the greatest particle and atomic SNR in this work, an error bar of ± 0.13 and ± 0.35 (std) can be assigned to all calculated particle and atomic SNR, respectively.

3. Results

3.1. Demonstration of Virtual Imaging for In Situ Stages

In situ stage design has been aimed at making the environmental chamber as electron transparent as possible while withstanding the vacuum environment of the electron microscope [41]. By optimizing electron transparency, the loss in signal-to-noise (SNR) is minimized thereby enhancing both attainable contrast and resolution. In this study, various fluid-stage holders consisting of silicon nitride windows separated by spacers of different height are considered. Relationships between cell design and resulting SNR have been determined with image simulation.

Calculated virtual images for a spherical 5 nm galena nanoparticle under vacuum conditions, within a gas-stage holder consisting of two window membranes, and within a fluid-stage holder consisting of two window membranes and liquid water are shown in Figure 1. Window membrane thickness is 50 nm as in the experimental study of galena nanoparticle growth [1], and gas/fluid path length is 50 nm. Atomic resolution is observed in all cases with quantitative contrast features depending on the environment. It is also found that calculating just the central $1 \times 1 \text{ nm}^2$ area of the nanoparticle image gives all the information needed for determination of particle and atomic SNR. Imaging only this central area reduces the required computation time by a factor of 36, and this saved time can in turn be used to carry out more accurate simulations through the use of a more detailed and computationally expensive virtual beam probe.

3.2. Effect of Window Thickness

Window thickness has a very significant influence on image quality. Figure 2 shows virtual images obtained with window thicknesses in the range of 5 to 100 nm in a holder containing a 100 nm fluid path length and a 5 nm spherical galena particle deposited at the top window. Results for the vacuum condition (i.e. an isolated nanoparticle, shown as window thickness = 0 nm in Figure 2), which are expected to be similar to results obtained for deposition on a thin membrane, are included. Over all tested conditions, image interpretation does not change; contrast maxima are clearly located at the corresponding position of atomic columns.

Although image interpretation does not change, very significant relationships between window thickness and particle/atomic SNR exist, and they resemble exponential decay. As window thickness increases, the particle/atomic SNR is reduced due to increasing Poisson noise and decreasing specimen signal. Comparing the results for a fluid-stage holder with 50 nm windows to the results in vacuum, the use of a fluid-stage holder causes particle SNR (i.e. particle resolution) to decrease by a factor of 1.6 and atomic SNR (i.e. atomic resolution) to decrease by a factor of 2.1. As window thickness increases, atomic resolution relatively decreases much more than does particle resolution. This loss in atomic resolution is mostly due to decreasing image contrast maxima, but image contrast minima do significantly increase as well.

At the lowest window thickness of 5 nm, both particle and atomic SNR are only 7% lower than that found under vacuum conditions. This is a promising result for the potential

resolution capabilities of the fluid-stage. Note that these results do not include bowing effects [42, 43], which effectively increase the fluid path length to an extent that depends on the holder's window dimensions. An increase in fluid path length always decreases particle/atomic SNR, as discussed in Section 3.3.

3.3 Effect of Fluid Path Length

Fluid path length can significantly influence image quality. Calculations have been performed for fluid-stage holders with 50 nm thick windows and fluid path lengths of 50, 250, and 500 nm and a 5 nm spherical galena particle deposited at either the top or bottom window. Qualitative image contrast features are the same as those found in the previous section; image interpretation does not change.

On the other hand, atomic SNR is sensitive to fluid path length. Atomic SNR is found, for a particle deposited at the top window, in order of increasing fluid path length, to be 14.2, 12.8, and 12.0. Increasing Poisson noise is mainly responsible for this reduction in SNR. It should be noted that the simulations do not accurately account for attenuation of the HAADF signal (i.e. scattering of electrons specifically out of, not into, the detector's annular range) caused by the fluid path and windows. Hence, the given values for atomic SNR are likely overestimated. For a particle deposited at the bottom window, atomic SNR is found, in order of increasing path length, to be 12.5, 7.7, and 4.4. With the large fluid path length of 500 nm, the atomic SNR is three times smaller when the particle is deposited at the bottom window. Bottom-window deposition always results in reduced SNR due to a poorer specimen signal. Current simulation findings suggest that particle SNR follows a similar trend.

These findings can be understood in terms of the evolution of the electron beam probe. The result that the fluid path length influences resolution much more when the particle is deposited at the bottom window suggests that the influence of pre-specimen beam spreading is more significant to image quality than post-specimen beam interactions with amorphous or liquid material. Specimen images can thus be expected to have much better quality when the specimen is deposited at the top window, with the relative difference increasing with an increasing fluid path length. Bowing effects likely further increase this relative difference.

3.4. Effect of Defocus

The relationship between defocus and image quality is shown in Figure 3 for defoci ranging from $f = -20$ nm to $f = 12$ nm (f is defined relative to the center of the nanoparticle with a positive defocus indicating a defocus plane above the center). Images were calculated with 50 nm window thickness, a 50 nm fluid path length, and a 5 nm spherical nanoparticle deposited at the top or bottom window. Out of the nine defoci tested, the optimum defocus is found to be $f = -4$ nm. This is the same optimum defocus as found for a galena nanoparticle in vacuum, indicating that the fluid-stage environment does not significantly change the optimum defocus.

Defocus plays a very critical role on image quality. A defocus that is 8 nm away from optimal conditions results in an atomic SNR that is about half the optimal result. This can be seen in the calculated images as the apparent development of signal intensity within the electron channels, and this is mostly due to decreasing contrast maxima (see linescans). Particle SNR (not shown), however, is reduced by a much smaller factor of 1.2. As expected, atomic resolution capabilities are more at risk than detection limits when a poor defocus is used for imaging. At a defocus that is 16 nm away from optimal, atomic SNR is reduced by a factor of 9, and the image formed is hard to interpret. Chances of observing atomic-level detail in the fluid-stage microscope are thus highly dependent on the defocus

being close to optimal. Virtual simulations can help identify the ideal defocus conditions needed in an *in situ* experiment.

It is interesting to note that, over each defocus series, variation in the average pixel intensity (i.e. the average intensity over all pixels in the image) is at least nine times smaller than the variation in specimen signal. In other words, defocus does not significantly influence the average contrast over the entire image. This is in concurrence with the notion that integrated intensities can be effective for quantitative comparisons between simulation and experiment when defocus is not precisely known [29].

3.5. Effect of Particle Size

The influence of particle size on particle/atomic SNR has been determined. Simulations of a fluid-stage holder having 50 nm window thickness and 50 nm fluid path length have been carried out for top-window deposited spherical nanoparticles with sizes of 1.3, 1.8, 2.3, 3.8 and 5.0 nm. In all tests, the defocus plane is set such that atoms located at the top of the nanoparticle are at Scherzer defocus. Particle SNR (see Section 2.3 for its statistical definition) is found to observe a quasilinear relationship with particle size, increasing from 6 to 26. Assuming the Rose criterion for particle observation (i.e. $\text{SNR} \geq 5$), this suggests a detection limit for spherical galena nanoparticles of ~ 1 nm. This is in good agreement with the smallest galena nanoparticles previously observed in experiment [1]. Atomic SNR also shows a quasilinear relationship, increasing from 2 to 14. In order to achieve an atomic SNR greater than 5, a particle size greater than ~ 2 nm is required.

Considering the statistical relationship between atomic SNR and particle size, linear fitting returns an atomic SNR of -2.5 when the particle size is extrapolated to 0. This negative SNR can be partially attributed to the inclusion of surface relaxation effects and variable Debye-Waller factors in the model for the galena nanoparticle. Atomic SNR is highest when contrast contributions are made by highly ordered atomic columns in a specimen. Atoms in the surface layer of the nanoparticle, however, contribute variable HAADF-STEM intensities at off-column positions. Hence, surface layers give significantly less contribution to atomic SNR than bulk-like internal layers of the particle, and the fraction of the particle atoms that are at the surface increase as particle size decreases. Thus, a linear fit extrapolated to zero particle size should give a negative SNR. This suggests that inclusion of surface effects in the simulation model can be significant to the calculation of resolution limits.

4. Conclusions

Much work remains to be done in improving the efficiency of the presented image simulation technique. Although complicated beam spreading effects induced by atoms above the specimen are very significant to image quality, the influence of atoms below the specimen only slightly influence image contrast features. Very little information is gained by the post-specimen part of the multislice simulation, and a simple mathematical treatment to account for post-specimen beam interactions may be more practical and possibly even more accurate. This could greatly reduce the size of the simulation problem and permit researchers to more rapidly investigate how slight variations in experimental setup will affect downstream imaging quality. Additionally, the design of structural atomistic models used in image simulation is an ongoing subject of study.

Nonetheless, the virtual simulations of galena nanoparticles in the fluid-stage presented here have already determined many relationships important to the achievement of atomic resolution in the *in situ* environments provided by a fluid-stage holder:

1. With an aberration-corrected microscope, the (ideal) fluid-stage holder does not change image interpretation; image quality degradation can be completely described in terms of reduced SNR. Virtual simulations have determined that reduction in SNR, thus providing a means for predicting the resolution capabilities of *in situ* electron microscopy. It has been predicted that the (220) lattice fringes of PbS are able to be resolved in the fluid-stage with good particle/atomic SNR, agreeing with experimental findings.
2. Particle SNR and atomic SNR decrease with increasing window thickness. This dependency on window thickness must be taken into account when designing fluid-stage experiments, as the fluid-stage environment may not provide adequate resolution. Holders particularly consisting of thick window membranes cause a great loss in resolution. In the case of holders with small window thickness (i.e. ≤ 10 nm), however, atomic resolution can be very comparable to that obtained with deposition on a thin membrane.
3. Images are always of higher quality when the specimen is deposited at the top window as opposed to the bottom window. Beam spreading effects, which reduce image quality, are much more significant when the particle is deposited at the bottom window. This reduction in image quality is more significant at larger fluid path lengths.
4. Defocus plays a major role in atomic resolution, and defocus optimization may be the most significant factor in the quality of fluid-stage images. Small deviations in defocus from optimal conditions can greatly reduce atomic resolution capabilities.
5. Particle SNR and atomic SNR increase quasilinearly with particle size. Minimum particle sizes for particle observation and atomic resolution have been calculated. Additionally, surface effects appear to have a significant influence on calculated contrast. Therefore, future simulations, particularly of small particles, should take into account surface relaxation and variable Debye-Waller factors.

It must be recognized that not all effects that degrade image resolution have been considered in this study. Additional effects that must be considered on a per system basis are: specimen stability, specimen motion and orientation, charging effects, window defects, bubble formation, electrolyte impurities, and thickness effects not accurately accounted for in image simulation. Although atomic resolution has been shown to be possible for some specimens of interest, the limitations on *in situ* imaging caused by these effects are not yet fully understood.

Fluid-stage microscopy is engaged in an ongoing, evolutionary process with the design of its sample holders. Hence, the described virtual technique can work in parallel with the design of new fluid-stage holders to understand various implications for image quality including resolution limits, detection limits, general contrast features (i.e. the overall structure-image relationship), and complicated chemical events unique to *in situ* microscopy such as beam-initiated reaction kinetics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Image simulation has been performed to understand *in situ* electron microscopy experiments.
- Experimentally observed resolution of *in situ* grown PbS nanoparticles has been virtually reproduced.
- General relationships between image resolution and *in situ* holder design, defocus, and particle size have been determined.
- The presented image simulation technique can predict the obtainable resolution of future experiments.

Figure 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 $1 \times 1 \text{ nm}^2$ 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.

Figure 2.

Images formed with variable window thicknesses ((a) = 0 nm/vacuum, (b) = 10 nm, (c) = 30 nm, and (d) = 50 nm) along with corresponding diagonal linescans. Particle/atomic SNR decreases with increasing window thickness. Fluid path length is 100 nm.

Figure 3. Relationship between defocus and resolution. Calculated images are shown for a defocus of (a) optimal defocus [-4 nm], (b) -12 nm, and (c) -20 nm. Corresponding diagonal linescans are shown. Calculations are for a holder with 50 nm window thickness and 50 nm fluid path length and a 5 nm spherical galena particle deposited (unless otherwise stated) at the top window. Note that random simulation error in atomic SNR is ± 0.35 .