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Nanometer-resolution electron microscopy through micrometers-thick water layers

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Abstract

Scanning transmission electron microscopy (STEM) was used to image gold nanoparticles on top of and below saline water layers of several micrometers thickness. The smallest gold nanoparticles studied had diameters of 1.4 nm and were visible for a liquid thickness of up to 3.3 μm . The imaging of gold nanoparticles below several micrometers of liquid was limited by broadening of the electron probe caused by scattering of the electron beam in the liquid. The experimental data corresponded to analytical models of the resolution and of the electron probe broadening, as function of the liquid thickness. The results were also compared with Monte Carlo simulations of the STEM imaging on modeled specimens of similar geometry and composition as used for the experiments. Applications of STEM imaging in liquid can be found in cell biology, e.g., to study tagged proteins in whole eukaryotic cells in liquid, and in materials science to study the interaction of solid:liquid interfaces at the nanoscale.

1. Introduction

The ultrastructure of cells has traditionally been studied with transmission electron microscopy (TEM) achieving nanometer resolution on stained and epoxy/plastic embedded thin sections, or on cryo sections [1–3]. Disadvantages are that the cells are not in their native liquid state and not complete. TEM imaging of whole, vitrified cells is possible, but restricted to the very edges of a cell. Ever since the early days of electron microscopy it has been a goal to achieve nanometer resolution on whole cells in liquid [4]. Two scientific advances from the last decade, the introduction of gold nanoparticles serving as specific protein labels [5] and the development of silicon nitride membranes used as electron-transparent windows in a liquid compartment [6], have led to the introduction of a novel

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concept to achieve nanometer resolution on tagged proteins in eukaryotic cells [7]. A liquid specimen is placed in a micro-fluidic compartment with electron-transparent windows (Fig. 1) and imaged with a scanning transmission electron microscope (STEM) using the annular dark field (ADF) detector. The contrast mechanism for imaging with the ADF detector is sensitive to the atomic number Z of the specimen [8], which can be used to image high- Z nanoparticles in thick solid- [9], or liquid samples [7]. Nanoparticles specifically attached to proteins [5] can then be used to study protein distributions in whole cells in liquid [7], similar as in fluorescence microscopy, where proteins tagged with fluorescent labels are used to study (dynamic) protein distributions in cells [10]

Here, we report on the resolution achievable with STEM imaging in liquid depending on the vertical position z of the nanoparticle and on the thickness T of the liquid. Two specimen configurations can be distinguished. 1) The nanoparticles are located in the top layer of the liquid with respect to the electron beam entrance, such that imaging occurs with an unperturbed electron probe. 2) The nanoparticles are at a position z deeper in the liquid, for which beam broadening plays a role. We measured the resolution on gold nanoparticles placed above and below water layers with $1.3 < T < 13 \mu\text{m}$, representing a size range from bacterial cells to eukaryotic cells. We describe a theoretical model of the resolution and present Monte Carlo simulations of the STEM experiments. The Monte Carlo simulations were used to calculate the resolution achievable on gold nanoparticles in the middle of the liquid as function of z for $T = 5 \mu\text{m}$.

2. Experimental

A series of test samples was imaged with STEM to determine the resolution of imaging gold nanoparticles in a saline water layer. The micro-fluidic compartments for liquid STEM imaging consisted of two silicon microchips supporting silicon nitride windows. The windows had dimensions of $50 \times 200 \mu\text{m}$, or $70 \times 200 \mu\text{m}$, and 50 nm thickness (Protochips Inc, NC). The microchips were made hydrophilic by coating with poly L-lysine. Gold nanoparticles of average diameters of $d = 1.4, 5, 10, 30$, and 100 nm were applied from solution, by applying a droplet of about $0.5 \mu\text{l}$ with a micropipette. Different liquid compartments were made with polystyrene microspheres in the range of 2 to $10 \mu\text{m}$ diameter, serving as spacers and thus forming a micrometer-sized gap of defined thickness between the windows. The chips were loaded in the STEM using a fluid specimen holder (Hummingbird Scientific, WA) [7]. A flow of 10% phosphate buffered saline (PBS) in water with a flow rate of $1\text{--}2 \mu\text{l/minute}$ was used. The STEM (CM200, Philips/FEI company, OR) was set to 200 kV , a probe current I = between 0.47 and 0.58 nA (the probe current was different after a tip change; for the calculations we used the average of 0.53 nA), and a beam semi-angle $\alpha = 11 \text{ mrad}$. Under these conditions the theoretical probe size containing 50% of the current is $d_{50} = 0.5 \text{ nm}$ [11]. The real probe size was estimated to be $d_{50} = 0.6 \text{ nm}$. ADF detector (Fishione Instruments) semi-angles of $\beta = 70$ and 94 mrad were used. The outer angle β_2 was 455 and 611 mrad for $\beta = 70$ and 94 mrad , respectively (for the Monte Carlo calculations a value of 500 mrad was used). Image processing was done with imageJ. Contrast and brightness were adjusted for maximum visibility, and a convolution filter with a kernel of $(1, 1, 1; 1, 5, 1; 1, 1, 1)$ was applied to reduce the noise for the STEM images, while the data analysis via line scans and the resolution determination was performed on the original, unfiltered data.

3. Results and Discussion

3.1. STEM imaging of gold nanoparticles on top of a water layer

Fig. 2a shows a STEM image of gold nanoparticles with $d = 1.4 \text{ nm}$ on top of a water layer. The image was recorded at the edge of a $50 \times 200 \mu\text{m}$ window in a flow cell made with a

spacer of 5 μm polystyrene microspheres. For this particular sample the nanoparticles were positioned onto the vacuum side of the window to prevent the particles from dissolving in the liquid during imaging, which occurred at the higher magnifications, here 480,000. Remark that nanoparticles only dissolved in the liquid during imaging at the highest magnification range in this study. The gold nanoparticles are visible as bright yellow spots on a blue background and are individually resolved. Several nanoparticles were selected that were visible above the background noise. A line-scan over one of the smallest nanoparticles (Fig. 2b) had a full-width-at-half-maximum (FWHM) of $d_{\text{FWHM}} = 1.5 \text{ nm}$ of the peak above the background level. The background level was determined from the average signal of horizontal positions 0–10 nm. The signal-to-noise ratio (SNR) was 5.3, as determined from the ratio of the peak height (the maximal signal – the average of the background signal) and the standard deviation of the background. The average of five of the smallest nanoparticles gave $d_{\text{FWHM}} = 1.2 \pm 0.2 \text{ nm}$ (the error is the standard deviation) for $SNR = 5 \pm 1$. The factor of 5 is consistent with the Rose criterion [12–14] stating that $SNR \geq 5$ for pixels to be visible in an image containing background noise. T was calculated to be 3.1 μm from the measured fraction $N/N_0 = 0.26$ (and using Eq. (1) as described elsewhere [7]). As a second measurement of T we recorded an image at a lower magnification, with nanoparticles at the bottom window visible, and an image with the sample tilted by 24.6° (Fig. 2c). From the parallax equation it followed that $T = \Delta L / \sin \phi = 3.3 \pm 0.3 \mu\text{m}$. From measurements of 11 different positions on a total of 6 different samples it was found that both methods to determine T were equal within a standard deviation of 20%. The value of 3.3 μm is smaller than the diameter of the polystyrene microspheres of 5 μm used for spacing; presumably compression of the beads and/or deformation of the chips occurred. STEM imaging at the position of a corner of this window resulted in $d_{\text{FWHM}} = 1.0 \pm 0.3 \text{ nm}$ for $SNR = 5$ and $T = 1.7 \mu\text{m}$.

To study the imaging in thicker water layers, a flow cell was made with 10 μm polystyrene microspheres. Gold nanoparticles of 5, 10 and 30 nm were placed from solution at the liquid side (thus inside the flow cell during STEM imaging) of a $70 \times 200 \mu\text{m}$ large top window. The 70 μm wide silicon nitride windows were found to bulge outward into the vacuum by a maximum of 3 μm per window (the bulging was 1 μm for the 50 μm wide windows). The liquid STEM image obtained at a position in the middle of the window is shown in Fig. 2d, with $T = 13 \mu\text{m}$ (from $N/N_0 = 0.50$), from 6 μm total bulging, and compression of the 10 μm microsphere spacer. The images for this sample were recorded at a lower magnification than for Fig. 2a, and the gold nanoparticles, now in contact with the liquid, remained at their positions during imaging. The detector was set to $\beta = 94 \text{ mrad}$ for optimal visibility of the nanoparticles in the background signal. Gold nanoparticles of $d = 10$ (e.g., at arrow #3) and 30 nm are clearly visible above the noise. At the arrow #4 a smaller nanoparticle is just visible with $d_{\text{FWHM}} = 4 \text{ nm}$ and $SNR = 4$. The average of 8 of the smallest nanoparticles (some outside the cropped area shown in Fig. 2d) gave $d_{\text{FWHM}} = 5 \pm 1 \text{ nm}$ for $SNR = 5 \pm 1$. Further images were recorded with $\beta = 94 \text{ mrad}$ at the edge of the window of a third sample. The average of 4 of the smallest nanoparticles gave $d_{\text{FWHM}} = 3 \pm 1 \text{ nm}$ for $SNR = 5$. The determined thickness was $T = 7.5 \mu\text{m}$. In our prior work we demonstrated a resolution of 4 nm for 10 nm gold nanoparticles in the top layer of 7 μm of water [7], consistent with the results presented here within the error margin. Note that the 20–80% rising edge width r_{20-80} was used in this previous work as measure of the resolution and not the d_{FWHM} , because the nanoparticles used in that study were larger (10 nm diameter) than the minimum detectable size, and for that situation the r_{20-80} is the most accurate measure of the resolution.

3.2. Theoretically achievable resolution of STEM imaging of nanoparticles in the top layer of a liquid

The number of electrons N scattered into the ADF detector, i.e., by an angle larger than β , is calculated from the partial cross section for elastic scattering $\sigma(\beta)$ as [15]:

$$\frac{N}{N_0} = 1 - \exp\left(-\frac{T}{l(\beta)}\right), l(\beta) = \frac{W}{\sigma(\beta)\rho N_A}, \quad (1)$$

with the number of incident electrons N_0 , mean-free-path length for elastic scattering $l(\beta)$, mass density ρ , the atomic weight W and Avogadro's number N_A . For the screened Rutherford scattering model based on a Wentzel potential the partial cross section for elastic scattering is given by [15]:

$$\sigma(\beta) = \frac{Z^2 R^2 \lambda^2 (1 + E/E_0)^2}{\pi a_H^2} \frac{1}{1 + (\beta/\theta_0)^2}; \quad (2)$$

$$E_0 = m_0 c^2; \lambda = \frac{hc}{\sqrt{2EE_0 + E^2}}; \theta_0 = \frac{\lambda}{2\pi R}; R = a_H Z^{-1/3}; E = Ue, \quad (3)$$

with electron accelerating voltage U (in V), atomic number Z , a_H the Bohr radius, m_0 the rest mass of the electron, c the speed of light, h Planck's constant, and e the electron charge. For the gold nanoparticles, $l_{\text{gold}} = 73.2$ and 122 nm for $\beta = 70$ and 94 mrad, respectively. From the quadratic average Z of water of $\sqrt{(2/3 \times 1^2 + 1/3 \times 8^2)} = 4.69$ (close to experimental estimates [16]), it follows that $l_{\text{water}} = 10.4$ and 18.5 μm , for $\beta = 70$ and 94 mrad, respectively [7]. Here, inelastic scattering, typically occurring at smaller angles is neglected. Multiple scattering is also neglected.

The value of d_{FWHM} represents the noise-limited resolution for the configuration of nanoparticles in the top layer of the liquid, where the electron probe is smaller than the nanoparticles. The contrast is generated from the difference between the number of detected electrons N_{signal} for a pixel with the electron beam at the position of a nanoparticle and the background signal N_{bkg} . Both are given by [7,17]:

$$\frac{N_{\text{signal}}}{N_0} = 1 - \exp\left[-\left(\frac{d}{l_{\text{gold}}} + \frac{T-d}{l_{\text{water}}}\right)\right], \frac{N_{\text{bkg}}}{N_0} = 1 - \exp\left[-\frac{T}{l_{\text{water}}}\right]. \quad (4)$$

Following the same definition of the SNR as used for the experimental data, assuming 100% detection efficiency, assuming $N_{\text{signal}} \cong N_{\text{bkg}}$, and assuming Poisson statistics the SNR can be approximated by:

$$SNR = \frac{N_{\text{signal}} - N_{\text{bkg}}}{\sqrt{N_{\text{bkg}}}} \quad (5)$$

The SNR should be at least 5 to be able to detect one pixel in a noisy image [12–14]. Eq.(5) was solved numerically (Mathematica, Wolfram Research, Inc.) for 6.6×10^4 electrons per pixel yielding d , the smallest nanoparticle visible for $SNR = 5$ [12]. Fig. 3 shows that the

calculated resolution is somewhat higher (values of d lower) than the experimental resolution. Yet, the values agree within the experimental error margin, except for a small mismatch at the largest water thickness. From this agreement it can be concluded that the model of a noise-limited resolution is valid, and that inelastic scattering and multiple scattering can be neglected for the STEM imaging of gold nanoparticles on top of a water layer of several micrometers thickness. The thicker liquids are preferentially imaged with $\beta = 94$ mrad, while there is no preference for either 70, or 94 mrad for $T < \sim 7 \mu\text{m}$. The calculation can possibly be improved by using a more precise model for scattering than the Rutherford model. An analytic expression of the resolution can be derived for $T \ll l_{\text{water}}$ and $d \ll l_{\text{gold}}$, such that the Taylor expansion of Eq. (5) yields:

$$d = 5 l_{\text{gold}} \sqrt{\frac{T}{N_0 l_{\text{water}}}}. \quad (6)$$

Note that we used a different definition of the SNR in our previous work [7], resulting in a factor of $\sqrt{2}$ larger values of d obtained with Eq. (6). As can be seen in Fig. 2d Eq. (6) fits the experimental data for smaller T , but becomes inaccurate for $T > \sim \frac{1}{4} l_{\text{water}}$. This equation may serve as a first estimate of the noise-limited resolution of STEM on heavy nanoparticles on a thick layer of a light material.

3.3. Electron dose

For biological applications the electron dose should be taken into account. Most images were recorded at magnifications $\leq 160,000$ for which the pixel size was 0.87 nm or smaller. With $d_{50} = 0.6$ nm and a pixel time of 20 μs , the electron dose was approximately 1.0×10^5 electrons/ nm^2 , which is a factor of 4 smaller than the limit of radiation damage used for conventional thin sections containing fixed cells [18], an order of magnitude larger than the radiation limit used for the imaging of cells in cryosections [19], and two orders of magnitude larger than the radiation limit used for single particle tomography on samples in amorphous ice [20]. The radiation limits for STEM imaging in liquid are not yet determined; we found a dose of 7.0×10^4 electrons/ nm^2 to be compatible with the imaging of gold nanoparticles used as molecular labels on fixed cells in flowing saline water [7]. For the highest magnifications used in this study, i.e., Fig. 2a with 480,000, the pixel size was 0.3 nm, considerably smaller than d_{50} , and thus the electron dose for those experiments was 8.0×10^5 electrons/ nm^2 , too high even for conventional thin sections. We found nanoparticles to dissolve in the liquid at this electron dose, while they remained on their support for the electron dose of 1.0×10^5 electrons/ nm^2 .

From Eq. (6) follows that the achievable resolution depends via N_0 on the specific radiation limit of the sample under investigation. In case the pixel size is larger than the probe size, the settings of the microscope can be optimized to achieve a reduced electron dose by increasing the probe size of the STEM via either increasing, or decreasing the semi-angle of the electron probe. A second way to optimize for low-dose imaging is by applying noise filtering, and/or using pattern recognition, such that nanoparticles can be discerned from the noise background with a lower electron dose than needed on the basis of the Rose criterion [12]. It should also be noted that regions of the sample positioned deeper in the liquid will be imaged with a broadened probe (see below), and thus with a lower electron dose.

3.4. Monte Carlo simulations

As additional evaluation of the achievable resolution in liquid we used Monte Carlo calculations to simulate electron trajectories for given irradiation schemes and sample geometries. The Monte Carlo calculations have the advantage over the analytical model

presented in the above of including beam blurring and multiple scattering. The calculation model was based on the software CASINO [21], modified to simulate STEM optics, including a conical electron beam, Poisson statistics of the electron source, and the ADF detector. The total elastic scattering cross section was determined using the software ELSEPA [22]. A model sample was programmed containing gold nanoparticles of diameters 0.8 – 10 nm on top of water layers with $T = 1 - 12 \mu\text{m}$, enclosed between two sheets of silicon nitride of 50 nm thickness each. For each water thickness line scans were simulated over the nanoparticles, with a pixel size of 0.1 nm and 6.6×10^4 electrons per pixel, from which the smallest nanoparticles visible with $\text{SNR} = 5 \pm 1$ were determined as measure of the resolution. The FWHM values were determined for those nanoparticles as measure of the resolution. The detection efficiency was assumed to be 100%. The semi-angle of the ADF detector was set to a value of $\beta = 94 \text{ mrad}$. Fig. 3 shows a good agreement of the resolution values obtained with the Monte Carlo simulations with those from the experimental data. The Monte Carlo calculations confirm the capability of liquid STEM to achieve nanometer resolution on gold nanoparticles in water layers with thicknesses in the micrometer range.

3.5. STEM imaging of gold nanoparticles below a water layer

The imaging of nanoparticles below a liquid layer takes place with an electron probe broadened by electron-sample interactions and it is thus not correct to assume that the resolution is noise limited. In case the probe is broadened to a width larger than the size of the nanoparticles the electron probe limits the resolution. Two point objects imaged by a Gaussian probe with d_{FWHM} and spaced by $1.14 \times d_{\text{FWHM}}$ would result in a line scan with two peaks and a 20% signal dip in their middle, which is the Raleigh criterion of resolution. As lower limit of the resolution we have thus used the d_{FWHM} . The resolution below the liquid was measured for 8 different positions with varying T on 4 samples containing gold nanoparticles with $d = 5, 10, 30$, and 100 nm . The value of T was determined from the average result of the two methods described above, i.e., tilting the specimen, and measuring the detector current. Fig. 4a shows several gold nanoparticles at the bottom of a $1.3 \mu\text{m}$ thick liquid layer (the same data used for this figure was used for the supporting information in [7]). Arrow #1 points to a nanoparticle with $d = 10 \text{ nm}$. A smaller nanoparticle is visible at arrow #2, presumably with $d = 5 \text{ nm}$ exhibiting $d_{\text{FWHM}} = 8 \text{ nm}$ in the line scan shown in Fig. 4b. The average of 4 small nanoparticles gave $d_{\text{FWHM}} = 9 \pm 1 \text{ nm}$. The nanoparticle at arrow #3 has $d = 30 \text{ nm}$ and the signal intensity is clipped. The background signal varies within the image (see e.g. the increased scattering at the right upper corner) due to the bulged silicon nitride windows, leading to a changing water thickness as function of the lateral position. The d_{FWHM} is plotted as function of T for all 8 samples in Fig. 4c.

3.6. Calculation of the beam broadening

The broadening of the electron probe due to beam-sample interactions can be calculated from the elastic scattering cross section [15]. In a simplified model, it is assumed that all blurring occurs as single scattering event in the middle of the sample at $z = T/2$ [23]. Electron probe broadening then follows from the un-scattered fraction of the electron probe:

$$\frac{N_{\text{not-scattered}}}{N_0} \cong \exp\left(-\frac{T/2}{l(\beta)}\right). \quad (7)$$

The angle containing a certain fraction of the current translates to a probe diameter as $d = 2(T/2)\beta$. In the original papers [23,24] the d_{90} (the diameter containing 90% of the current) was used to represent the resolution in X-ray analysis of elements in a thin foil. Also, Monte Carlo based estimation methods of the beam broadening use the d_{90} [25]. However, for

STEM imaging the d_{90} is an inaccurate measure of the resolution, because it is dominated by the beam tails formed by infrequent high-angle scattering events [26]. The high-resolution electron probe is maintained on a background signal formed by the beam tails, as discussed also for the imaging in vapor with a scanning electron microscope [27]. For our study we used the d_{25} in solving Eq. (7). Fig. 4c shows agreement of the theoretical model of d_{25} with the experimental data within the error margin. It should be noted that the contribution of the beam divergence [24] was neglected here, because the probe diameter in vacuum was much smaller than the d_{25} for T in the experiment. Fig. 4c also shows that for $T < 1 \mu\text{m}$ the use of d_{25} as measure of the broadening is not accurate. In this thickness regime a transition occurs from the resolution being limited by noise to being limited by beam broadening. For $T > 2 \mu\text{m}$ the solution of Eq. (7) can be approximated by (Fig. 4c):

$$d_{25} = 1.2 \times 10^3 T^{3/2} \frac{Z}{U} \sqrt{\frac{\rho}{W}}, \quad (8)$$

This equation is similar as the 25–75% edge width for beam blurring derived by others [15].

We have also calculated the resolution of STEM imaging on gold nanoparticles below a water layer with Monte Carlo simulations using $\beta = 94 \text{ mrad}$. For water thicknesses between 0.1 and 8 μm the smallest nanoparticles with a $SNR = 5 \pm 1$ were determined. For the larger water thicknesses we found that the $FWHM$ of a peak at the position of a nanoparticle was much larger than its diameter, demonstrating the beam broadening effect. Fig. 4c shows that the values of d_{FWHM} match within a factor of two with the experimental results. Our results compare with studies of others on polymer films coated with nanoparticles. 200 KV STEM images of 6.4 nm diameter gold nanoparticles at the bottom of a film with a thickness of $1.1 \pm 0.1 \mu\text{m}$ exhibited a $FWHM = 9 \pm 3 \text{ nm}$ [26], similar to the value we measured for water. In another study, performed with a 300 kV STEM, gold nanoparticles of a diameter of 50 nm were visible below a 4 μm thick nanocomposite polymer filled with carbon black [28].

3.7. Resolution as function of the vertical position of nanoparticles

Since the Monte Carlo simulations agree with the STEM experiments we can use the Monte Carlo method to predict the achievable resolution for various sample geometries and materials. An important question is how the resolution changes with the vertical position of the nanoparticle in the liquid. The resolution was determined for gold nanoparticles in a water layer with $T = 5 \mu\text{m}$, representative for the imaging of thin eukaryotic cells. Fig. 4c shows the resolution as function of z . The resolution is noise-limited at $z = 0 \mu\text{m}$. For $0 < z < 4 \mu\text{m}$ the effect of beam broadening increasingly influences d_{FWHM} . For $z \geq 4 \mu\text{m}$ d_{FWHM} follows the same curve as the Monte Carlo calculation of the resolution obtained on nanoparticles below the liquid. A resolution better than 10 nm can be achieved for the top 1 μm of the specimen. The resolution achieved on nanoparticles positions deeper in the liquid can possibly be improved by applying deconvolution algorithms, by using particle recognition techniques, and by simply turning the specimen holder by 180° to image the specimen from the other side.

4. Conclusions

We have determined that the resolution obtained on gold nanoparticles in the top layer of water is noise-limited. The smallest nanoparticles in this study had diameters of 1.4 nm and were visible above the noise for water thicknesses of up to 3.3 μm . The imaging of nanoparticles below micrometers of water is limited by electron probe broadening. Analytic models and Monte Carlo simulations agreed with the experimental data within the error margin. The equations for the resolution provided here are generally applicable to other

materials than water and gold. Considering that individual proteins have sizes in the range of several nanometers, it seems feasible to study protein distributions in cells with over an order of magnitude higher resolution than recently developed nanoscopy techniques [29]. STEM imaging can also be used to study whole vitrified cells with labeled proteins, thus avoiding the often-difficult step of sectioning. Further applications can be found in materials science to study the interaction of solid:liquid interfaces at the nanoscale [30], in e.g., micro-batteries, or fuel cells.

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Fig. 1. Principle of operation of scanning transmission electron microscopy (STEM) of nanoparticles in a liquid enclosed between two electron-transparent silicon nitride windows. The enclosure is placed in the vacuum of the electron microscope. Images are obtained by scanning the electron beam over the sample and detecting elastically scattered transmitted electrons with the annular dark field detector. The dimensions and angles are not to scale.

Fig. 2.

STEM imaging of nanoparticles on top of a water layer. (a) Image of gold nanoparticles with an average diameter $d = 1.4$ nm on a liquid with a thickness $T = 3.3$ μm , recorded at a detector semi-angle $\beta = 70$ mrad, magnification $M = 480,000$, with a pixel size of $s = 0.29$ nm, and a pixel-dwell time of $t = 20$ μs . The signal intensity was color-coded. (b) Line-scan, signal S versus horizontal position x , over the nanoparticle indicated with the arrow in (a) from the unfiltered data. The dashed line represents the background level. (c) Overlay of two images recorded at the same position as (a), but at $M = 24,000$ and with the stage tilted by 24.6° for one image. Arrow #1 connects the shift of 0.54 μm of a nanoparticle with $d = 30$ nm on the top window. Arrow #2 connects a 0.82 μm shift in opposite direction of a nanoparticle with $d = 100$ nm on the bottom window. The arrows are not entirely parallel due to stage drift during tilting. (d) Image of a sample with $T = 13$ μm recorded at $M = 160,000$, $s = 0.87$ nm, $t = 20$ μs , and $\beta = 94$ mrad. Arrow #3 points to a gold nanoparticle of 10 nm and arrow #4 to one of a 5 nm.

Fig. 3.

Resolution of STEM in the top layer of a liquid, d_{FWHM} as function of T . The experimental data is compared with numerical calculations for $\beta = 94$ mrad detector angle (black solid line) and $\beta = 70$ mrad (black dotted line), with an analytic model for $\beta = 94$ mrad (dotted blue line), and with minimum observable d determined with Monte Carlo simulations for $\beta = 94$ mrad (green solid line).

Fig. 4.

STEM imaging of gold nanoparticles below a water layer. (a) Image of gold nanoparticles of $d = 5, 10, 30$ nm below a water layer of $T = 1.3 \mu\text{m}$, recorded with $\beta = 94$ mrad, $M = 160,000$, $s = 0.87$ nm, and $t = 20 \mu\text{s}$. The signal intensity was color-coded. Arrows #1, #2, and #3 point to nanoparticles with respective diameters of 10, 5 and 30 nm. (b) Line-scan over the nanoparticle at the arrow #2 in (a) of the unfiltered data. (c) Resolution as function of T . Experimental data is compared with a numerical calculation (black solid line), with an analytic expression (blue dotted line), and with Monte Carlo simulations (green line and triangles). The resolution versus the vertical position of nanoparticles in the liquid z for $T = 5 \mu\text{m}$ from Monte Carlo calculations is also included (red line, here the horizontal axis represents z).