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Chemical and Bonding Analysis of Liquids Using Liquid Cell Electron Microscopy

Peter Ercius, Jordan A. Hachtel, Robert F Klie*

Liquid cell transmission electron microscopy (TEM) has become an essential tool for studying the structure and properties of both hard and soft condensed matter samples, as well as liquids themselves. Liquid cell sample holders, consisting often of two thin window layers separating the liquid sample from the high-vacuum of the microscope column, have been designed to control in-situ conditions, including temperature, voltage/current or flow through the window region. While high-resolution and time-resolved TEM imaging probes the structure, shape and dynamics of liquid cell samples, information about the chemical composition and spatially-resolved bonding is often difficult to obtain due to the liquid thickness, the window layers, the holder configuration or beam induced radiolysis. In this article, we provide an overview of different approaches to quantitative liquid cell electron microscopy, including recent developments to perform energy dispersive X-ray (EDS) and electron energy-loss spectroscopy (EELS) experiments on samples in a liquid environment or the liquid itself. We will cover graphene liquid cells and other ultra-thin window layer holders.

Keywords: transmission electron microscopy (TEM), liquid, in situ, spectroscopy

1. Introduction

Liquid cell transmission electron microscopy (TEM) has a long history in quantitative materials science and life-science microscopy and has evolved significantly since the early work by prominent pioneers of the field, such as Ruska and Marton, in the early 1930s [1] and 1940s [2-4]. Early liquid cell TEM required dedicated instruments, where the liquid sample was either directly exposed to the vacuum (open-cell) or separated by a thick window layer (usually 100's of μm thick metal foil, such as Al, or thick plastic layers) capable of withstanding the pressure differential (closed-cell). Over the last 20 years, these approaches have been significantly improved and were used to successfully characterize the growth of Si nanowires from a liquid phase [5] and identify the dynamics of Cu plating on Au.[6] However, both open- and closed-cell approaches severely limit sample choice; either to low-vapor pressure liquids that can withstand the vacuum in the TEM, or high-contrast samples with sufficient signal to overcome the thick liquid and window layers. Resolution was also limited by multiple scattering. Furthermore, conventional analytical approaches, such as energy-dispersive X-ray spectroscopy (EDS) or electron energy-loss spectroscopy (EELS), are also precluded in dedicated liquid cell instruments due to the small differential pumping apertures in an open-cell microscope and the thick window layers blocking most of the photons or inelastically scattered electrons.

The development of Si/SiN_x in-situ heating/biasing holders in the early 2000s [7] (see timeline of selected liquid cell development in Figure 1) spurred the implementation of liquid cells using modular side-entry stages in conventional and

aberration-corrected TEMs. The resolution of these in-situ liquid cell experiments, starting in 2009, reached 1 nm as the result of significant reduction in the SiN_x window layer thickness (~25-50 nm) and the dramatic reduction in the liquid layer thickness.[8] In addition to higher resolution imaging of colloidal nanoparticles in solution, the ability to flow and mix solvents also allowed for the observation of growth dynamics and imaging of whole cells in a liquid environment.

The need for more analytical capabilities was met by redesigning the window shape to support ultra-thin SiN_x layers. The thinner window layers now also allowed for selective EELS quantification of samples, including energy-filtered TEM, for materials which avoid the silicon (Si) and nitrogen (N) core-loss excitations (~100 eV and ~400 eV respectively). When combined with the ability to apply a bias and using continuous liquid flow, the plating and stripping behaviors of anodes in rechargeable batteries became a focus of in-situ electrochemical characterization in a TEM (see Ref. [9] as one of many examples). Yet, the window layer thickness and sensitivity of the liquids to the electron beam did not allow for atomic-resolution imaging and spectroscopy.

The development of ultra-thin windows layers, including graphene and BN, significantly reduce liquid layer thickness and largely eliminated the scattering by the window-layer resulting in both higher resolution imaging and spectroscopy of in-situ imaging. In addition, ultrathin graphene seems to increase the tolerance of the encapsulated materials to the high-energy electron beam and reduce the formation of bubbles from radiolysis.[10, 11] The ability to perform electron energy-loss spectroscopy (EELS) or EDS during in-situ experiments has the

potential to overcome many of the current limitations for quantitative in situ TEM, providing information about beam-sample interactions and local reactions during imaging. In this article, we will review recent developments in quantitative and analytical liquid-cell TEM with a focus on quantitative chemical and bonding analysis enabled by innovations in holder and window layer designs, as well as new detector and electron source technologies. We will also present our vision for next generation analytical liquid phase microscopy exploiting parallel electron microscopy breakthroughs in tomography, direct electron detection, and monochromation to probe new aspects and dimensions of novel materials in liquid cell samples.

2. EDS and EELS in SiN-based liquid cells

While high-resolution and dynamic imaging of nanoparticles and whole cells flourished using liquid cell TEM, chemical and bonding analysis in SiN_x liquid cells faced several challenges. EELS was primarily used to measure the relative total sample thickness and to quantify the extent of window bowing.[12] Examples of early quantitative measurements include low-loss valence-EELS, as well as core-loss spectroscopy of the O *K*- and Fe *L*-edges, to examine the de/intercalation of Li-ion battery cathodes in electro-chemical cells.[13]

Until recently, the limitations to EDS in liquid cells were primarily due to geometric constraints of the Si chips used to support the SiN_x window layers and the TEM holder. Specifically, the cutouts in the Si chips produced by KOH and HF etching produce a 52° take-off angle, blocking the majority of X-rays generated by the

primary electron beam in the liquid from reaching the EDS detector. Furthermore, the need to apply pressure to seal the chips required significant hardware surrounding the cell which also blocked line of site to the x-ray detector. However, advances in fabrication of Si chips and environmental holders have removed material from key regions improving the effective X-ray detector solid angle without sacrificing liquid cell functionality. Initial work demonstrated that 10 nm resolution in EDS elemental mapping using a probe-corrected STEM was possible.[14]

Recent developments in large solid-angle X-ray detectors and high sensitivity EEL spectrometers (see section 4.1) have vastly improved the speed and sensitivity of chemical and bonding analysis in normal analytical S/TEM, and has significant potential for liquid phase microscopy. Yet, even with the novel holder/chip geometries and reduced windows/liquid layer thicknesses, high electron doses are required for both EDS and EELS to overcome unwanted inelastic scattering from the liquid cell itself. Such heavy electron irradiation induces radiolysis in the liquid which can radically alter chemistry inside the cells and obfuscate the desired reactions and processes.[15, 16] Additionally, for beam-sensitive polymers, biomolecules and energy materials, the electron beam irradiation can inject huge amounts of energy to break bonds and modify or even ablate material.[17] Novel acquisition schemes, including reduced sample thickness (window and liquid), leapfrog scanning of the samples, direct electron detectors (DED) for EELS acquisition and modifications to the liquid to delay the onset of bubble formation are now all considered in an effort to improve the quality of the acquired results.

3. 2-D window-layer cells for high spatial/energy resolution

Since all material in the beam path interacts with the electron beam, often overwhelming the signal from the material of interest due to multiple scattering, a critical problem with *in situ* liquid-cells is the window thickness and the large volume of liquid trapped between them. However, thinner windows can also bulge out far larger than the expected thickness.[12] One of the most effective methods to side-step this problem is to encapsulate the liquid between sheets of 2D materials,[18-20] simultaneously reducing the window and liquid thicknesses while also allowing the material of interest to comprise a much higher volumetric fraction of the liquid cell. The advent of graphene and boron-nitride liquid cells (GLCs and BNLCs respectively) has resulted in an outpouring of atomistic structural analysis of dynamic processes in colloidal nanoparticles, [16, 21-25] the compositional analysis of hydrated biological specimens and soft matter outside of cryogenic conditions,[26-32] and the direct analysis of liquids and their various phases.[15, 30, 33-35]

Perhaps the most critical advantage of the nanoscopic volume of supporting material in the 2D layer liquid cells is the opportunity to use low-dose techniques to mitigate and control beam damage and radiolysis in liquid cells. Several approaches for efficient high-resolution imaging and spectroscopy in encapsulated liquid cells are highlighted in Figure 2. A dark field image of ferritin molecules in a GLC is shown in Fig. 2a. If the instantaneous dose rate can be kept below $10 \text{ e}^-/\text{\AA}^2/\text{s}$ nanobubbles generated by radiolysis are prevented from forming, and the Fe *L*-edge and O *K*-edge fine-structure can be measured and mapped during a

continuous scan (Fig. 2b).[26] Furthermore, instead of preventing radiolysis, it can be controlled by adjusting the instantaneous electron dose rate. Figs. 2c and 2d show nanobubbles generated by radiolysis in liquid water BNLCs under low-dose rate conditions with $3 \text{ e}^-/\text{\AA}/\text{s}$ (Fig. 2c) and higher-dose rate (Fig. 2d) conditions of $>10 \text{ e}^-/\text{\AA}/\text{s}$. It is also important to highlight the effects of the window layer material on the bubble formation threshold. While a dose rate of $3 \text{ e}^-/\text{\AA}/\text{s}$ in GLCs does not result in the formation of visible bubbles, they are clearly formed in water encapsulated in a BNLC (see Fig. 2b). This effect has been attributed to the high electron mobility in graphene or a catalytic reaction at the graphene/bubble interface.[11] It is also observed that the low-dose bubbles in GLCs reabsorb into the liquid, while the high-dose/larger bubbles in both GLC and BNLC remain or grow (Fig. 2e), indicating the potential to directly control radiolysis in liquid cells using the electron beam to manipulate the chemistry or pH [15]. Lastly, one can avoid direct electron beam irradiation entirely [36] while still effectively probing the sample by placing the beam $\sim 30 \text{ nm}$ away from the sample and allowing the beam to couple to low-energy excitations in the sample without the high energy electrons ever directly interacting with the liquid in the cell (the so called ‘aloof’ configuration).[37, 38] Spatial resolution is governed by delocalization of inelastic scattering where the interaction volume is inversely proportional to energy loss. [39] Next-generation electron monochromators, which can possess energy resolutions as low as 4.2 meV and significantly reduced background in the infrared,[40] allow for high-sensitivity, high precision measurements of phonons and molecular vibrations of organic molecules and other beam-sensitive

samples.[41-45] In Fig. 2f, the difference between a non-monochromated (black) and monochromated (red) EEL spectrum is shown in the infrared energy range, demonstrating the improved energy resolution and reduced background. In Fig. 2g, the aloof monochromated EEL spectrum of liquid-water in a BNLC is shown demonstrating the ability to sample the O-H molecular vibration without direct irradiation.

4. New detectors and approaches

Many of these new experiments and results are facilitated by tangential breakthroughs in non-liquid-cell electron microscopy techniques. Here, we outline more recent advancements, and provide perspective on their influence in liquid phase TEM.

4.1. Detectors

In situ liquid cell experiments are often focused on the dynamics of the systems of interest, meaning that time resolution is an important metric. Previously, charged coupled detector (CCD) technology limited the capabilities of in situ TEM to at best 30 frames per second (fps), but much lower for HR-TEM in the 1 frame per second range. The main limitation was in the CCD readout rate, which also required the detector was “blanked” during readout reducing the duty cycle significantly. Blanking was often done below the sample while the sample was still illuminated and potentially changing without adding to the image signal. We are now in a new era of direct electron detectors (DED) which can be fully readout on the millisecond or less time scale with no blanking. [46] The detector point spread function (PSF) has also been improved producing sharper images [47] and enhanced sensitivity for

spectrometers.[48] DEDs are revolutionizing several aspects of TEM, such as cryo-EM 3D bio-structural imaging,[49, 50] 4D-STEM,[51] EELS,[52] and in situ TEM is no exception. Faster data (image or spectra) captured with higher quality improves traditional imaging experiments [53, 54] while also allowing new capabilities such as tomography discussed later. The speed and size of DEDs is still increasing, growing data set sizes exponentially, where 2.5 TB of data can now be collected per minute.[55] However, even higher readout speed could achieve the ultimate in in-situ imaging and analytical analysis by utilizing electron counting [48] at the dose rates needed for in situ TEM to record only electron strikes and reduce the noise in TEM images and low-loss spectra to the ultimate limit of Poisson noise. This will simultaneously improve the resolution and contrast in in situ TEM movies while also compressing the data 100x.

4.2. Beam sensitive materials

TEMs are now capable of resolving most atomic spacings in materials due to aberration correctors. The limiting factor is now the electron dose applied to the sample in both materials science and biology.[56] New developments in both liquid-cell window layer materials and faster detectors (mentioned earlier) are providing new opportunities for imaging beam sensitive materials and biostructures in the native buffer solution with higher contrast and resolution. In situ TEM imaging of biological materials while hydrated in a native buffer solution has been achieved using SiN windows using SEM, TEM and STEM.[57] The biological structures studied range from whole cells to viruses. De Jonge, for example, used STEM and Au nanoparticles as high contrast tags to image whole cells at 4 nm

resolution while also providing dynamical information.[58] It has also been shown in several different ways that GLCs can reduce the effects of beam damage when imaging beam sensitive materials, [59] which is critically important as resolution in TEM is now more limited by applied dose than microscope resolution.[60, 61] Chemical bonding information was obtain using EELS from beam sensitive materials, such as polyphosphate nanoparticles encapsulated in sterically stabilized liposomes from samples encapsulated in graphene liquid cells.[27] Hybrid graphene/SiN liquid cells have also been used for correlative experiments in the TEM as well as photoluminescence. The ability of graphene to be an inert conformal covering provides the ability to interface GLC technology with live cell imaging by keeping the cells hydrated in the TEM vacuum. Park *et al.* utilized several layers of graphene to encapsulate live hydrated cells and influenza viruses thus reducing the thickness of the window material and the liquid encapsulating layer to achieve nanometer scale resolution at room temperature.[62] Confirmation of the wet conditions were confirmed by noting the motion of Au nanoparticles inside the cells. Correlative studies with photoluminescence confirmed that the cells survived the process.

4.3. Tomography

Fast and sensitive detectors paired with the GLC technology [19] has recently enabled 3D reconstruction of nanoparticles suspended in liquid. Yuk *et al.* [19] had previously shown that using graphene sheets as the window material could significantly improve image contrast and resolution in aberration corrected in situ liquid cell experiments. The technique was limited to a few frames per second due

to the speed of the CCDs at the time and the dose required to image the atomic structure. Early on, the group utilized an early version of a DED [48] to achieve 50 frames per second while maintaining the resolution and contrast required to achieve atomic-scale resolution, as shown in Figure 4A-B where tentative atom positions in cyan, black and magenta are overlaid on the density isosurface.[21] Recently, utilizing modern DEDs and improvements in reconstruction algorithms allowed the team to achieve true atomic resolution imaging in 3D to determine the coordinates of 9 Pt nanoparticles with approximately 2 nm diameters.[22] A nearly perfect NP and a NP with a defect are shown in Figure 4C-D and 4E-F, respectively. The atoms were located with a precision of +/- 19 pm and provide information about the structural heterogeneity of nanoparticles in solution and their internal structural heterogeneity. Identifying the positions of atoms with high spatial sensitivity in 3D provides the ability to determine the NPs' properties by ab initio quantum mechanical simulations. Next steps utilizing next generation DEDs and spectrometers could expand this capability to include compositional as well as structural 3D information.

4.4. Aloof Monochromated EELS

Aloof EELS, as shown in Figure 2f and 2g, allows for virtually damage free spectroscopy of even the most beam sensitive materials. This is possible due to the fact that the aloof coupling strength is much higher for low-energy infrared (IR) excitations, such as phonons and molecular vibrations, than it is for damaging high-energy (UV) excitations, needed for core-loss EELS. This can be understood by considering the radial dependence of the aloof scattering probability, $P_E(r) \propto$

$\frac{e^{-2r\omega/v}}{r^2}$, where r is the radial distance from the probe, ω is the frequency of the energy loss being considered, and v is the velocity of the primary electron [63]. For $r > v/\omega$ the interaction strength exponentially decays, meaning the intensity of damaging UV excitations attenuates long before the damage-free IR excitations. We can use this probability to estimate the maximum potential damage-free spatial-resolution achievable with monochromated EELS.

Vibrational spectroscopy is capable of reaching atomic-resolution, the same as conventional core-loss EELS, by using experimental geometries that emphasize highly-localized impact scattering in the collected EELS signal [64-66], but these techniques all require direct irradiation and long acquisition times and, thus, are not suitable for beam-sensitive liquid-cell samples. For damage-free aloof EELS, we can follow the formalism put forth by Egerton [63], where we imagine the probe at some impact parameter, b , away from a truncated slab and calculate radial distance from which the majority of the aloof signal originates as a function of the aloof scattering probability. Figure 3 shows this calculation for two different energy losses at 0.42 eV (O-H stretch mode in water [30]) and at 4.2 eV (where absorption in organic molecules begins to break bonds [67]), and two different impact parameters (10 nm and 30 nm). We can see that at 10 nm, the majority of the IR signal comes from a nano-sized region surrounding the probe, but that there is significant UV excitation in this area as well. By moving the probe back to a 30 nm impact parameter, the spatial resolution for the IR excitation is not significantly reduced (< factor of 2), but the ratio of IR excitation to UV excitation is improved by an order of magnitude. Thus, we can define a regime, at impact parameters of

~30 nm, where nanoscale spatial-resolution is achievable for vibrational analysis of beam-sensitive materials in liquid cells with little-to-no damage in the sample, this estimate agrees with experimental monochromated EELS analysis of organic molecules, which only show beam damage after long exposures at small impact parameters (~10 nm).[68]

4.5. Nano-reactors

Static liquid cell configurations that are optimized for spatial resolution or spectroscopy do not allow to define and control the exact chemical reaction and environmental conditions during an experiment. It often takes tens of minutes at best between completion of the cell assembly, loading it into the TEM and subsequent analysis. Mixing and controlling the reactants in flow cells is also notoriously difficult, as the thickness and composition of the surrounding liquid are hard to control and measure during rapidly changing and dynamical experiments.[12, 69, 70] Thus, encapsulating materials inside nanoreactors, such as liposomes,[71] with a well-defined size and spherical shape provides a way to accomplish many of the requirements of quantitative in situ TEM, which mirror the capabilities of lipid vesicles in biology. A nanoreactor provides a way to control or at least directly measure zeptoliter amounts of a material to react and even time the reaction.[72] Several different types of nanoreactors can theoretically be mixed and then opened (e.g. using the electron beam) inside a liquid cell to initiate a reaction. Alternatively, the electron beam can be used to change the local composition (or concentration of OH^- in water) to initiate a reaction. The high dose-rate tolerance

of samples in GLCs, or the use of damage-free aloof EELS, will then allow these reactions to be observed without negatively affecting the reaction pathways.

5. Outlook

This is an exciting time for quantitative liquid-cell microscopy, with new capabilities, better resolution and higher sensitivity being reported at an increasing pace. While liquid cell configurations with ultra-thin window and liquid sample layers have enabled EELS, EDS and imaging at unprecedented resolutions, GLC or BNLCs will need to overcome several shortcomings before finding more widespread use. First, the control of the liquid sample composition, the density of liquid cell pockets and their location cannot be controlled using the current sample preparation approaches. A promising development was reported by Kelly *et al.*, [20] where the top and bottom graphene window layers are separated by a thin layer of hexagonal boron nitride (hBN) with fabricated wells that can be filled with sample solutions. While this approach allows for control over the sample morphology, location and composition, it is currently not possible to access the liquid samples to apply external stimuli, such as current/voltage or temperature, or flow reactants into the sample region. Here, a hybrid approach, similar to the first liquid cell design by Abrams and McBain [3] could be considered, where the 2-D material is used to cover the top and bottom apertures of a flow cell. If sufficient mechanical stability and reliability can be achieved, this approach could also be used to apply a bias to the sample or to perform heating or cooling experiments. Furthermore, self-assembled structures based on bio-inspired liposomes[73] and vesicles, as well as polymeric shells can be used as self-assembled nanoreactors to

contain and control reactions. Currently, some of the limitations that need to be overcome include the dose sensitivity of the encapsulated materials, the stability of the shells, which tend to rupture at interfaces and the ability of the nano-reactor shells to contain the desired materials.

Second, sample contamination appears to be affecting the formation of liquid cells as well as the behavior of the encapsulated liquid samples. The synthesis of the 2D window materials or the process of lifting the window materials off their support often results in organic or residual metallic clusters on the graphene or BN layers. New sample preparation processes need to be developed to allow for cleaner window layers and better approaches of depositing the liquid samples between the two window layers.[74]

Third, one should consider moving beyond graphene and BN, and exploit other 2D materials to extend the analytical range of the encapsulated liquid cells. Developing other ultra-thin window layers has critical implications for vibrational spectroscopy in encapsulated liquid cells, as it will allow for careful selection of spectral transparency regimes in the encapsulating material. For instance, hBN has a strong phonon response between 160 meV and 200 meV meaning the analysis of critical molecular vibrational modes (such as O-H bending modes in water [75], C-C stretching modes in carotenes [76], C-O stretching mode in amino acids [77]) would be obscured by phonons in the encapsulating material. Alternatively, transition metal dichalcogenides, such as MoS₂ or WSe₂, have their phonon response in the ~50 meV spectral regime [78], providing a diverse range of well-studied materials for encapsulated liquid cells with tunable spectral transparency.

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Figure Captions

Figure 1: Timeline of selected liquid-cell holder developments over the last 80 years. The extent of the liquid and window layers are indicated by the blue and green lines, respectively.

Figure 2: Dose and Damage Control in Encapsulated Liquid Cells. (a,b) High spatial resolution EELS of ferritin particles in a GLC acquired under a continuous low-dose scan. (c-e) The manipulation of radiolysis generated nanobubbles that can be tailored to be reabsorbed over time or stay as nanobubbles. Reproduced from Ref. 16 (f,g) Monochromated EELS radiolysis/damage-free analysis of the vibrational spectrum of liquid water in BNLC. Reproduced from Ref. 30.

Figure 3: Aloof Spatial Resolution with Monochromated EELS. Maps of the normalized aloof scattering probability for different impact parameters and energy losses. Demonstrating capacity for efficient nanoscale excitations of IR vibrational modes without creating damaging UV+ excitations.

Figure 4: The progress in analytical in situ liquid cell electron tomography utilizing GLCs. The A) isosurface density map and B) tentative atom positions (respectively) of an atomic-scale reconstruction of a Pt nanoparticle. Scale bar 0.5 nm. [79] In C-F), the process was optimized using GLCs, advanced DEDs and algorithms to achieve true atomic resolution in Pt NPs with C-D) a nearly perfect crystal structure and E-F) a dislocation. Scale bar 1 nm. [22]

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Peter Ercius is a staff scientist at the National Center for Electron Microscopy (NCEM) facility within the Molecular Foundry Division at Lawrence Berkeley National Laboratory. He received his Bachelors of Science and PhD at Cornell University from the Applied and Engineering Physics Department in 2003 and 2009, respectively. He subsequently finished a postdoc position at the NCEM before being hired as a staff scientist, which is his current position. He is the main staff contact for the TEAM 0.5 aberration corrected instrument. His research focuses on atomic resolution electron tomography, scanning nanodiffraction (4D-STEM), *in situ* liquid cell electron microscopy and 2D/3D image analysis.

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Figures

Figure 1:

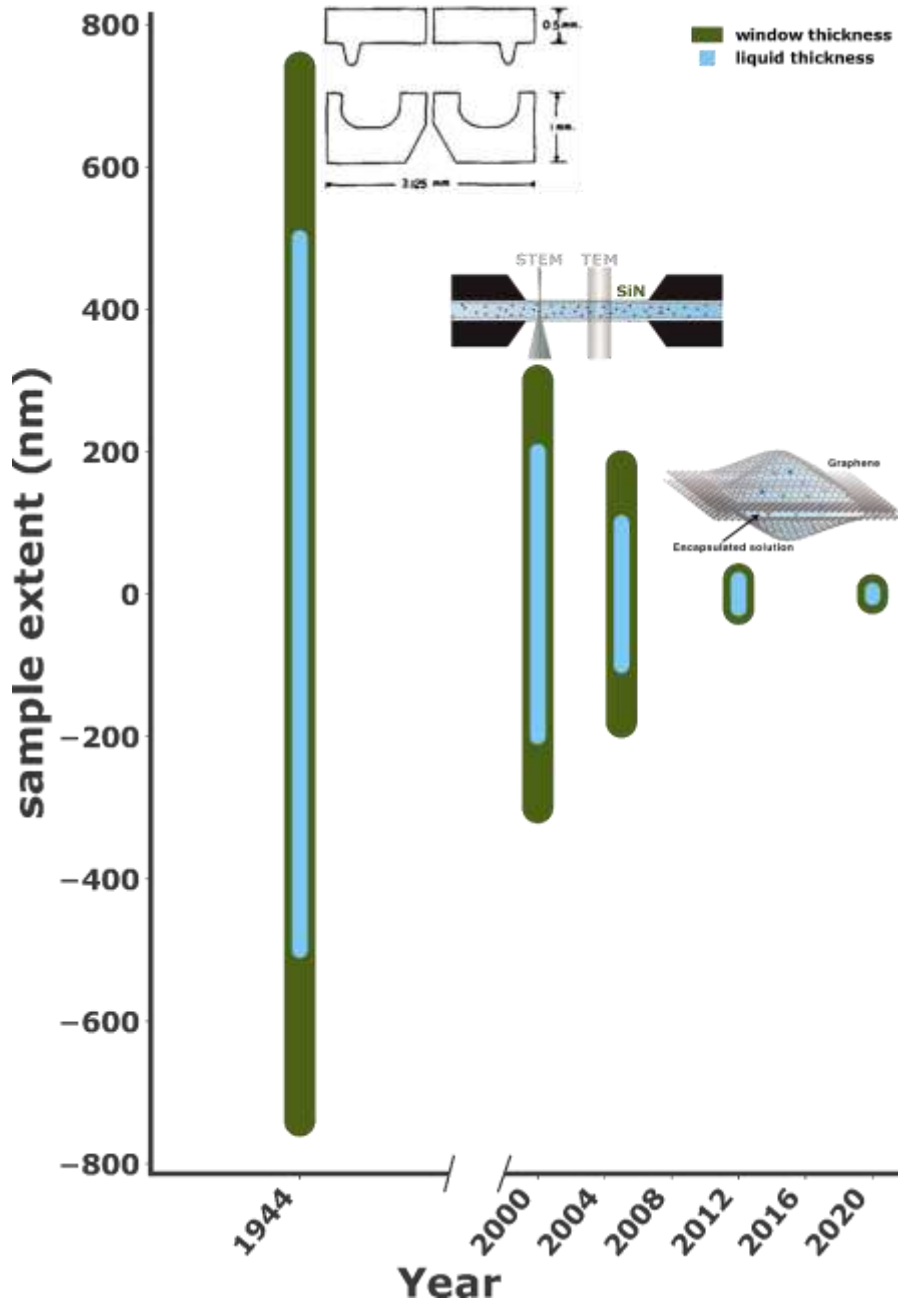


Figure 2:

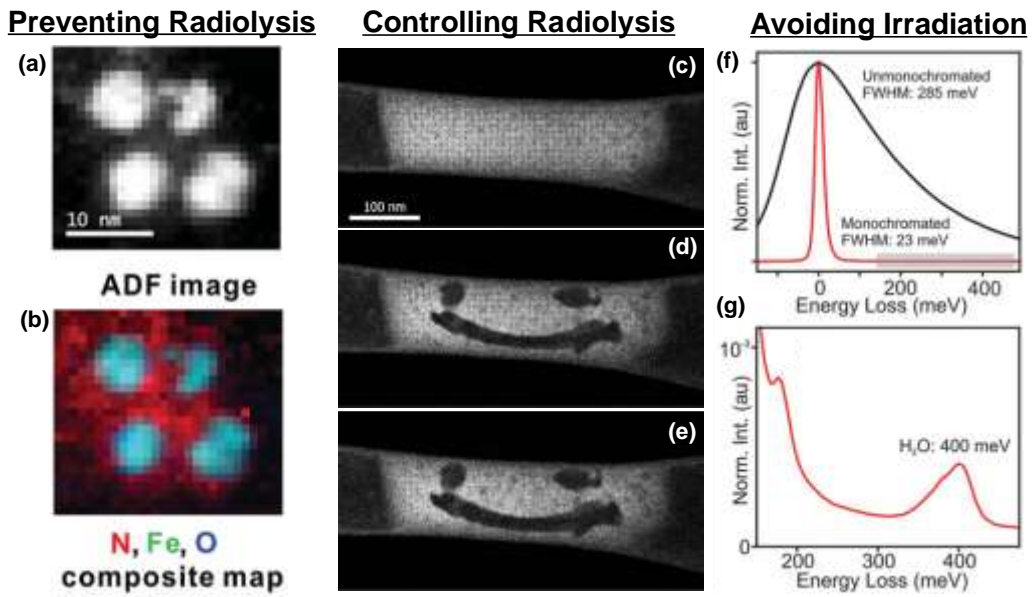


Figure 3:

