Mapping and Controlling Liquid Layer Thickness in Liquid-Phase (Scanning) Transmission Electron Microscopy

Hanglong Wu, Hao Su, Rick R. M. Joosten, Arthur D. A. Keizer, Laura S. van Hazendonk, Maarten J. M. Wirix, Joseph P. Patterson, Jozua Laven, Gijsbertus de With, and Heiner Friedrich*

Liquid-Phase (Scanning) Transmission Electron Microscopy (LP-(S)TEM) has become an essential technique to monitor nanoscale materials processes in liquids in real-time. Due to the pressure difference between the liquid and the microscope vacuum, bending of the silicon nitride (SiNx) membrane windows generally occurs. This causes a spatially varying liquid layer thickness that makes interpretation of LP-(S)TEM results difficult due to a locally varying achievable resolution and diffusion limitations. To mediate these difficulties, it is shown: 1) how to quantitatively map liquid layer thickness for any liquid at less than 0.01 e− Å−2 total dose; 2) how to dynamically modulate the liquid thickness by tuning the internal pressure in the liquid cell, co-determined by the Laplace pressure and the external pressure. It is demonstrated that reproducible inward bulging of the window membranes can be realized, leading to an ultra-thin liquid layer in the central window area for high-resolution imaging. Furthermore, it is shown that the liquid thickness can be dynamically altered in a programmed way, thereby potentially overcoming the diffusion limitations towards achieving bulk solution conditions. The presented approaches provide essential ways to measure and dynamically adjust liquid thickness in LP-(S)TEM experiments, enabling new experiment designs and better control of solution chemistry.

1. Introduction

(Scanning) Transmission Electron Microscopy ((S)TEM) is a key technique for morphological and compositional characterization of materials from (sub) nanometer to micrometer scales.[1–4] For beam stable thin and solid samples, sub-Ångström resolution can be achieved, while for beam sensitive cryogenic samples, such as nanoparticles in vitrified liquids, (sub-) nanometer resolution is possible.[5] To directly image nanoscale materials formation and transformation processes in solution in real-time, liquid-phase (LP)(Scanning) transmission electron microscopy (LP-(S)TEM) can be employed with nanometer spatial resolution.[6,7] The benefits of LP-(S)TEM over conventional dry (S)TEM (i.e., preserving the native aqueous state) or cryogenic approaches (i.e., preserving the dynamics of the sample) in materials science and life science have recently been discussed extensively.[8–10]

The most common LP-(S)TEM setup is a closed-cell in which the liquid is confined between two electron-transparent silicon nitride (SiNx) membranes.[11] To define the thickness of the liquid layer and to guide the liquid flow, spacers with a thickness of between 50 nm to 5 µm are added around the membrane.[8] However, the observed thickness of the liquid layer is not only determined by the spacers,[12,13] but also depends strongly on the

DOI: 10.1002/smtd.202001287
SiN₆ layer thickness and its lateral dimensions, the employed procedure of cell assembly, and the pressure inside the liquid cell.[14,15] Notably, the pressure difference between the cell interior (1 bar) and microscope column (close to 0 bar) leads to the SiN₆ windows bending outwards, causing the liquid layer thickness to vary across the viewing area. There are two principal but unfortunately competing requirements on liquid layer thickness. On the one hand, liquid layers should be as thick as possible to avoid confinement effects from diffusion limitations or (charged) surfaces[16,17] to be able to translate LP-(S) TEM observations into mechanisms that actually occur on a laboratory scale. On the other hand, the liquid layer should be as thin as possible to avoid spatial broadening of the electron beam and broadening of the energy spread due to inelastic scattering events in the liquid cell to be able to follow, for example, nucleation, growth and assembly processes with atomic resolution.[6,18] Clearly, both requirements cannot be fulfilled at the same time, thus being able to dynamically control the liquid layer thickness in LP-(S)TEM experiments would be an attractive solution. Rapid dynamic control of liquid layer thickness enables a new way of performing LP-(S)TEM, which is carrying out the largest part of the experiment using a thick liquid layer to avoid confinement effects and achieving thin liquid layers whenever needed for imaging at higher resolution.

Another key challenge for LP-(S)TEM is to minimize and/or model and account for electron beam effects, such as the radiolysis of water.[19] For instance, OH⁻ and H⁺ species will locally change the pH in the illuminated area and beyond, while solvated electrons (eaq) can reduce metal ions, thus, inducing significant changes to the observed solution chemistry.[16,20] Therefore, in addition to dynamic control also mapping of the liquid layer thickness throughout the entire viewing area is essential for progress in LP-(S)TEM towards achieving bulk laboratory-scale solution conditions.

Employing electron energy-loss spectroscopy (EELS) to estimate the thickness of the liquid layer for the entire imaging area before each experiment is considered to be too time-consuming or, due to lack of an energy filter, unfeasible in many laboratories.[12–14,23–25] Furthermore, EELS and energy-filtered TEM (EFTEM) thickness measurements require high electron doses, which might lead to significant beam damage of the liquid samples prior to the actual experiment.[19] Alternatively, the use of the measured current density with and without a sample in TEM and STEM has been employed to measure the liquid thickness at a few points across the viewable area, again mainly carried out high magnification and at high electron doses.[16,26] Thus, a simple low-dose method to map the liquid layer thickness throughout the entire viewing area is needed to avoid changes to the chemical environment but still provide the necessary information to: 1) estimate which resolution can be reliably achieved,[15,27] 2) model beam effects,[19] and 3) be able to control liquid thickness throughout the experiment.[28]

In summary, to improve LP-(S)TEM methodology, accurate and dynamic control of the liquid layer thickness is required, which has to be achieved by modulating the pressure in the liquid cell.[14,15] Furthermore, mapping the liquid layer thickness throughout the entire viewing area at minimal electron dose is an essential prerequisite that also aids in modeling beam effects and contrast/resolution simulations to assess the interpretability of obtained data.[8,10,11,19,27]

In this manuscript, we first present a simple low dose method requiring less than 0.01 e⁻ Å⁻² to acquire a map of the liquid layer thickness throughout the entire viewing area of any liquid cell and with minimal radiolysis effects even during repeated measurements. With this tool at hand, it is shown that thin liquid layers, corresponding to inward bulging of the SiN₆ membranes, can be realized by using the Laplace pressure, that is, via changing the filling state of the liquid cell and modulating the relative humidity in the surroundings. Finally, we show that dynamic control over the liquid thickness, SiN₆ bulging, and total volume of liquid in the cell can be obtained by modulating the inlet and outlet pressure of the cell with pressure pumps. The presented approaches offer additional flexibility in designing LP-(S)TEM experiments towards overcoming confinement effects achieving bulk laboratory-scale solution conditions at increased resolution.

2. Results and Discussion

An in situ LP-(S)TEM holder was employed for the LP-TEM experiments (Figure 1a and Figure S1, Supporting Information). Two Si chips with a 400 μm x 20 μm SiN₆ window are assembled in a crossed configuration, thus forming a 20 μm x 20 μm sized imaging area. The thickness of the employed SiN₆ membranes is 50 nm. Two 2.6 mm x 2 mm chips without spacers are sealed with O-rings in the chamber of the tip, forming a liquid cell isolated from the vacuum in the TEM column. The groove in the holder tip, where the chips sit, can be considered as a cylindrical chamber with a diameter of 3 mm and a height of 1 mm (Figure S1, Supporting Information).

The pressure difference between the liquid cell and the microscope column results in a liquid layer that is flat in the region of the Si frame having a constant thickness t Bulg and varies in thickness h Bulg = f(x,y) in the SiN₆ window area (Figure 1b). Note that h Bulg here includes both the top and bottom bulged liquid layers. Since the thickness of the SiN₆ membranes is the same for the top and the bottom chip, we assume that an equivalent outward bulging occurs in the two directions in the window area (Figure 1c).

2.1. Low-Dose Thickness Mapping

To map the liquid thickness in the viewing area (Figure 1, Figures S2,S3, Supporting Information), two low magnification low-dose bright-field TEM images are acquired (typical electron flux: 0.001–0.01 e⁻ Å⁻² s⁻¹, 5–0.5 s exposure): one flat field image without sample yielding information on the electron flux, and one image containing information on the number of electrons that locally transmitted the viewing area. This implies that a thickness map of the whole viewing area can be acquired at a total dose of less than 0.01 e⁻ Å⁻². Note that, because imaging of the liquid is performed at low magnification (400–800 ×), low resolution and in focus, phase contrast contributions can
be neglected (see details in Section S4.2, Supporting Information). Thus, the liquid layer thickness throughout the imaging area can be approximated also taking the SiNx membrane thickness into account, by mass-thickness contrast considerations alone (MTC method). As shown in Equation (1), the liquid thickness is determined based on Lambert-Beer's law from the ratio between transmitted beam intensity $I_t$ and incident intensity $I_0$:

$$\frac{I_t}{I_0} = \exp \left( -\frac{t_i}{\Lambda_i} \right)$$

where $t_i$ is the thickness of the component $i$ ($i = \text{SiNx}$ and liquid) and $\Lambda_i$ is the elastic mean free path (EMFP) of each component, which can be calculated from the elastic electron scattering cross-section of the corresponding atomic components using well-established methods [30–33]. A detailed calculation of the liquid layer thickness, $t_{\text{liquid}}$, including considerations on effects from bulging, is provided in Sections S4.2 and S4.3, Supporting Information.

The thickness calculations were implemented in MATLAB 2017b (Mathworks, code provided in Supporting Information). Here we like to note again that all experiments have been carried out with a cross alignment of the rectangular windows resulting in a square viewing area. To simplify thickness calculations, the square viewing area is first rotated to align with a Cartesian coordinate system. An example image of an aligned and bulging liquid cell filled with water (EMFP: 430 nm) is shown in Figure 1d. Figure 1e shows the derived thickness map and the bulging map, respectively. Please note that the thickness of the cell might vary significantly in every experiment even using the same liquid with the same volume (Figure S2, Supporting Information). Based on the geometry of the crossed-window liquid cell (Figure S4, Supporting Information, see the comparison of configurations between crossed- and parallel-window liquid cell in Section S2, Supporting Information), $t_{\text{flat}}$ can be approximated as the liquid layer thickness at the corners of the viewing window area, while the thickness in the center of the window area, $t_{\text{liquid}}$, is the sum of $t_{\text{flat}}$ and $t_{\text{bulgC}}$, that is, $t_{\text{flat}} + 2t_{\text{bulgC}}$ (Figure 1b). From Figure 1e, we can estimate that $t_{\text{flat}}$ is 646 nm and $t_{\text{liquid}}$ is 926 nm. To adapt our method to any liquid and microscope, the corresponding EMFP can be approximated from the chemical composition of the liquid and the acceleration voltage of the employed microscope (see Sections S4.2–S4.4, Supporting Information). As an illustration, we show in Figure S3, Supporting Information, the thickness map of a 30 µm × 30 µm liquid cell filled with acetone (EMFP = 578 nm) under flow conditions, where $t_{\text{flat}}$ and $t_{\text{bulgC}}$ are calculated to be 427 and 152 nm, respectively.

We benchmarked our method with EELS thickness measurements on a water-filled liquid cell, employing the standard theoretical model [34,35] (Figure 1f) and employing a very recent approach [36] that is based on an adjusted average energy-loss term (Figure S9, Supporting Information). Our thickness measurements fall between both EELS approaches with our maximum liquid thickness being very close to the standard theoretical model and at lower thicknesses better matching the recent approach. Further details can be found in Section S4.5,
2.2. Thickness Control via Modulating the Internal Pressure

The window bulging is caused by a pressure difference between the internal pressure in the liquid cell and the vacuum in the microscope column. Therefore, gaining control of the internal pressure is crucial to alleviate bulging effects. In LP-(S)TEM experiments, three typical filling states of liquid cells can be obtained, as shown in Figure 2. The main difference between the first state and the last two states is the introduction of a flat (Figure 2a) or curved (Figure 2c) vapor-liquid interface at the edge of the overlapping silicon frames. It is known that the presence of the curved liquid-gas interface results in a Laplace pressure $P_{\text{Lap}}$. This means that, depending on the filling state of the liquid cell, the internal pressure $P_{\text{inter}}$ of the liquid cell is determined by both the external pressure $P_{\text{exter}}$ and the Laplace pressure $P_{\text{Lap}}$.

$$P_{\text{inter}} = P_{\text{exter}} + P_{\text{Lap}}$$  \hspace{1cm} (2)

where $P_{\text{exter}}$ is the atmospheric pressure (=1.0 bar) in most LP-(S)TEM experiments, but it can be regulated by changing the pressure at the inlet and outlet tubings.\textsuperscript{[14,15]} Here we assume $P_{\text{exter}}$ is constant at 1.0 bar. In a completely filled liquid cell (Figure 1a), $P_{\text{Lap}} = 0$, therefore $P_{\text{inter}} = P_{\text{exter}}$. This state (State I) is the most common in the liquid cells under flow conditions when no air bubble is trapped. Figure 2 (State II) exhibits a partially filled cell with a flat gas-liquid interface and liquid spill in the surrounding chamber. Still, $P_{\text{inter}} = P_{\text{exter}}$ as $P_{\text{Lap}} = 0$. But this is a metastable state and fluctuations, such as some evaporation, can easily transform this state into State III (Figure 2b), where a curved meniscus forms, leading to a negative $P_{\text{Lap}}$. The resulting $P_{\text{Lap}}$ can greatly reduce $P_{\text{inter}}$, depending on the formed curvature. So far, $P_{\text{Lap}}$ has never been considered in controlling the liquid thickness in LP-(S)TEM, and it is challenging to satisfy the formation conditions of the curved meniscus in such a confined environment. We describe in the following how $P_{\text{Lap}}$ can be used to achieve a thinner liquid cell. The Laplace pressure is given by

$$P_{\text{Lap}} = \gamma \left( \frac{1}{R} - \frac{1}{r} \right)$$  \hspace{1cm} (3)

where $\gamma$ is the surface tension of the liquid (e.g., for water, $\gamma = 7.3 \times 10^{-2}$ N m$^{-1}$ at 20 °C), and $R$ and $r$ are the largest and smallest principal radii of curvature, respectively. Here $R$ corresponds to approximately half the diameter of the overlapping chip area, therefore $R \gg r$. As $t_{\text{flat}} = 2\cos\theta$, Equation (3) can be written as

$$P_{\text{Lap}} = -\frac{\gamma}{r} = -\frac{2\gamma \cos\theta}{t_{\text{flat}}}$$  \hspace{1cm} (4)

Since $\gamma$ is normally constant in one LP-(S)TEM experiment, $P_{\text{Lap}}$ is dependent on $r$, that is, $t_{\text{flat}}$ and the contact angle $\theta$. As an upper bound to the maximum Laplace pressure $P_{\text{Lap max}}$ achievable, we consider the situation where the meniscus is just retracted fully into the gap between the chips. In this case, the curvature is similar to a cylinder that just fits in between the two chips with a radius of $t_{\text{flat}}/2$. Since the SiN$_x$ layer is hydrophilized by O$_2$ plasma treatments, we assume that $\theta$ is close to 0°. Therefore, we assume that

$$P_{\text{Lap max}} = -\frac{2\gamma}{t_{\text{flat}}}$$  \hspace{1cm} (5)

For example, when $t_{\text{flat}}$ of pure water layer is 400 nm, $P_{\text{Lap max}}$ is approximately $-3.65$ bar at 20 °C. Therefore, $P_{\text{inter}}$ is $-2.64$ bar according to Equation (2), which might result in an inward bulging membrane. When a critical $t_{\text{flat}}$ value is reached (1.4 µm), $P_{\text{inter}}$ is close to 0 bar, indicating the window should be flat without any outward bulging (Figure 2c).
So, how can we control the formation of the concave liquid meniscus in the liquid cell? The most straightforward solution is to load an ultra-low volume of liquid between the two chips to directly generate a liquid meniscus. Typical $r_{\text{real}}$ obtained in LP-(S)TEM experiments is in the range of 100 nm and 5 μm. For a 5 μm liquid layer over a 2 mm × 2.6 mm chip area, one needs only ~26 nL liquid to fill the cell, while for a 100 nm cell, less than 1 nL of liquid is needed. In contrast, static cells conventionally are prepared by direct drop-casting on the bottom chip with a liquid volume of ~500 nL.[7,37] This means that at least 474 nL of the liquid is used to fill the liquid cell chamber. Here we employed the SciTEM (Scienion AG, a CELLINK company, Germany), an automated piezoelectric-actuated ultra-low volume dispenser, to assist the liquid handling. The SciTEM is capable of patterning picoliter droplets of solutions onto the chip surface with a predefined array, and automatically loading the top chip to close the liquid cell (for details see Section S3, Figures S5–S7, and Movie S1, Supporting Information).[38,39] Thus, accurate liquid volume control and reproducible liquid cell assembly can be achieved. However, we found it challenging to deposit less than 20 nL water onto the hydrophilic bottom chip (contact angle $\theta \rightarrow 0^\circ$), while keeping the cell wet before being imaged in the TEM. Hence, we can conclude that by this direct loading strategy, State II is always obtained with the excess liquid being in the chamber. However, it is important to note that sometimes we can directly achieve an inward-bulged cell by using the SciTEM to load ~50 nL water due to unavoidable liquid evaporation during the cell assembly process (Figure S7, Supporting Information).

To transform State II to State III, one needs to evaporate liquid present between the two chips, leading to control over the formation of a concave meniscus (Figure 2). At equilibrium, the meniscus curvature can be described by Kelvin’s equation:[40]

$$r_k = \frac{2\gamma V_m}{RT \ln(RH)}$$

(6)

where $r_k$ is the Kelvin radius, $V_m$ is the molar volume liquid (e.g., for water, 18 × 10$^{-6}$ kg m$^{-3}$). $RH$ is the relative humidity, R is the universal gas constant (8.314 J mol$^{-1}$ K$^{-1}$) and T is the Kelvin temperature. If $r_k$ is smaller than $r$ (the actual radius of curvature in Equation (4)), liquid meniscus equilibrium cannot be obtained and consequently, the liquid will evaporate. In LP-(S)TEM experiments, $r_{\text{min}} = \frac{r_{\text{real}}}{2}$, and assuming $RH$ is 97% in the liquid cell, $r_k$ is ~35 nm. Since in most cases, $r_{\text{min}} > r_k$, a concave meniscus will form, and the liquid will evaporate. Therefore, altering the humidity in the liquid cell should be an effective way to tune the meniscus curvature.

As illustrated in Figure 2, when the $RH$ is controlled (either by flowing dry air into the cell, and/or by drying the excess liquid first before assembling the cell), a curved gas-liquid interface appears. Importantly, during evaporation, the curved gas-liquid interface might be pinned to the edge of the chips (Figure 2b–d). The radius of curvature is prone to decrease with time until the meniscus escapes the pinning positions (Figure 2e). During this process, $P_{\text{diep}}$ keeps increasing, leading to a decrease in $P_{\text{inter}}$ (Equation (2)), therefore no outward bulging (Figure 2c) but inward bulging membrane (Figure 2d,e) is expected. However, it is difficult to predict how long this process will take in such a small confined space and which state we have in the experiment (Figure 2f). It strongly depends on the surface homogeneity of the chip, the morphology at the edge of the chip, and the contact angle of the system.

To prove our point, we demonstrate how a trapped droplet between the chips could be slowly evaporated over time (Figure 3). To this end, 500 nL of pure water was first loaded into the cell by a pipette before the liquid cell assembly, thereby a droplet was encapsulated in the cell with excess liquid located in the surrounding chamber (Stage II, Figure 2a). The slow drying experiment was conducted by connecting the inlet tubing of the LP-(S)TEM holder to a pressure pump (Flui gent, Germany), which continuously supplied dry air at a constant overpressure (0.3 bar, relative to the atmospheric pressure), while the outlet tubing was kept open to the ambient pressure. The in situ LP-TEM images (Figure 3a, Movie S2, Supporting Information) and the corresponding intensity profile changes of the diagonal line across the window (Figure 3b) show within three minutes that the liquid cell experienced outward bulging ($t = t_0$) to nearly no bulging ($t = t_0 + 2.8$ min). Eventually, an inward-bulged cell ($t = t_0 + 3.2$ min) was obtained, consistent with what has been described in our model (Figure 2). More specifically, it was observed that the thickness of the central window area, $t_{\text{liquid}}$, changed from approximately 700 nm to close to 0 nm (Figure 3c), providing a large thin area for high-resolution imaging. Here it is important to note that the inward bulged cell could gradually transform back to the standard outward-bulged cell after the dry airflow was stopped (Figure 3d–f, Movie S3, Supporting Information), but this recovery process was ten times slower than the evaporation process. It was also found that the $t_{\text{flat}}$ decreased from 427 to 168 nm during the evaporation, and increased back slowly when there was no evaporation (Figure S8, Supporting Information), which we believe is related to the capillary condensation.

To illustrate how the signal-to-noise ratio of a real sample evolves upon changing the liquid thickness we measured 20 nm gold nanoparticles. While in thin liquid layers the particles are readily observed even at very low doses (0.3 e$^{-}$ Å$^{-2}$ s$^{-1}$), in thick liquid layers they can be hardly seen. Further details can be found in Section S5, Supporting Information.

### 2.3. Rapid Dynamic Control of Pressure and Liquid Layer Thickness

Recently, it has been shown that the liquid thickness in LP-(S) TEM can be significantly reduced in dependence of the static reduced pressure equally applied to both holder inlet and outlet via dry tubing functioning as vacuum lines.[14,15] Instead, our method uses liquid-filled tubing and independent pressure control for inlet and outlet via two pressure pumps which are required for realizing flow. We show how pressure cycling gives rise to rapid dynamic control over liquid thickness, thus providing an additional adjustable parameter for experiment design. Rapid dynamic control over liquid thickness is of key importance, in particular in bypass liquid cell systems, as it can help to overcome confinement problems such as diffusion limitations[27] or hindered Brownian motion.[41]

Here we demonstrate dynamic control over the liquid layer thickness using pressure pumps (Flui gent, Germany) that
modulate $P_{\text{inter}}$ while the liquid thickness was continuously mapped (Figure 4, Movie S4, Supporting Information) via the above method. The pressure pumps allow us to rapidly control the inlet and outlet pressure as well as the flow rate, therefore $P_{\text{inter}}$ can be programmed in a controllable way. In the experiment, pure water was flown at a rate of 6.2–6.8 $\mu$L min$^{-1}$ through the tubings of the liquid cell holder (State 1, $P_{\text{inter}} = P_{\text{exter}}$) and the inlet pressure was cycled between 1.0 and 3.0 bar (absolute pressure, unless stated otherwise), while the outlet pressure was simultaneously cycled between 0.4 and 1.0 bar. In

Figure 3. Reversible transformation of a liquid cell containing water from outward to inward bulging via slow evaporation. a) TEM image sequence (5-frame-average) showing the outward-to-inward bulging transition (Movie S2, Supporting Information). b) Intensity profiles and c) the corresponding thickness profiles taken from the diagonal dashed line across the viewing area shown in (a). Here $t_0$ indicates the time when the dry air started to flow into the LP-(S)TEM holder. d) TEM image sequence (5-frame-average) showing the inward-to-outward bulging transition (Movie S3, Supporting Information). e)Intensity profiles and f) the corresponding thickness profiles taken from the diagonal dashed line across the viewing area shown in (d). Here $t_1$ marks the time when the flow was stopped and both inlet and outlet tubings were opened to the lab environment at atmospheric pressure. Scale bars: 20 $\mu$m.
total, two cycles were performed to switch $P_{\text{exter}}$ between 0.4 to 3.0 bar. This resulted in $t_{\text{liquid}}$ in the viewing area being repeatedly modulated from approximately 600 to 850 nm (Figure 4a–c, Movie S4, Supporting Information). This proves that $t_{\text{liquid}}$ can be reduced by approximately 25% when $P_{\text{exter}}$ is reduced to 0.4 bar. During the pressure cycling, both $t_{\text{bulgC}}$ and $t_{\text{flat}}$ changed simultaneously (Figure 4d,e), with $t_{\text{bulgC}}$ responding more strongly than $t_{\text{flat}}$ (Figure 4e). Interestingly, when the lowest pressure (0.4 bar) was applied (State 1, 3, and 5, Figure 4e), $t_{\text{bulgC}}$ decreased to approximately 10 nm only at a total liquid layer thickness of 600 nm. This means that in this state bulging effects nearly disappeared and the window area was basically flat, a much-desired situation in LP-TEM as the resolution is now constant throughout the entire viewing area.\(^{14,24}\)

As shown above, in most cases $t_{\text{liquid}}$ is thus determined by $t_{\text{flat}}$, which means that changes of $t_{\text{flat}}$ lead to variations of the liquid volume that is present in the liquid cell. Therefore, pressure cycling of the liquid cell is similar to “breathing”, whereby water is brought in ($t_{\text{flat}}$ increases, State 1–2 or State 3–4) and flushed out ($t_{\text{flat}}$ decreases, State 2–3 or State 4–5) repeatedly. Following the results shown in Figure 4f, the liquid cell takes up a liquid volume of 0.72 nL (State 1–2) and in contrast, the liquid cell expels a liquid volume of 0.82 nL (State 2–3) and 0.64 nL (State 4–5) when $P_{\text{exter}}$ reaches the lowest pressure. As an aside, we note that the expelled volume in each cycle is always slightly larger than that of the volume taken up. The above observations bring us to a rather important point. As was calculated before, a 900 nm thick liquid cell contains 4.20 nL liquid. Assuming that the in and out liquid volume in each “breathing” action is the same, say, 0.70 nL, and that the liquid mixes immediately in the cell, ≈ 80% of the solution in this liquid cell will be refreshed after 10 “breathing” actions. Rapid dynamic control of liquid layer thickness is of
great significance for LP-(S)TEM experimental designs, as this "breathing" action is expected to provide an efficient way to refresh the solution chemistry in the liquid enclosure, such as ion concentrations and type of ion species. Furthermore, dynamic thickness control is seen as a simple means to mediate confinement effects,[19] particularly for the bypass LP-(S)TEM holder design.

To understand how the liquid thickness can be altered with an increased $P_{\text{inter}}$ in more detail, we mapped the liquid thickness at smaller increments of $P_{\text{inter}}$ values which were set manually (Figure 4b,g). It is found that $t_{\text{flat}}$ and the trapped liquid volume increased almost linearly with $P_{\text{inter}}$, indicating that it is possible to design a liquid cell with well-defined liquid volume by changing pressures of the inlet and outlet tubings, that is, $P_{\text{exter}}$. Furthermore, we also found there is no obvious difference in trapped liquid volume if we change the pressure slowly or rapidly, referred to as static or dynamic, respectively, in Figure 4h.

3. Conclusion

We have demonstrated approaches to map and control the liquid layer thickness in LP-(S)TEM experiments. First, we describe a low dose method (<0.01 e⁻ Å⁻³) to readily map the liquid layer thickness throughout the entire viewing area which only requires two low-magnification bright-field TEM images. The method is easily extendable to any liquid and acceleration voltage with electron beam effects on the liquid itself/liquid sample inside the cell being largely avoided and the influence of generating the map on the in situ observation minimized. By quantifying the liquid layer thickness, suitable experimental areas can be defined including estimates for the achievable resolution.[11,27,42] This “on-the-fly” measurement during LP-(S)TEM experiments opens the way to precisely control liquid-phase thickness maps. Second, we show how the Laplace pressure can be utilized to controllably modulate the liquid layer thickness. By controlling the filling state of the liquid cell, inward bulging has been achieved which facilitates electron diffraction and high-resolution imaging of samples in very thin liquid layers. Finally, it is demonstrated that by dynamically controlling the inlet and outlet pressures the liquid thickness can be rapidly adjusted in a programmed way. Therefore, rapid dynamic thickness control (via this “breathing” action) enables to refresh the chemical environment in the cell and to increase the achievable resolution during experiments if needed. With the above approaches at hand, new LP-(S)TEM experiment designs with liquid thickness tailored to the requirement of the imaged process come into reach.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

H.W. and H.S. contributed equally to this work. The authors gratefully acknowledge Shell Global Solutions and the Dutch Research Council (NWO) for funding H.S. through the CHIPP program. H.W. was supported by the EU H2020 Marie Skłodowska-Curie Action project "MULTIMAT" (676045). The authors thank Dr. Remco Fijneman and Jason Heinrichs (Eindhoven University of Technology) for the assistance with the slow evaporation experiments, and Joeri Opdam (Eindhoven University of Technology) for beneficial discussions and the help with creating 3D liquid cell images.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

(scanning) transmission electron microscopy, dynamic thickness control, liquid-phase, thickness maps

Received: December 23, 2020
Revised: March 18, 2021
Published online: May 5, 2021

[1] N. D. Browning, M. F. Chisholm, S. J. Pennycook, Nature 1993, 366, 143.
[2] Y. Cheng, T. Walz, Annu. Rev. Biochem. 2009, 78, 723.
[3] X. Zhang, E. Settembre, C. Xu, P. R. Dormitzer, R. Bellamy, S. C. Harrison, N. Grigorieff, Proc. Natl. Acad. Sci. USA 2008, 105, 1867.
[4] K. Gnanasekaran, R. Snel, G. De With, H. Friedrich, Ultramicroscopy 2016, 160, 130.
[5] J. P. Patterson, Y. Xu, M.-A. Moradi, N. A. J. M. Sommerdijk, H. Friedrich, Acc. Chem. Res. 2017, 50, 1495.
[6] D. Li, M. H. Nielsen, J. R. I. Lee, C. Frandsen, J. F. Banfield, J. J. De Yoreo, Science 2012, 336, 1014.
[7] H. Zheng, R. K. Smith, Y.-W. Jun, C. Kisielowski, U. Dahmen, A. P. Alivisatos, Science 2009, 324, 1309.
[8] F. M. Ross, Science 2015, 350, aaa9886.
[9] J. J. De Yoreo, N. A. J. M. Sommerdijk, Nat. Rev. Mater. 2016, 1, 16035.
[10] H. Wu, H. Friedrich, J. P. Patterson, N. A. J. M. Sommerdijk, N. Jonge, Adv. Mater. 2020, 32, 2001582.
[11] N. De Yonge, F. M. Ross, Nat. Nanotechnol. 2011, 6, 695.
[12] T. J. Woehl, T. Prozorov, J. Phys. Chem. C 2015, 119, 21261.
[13] K. L. Jungjohann, J. E. Evans, J. A. Aguilar, I. Arsalan, N. D. Browning, Microsc. Microanal. 2012, 18, 621.
[14] S. Keskin, P. Kunnas, N. De Jonge, Nano Lett. 2019, 19, 4608.
[15] Y. Inayoshi, H. Minoda, Y. Arai, K. Nagayama, Micron 2012, 43, 1091.
[16] A. Verch, M. Pfaff, N. De Jonge, Langmuir 2015, 31, 6956.
[17] R. Kröger, A. Verch, Minerals 2018, 8, 21.
[18] J. M. Yuk, J. Park, P. Ercius, K. Kim, D. J. Hellebusch, M. F. Crommie, J. Y. Lee, A. Zettl, A. P. Alivisatos, Science 2012, 336, 61.
[19] N. M. Schneider, M. M. Norton, B. J. Mendel, J. M. Grogan, F. M. Ross, H. H. Bau, J. Phys. Chem. C 2014, 118, 22373.
[20] H. Su, B. L. Mehdi, J. P. Patterson, N. A. J. M. Sommerdijk, N. D. Browning, H. Friedrich, J. Phys. Chem. C 2019, 123, 25448.
[21] J. M. Grogan, N. M. Schneider, F. M. Ross, H. H. Bau, *Nano Lett.* **2014**, *14*, 359.
[22] H. Zheng, S. A. Claridge, A. M. Minor, A. P Alivisatos, U. Dahmen, *Nano Lett.* **2009**, *9*, 2460.
[23] M. E. Holtz, Y. Yu, J. Gao, H. D. Abruiña, D. A. Muller, *Microsc. Microanal.* **2013**, *19*, 1027.
[24] D. J. Kelly, M. Zhou, N. Clark, M. J. Hamer, E. A. Lewis, A. M. Rakowski, S. J. Haigh, R. V. Gorbachev, *Nano Lett.* **2018**, *18*, 1168.
[25] K. L. Klein, I. M. Anderson, *Microsc. Microanal.* **2012**, *18*, 1154.
[26] M. J. Dukes, D. B. Peckys, N. De Jonge, *ACS Nano* **2010**, *4*, 4110.
[27] N. De Jonge, *Ultramicroscopy* **2018**, *187*, 113.
[28] N. de Jonge, L. Houben, R. E. Dunin-Borkowski, F. M. Ross, *Nat. Rev. Mater.* **2019**, *4*, 61.
[29] D. B. Williams, C. B. Carter, *Transmission Electron Microscopy: A Textbook for Materials Science*, Springer, New York **2009**.
[30] J. P. Langmore, M. F. Smith, *Ultramicroscopy* **1992**, *46*, 349.
[31] M. W. P. Van De Put, J. P. Patterson, P. H. H. Bomans, N. R. Wilson, H. Friedrich, R. A. T. M. Van Benthem, G. De With, R. K. O’reilly, N. A. M. Sommerdijk, *Soft Matter* **2015**, *11*, 1265.
[32] A. Ianiro, H. Wu, M. M. J. Van Rijt, M. P Vena, A. D. A. Keizer, A. C. C. Esteves, R. Tünier, H. Friedrich, N. A. J. M. Sommerdijk, J. P. Patterson, *Nat. Chem.* **2019**, *11*, 320.
[33] L. Reimer, H. Kohl, *Transmission Electron Microscopy: Physics of Image Formation*, Springer, New York **2008**.
[34] R. F. Egerton, S. C. Cheng, *Ultramicroscopy* **1987**, *21*, 231.
[35] T. Malis, S. C. Cheng, R. F. Egerton, *J. Electron Microsc. Tech.* **1988**, *8*, 193.
[36] M. N. Yesibolati, S. Laganá, S. Kadkhodazadeh, E. K. Mikkelsen, H. Sun, T. Kasama, O. Hansen, N. J. Zaluzec, K. Mølhave, *Nanoscale* **2020**, *12*, 20649.
[37] H. -G. Liao, L. Cui, S. Whitelam, H. Zheng, *Science* **2012**, *336*, 1011.
[38] M. A. Touve, C. A. Figg, D. B. Wright, C. Park, J. Cantlon, B. S. Sumerlin, N. C. Gianneschi, *ACS Cent. Sci.* **2018**, *4*, 543.
[39] J. P. Patterson, L. R. Parent, J. Cantlon, H. Eickhoff, G. Bared, J. E. Evans, N. C. Gianneschi, *Microsc. Microanal.* **2016**, *22*, 507.
[40] W. J. Moore, *Basic Physical Chemistry*, Prentice Hall, New York **1983**.
[41] M. N. Yesibolati, K. I. Mortensen, H. Sun, A. Brostrøm, S. Tidemand-Lichtenberg, K. Mølhave, *Nano Lett.* **2020**, *20*, 7108.
[42] N. De Jonge, N. Poirier-Demers, H. Demers, D. B. Peckys, D. Drouin, *Ultramicroscopy* **2010**, *110*, 1114.