

Chapter 4

Membrane Based Environmental Cells for SEM in Liquids

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Part 4.1 Introduction

4.1.1 Motivation and terminology

Environmental electron microscopy and Scanning Electron Microscopy (SEM) in liquids are among the most active research areas in modern electron microscopy and spectroscopy. Research interest in these techniques is broad as they enabling nanoscale research of dynamic systems in diverse fields such as materials science, biological, medical and environmental sciences.

Complementing conventional high vacuum SEM (Fig. 1a), three schemes enable electron microscopy in liquids and dense gases. These distinct instrumentation techniques include SEM microscopes that employ differentially pumped environmental cells (also often called as “open cells”). In such systems elevated vapor pressure or fluidic regions of interest are separated from the high vacuum of the microscope and detectors by multi-stage differential pumping through small apertures (Fig 1 b). Placement of the pressure limiting apertures into the column of the microscope allows elevated pressure inside the entire SEM sample chamber. Modern commercial environmental SEM (ESEM) employs the latter methodology, enabling instrument operation at elevated pressure sufficient to maintain up to a few kPa of gas or vapor near the sample^{1,2}. This class of instrumentation is known as ESEM, variable pressure SEM (VPSEM), or low vacuum SEM (LVSEM). By cooling water vapor within the instrument, nanoscale imaging of fluid is possible using ESEM/STEM within the microscope chamber³. An alternative approach uses molecular impermeable electron transparent membranes to isolate samples at atmospheric pressure or in liquids from the high vacuum of the microscope⁴. Such environmental cells (also often called as “closed cells”) filled with liquid, gases or fluids can be placed inside the microscope chamber enabling liquid SEM (LSEM) or wet scanning electron microscopy (WETSEM)⁵ (Fig 1c). Finally, the third method that enables electron microscopy instrumentation approach to imaging vapor or liquid specimens seals the entrance of the objective lens of the SEM with electron transparent membranes thus isolating the column and electron detectors from ambient atmosphere. This technique enables realization of true atmospheric pressure SEM (ASEM) or AirSEM, where the electron beam can probe a proximal object at ambient atmosphere conditions,⁶⁻⁸ as shown in Fig. 1d.

The historical development of the membrane based transmission electron microscopy (TEM) is intriguing and enlightening. More than seven decades of development of such liquid cells from early work on collodion membranes by Abrams and McBain⁴ until recent state of the art monolithic Si based chips for microfluidic electron microscopy⁹ are described comprehensively in Chapters 1 and 2 of this book. This chapter concentrates on the development, capabilities and applications of membrane based SEM in liquids, describing the advantages and limitations of the technique. This field is an increasingly active research area and, at this point in time, no versatile commercial product exists to meet the diverse experimental needs of the research community. The chapter thus discusses ongoing research efforts based upon custom fabricated instrumentation that improve the capabilities of membrane based environmental cells (E-cells). We will use the terms: liquid SEM, membrane SEM, WETSEM or ambient pressure scanning electron microscopy (APSEM) through the chapter to refer to this technique unless specified, implying that both dense gaseous and liquid media may be used with this technique.

4.1.2 Why SEM (differences, perspectives and limitations)

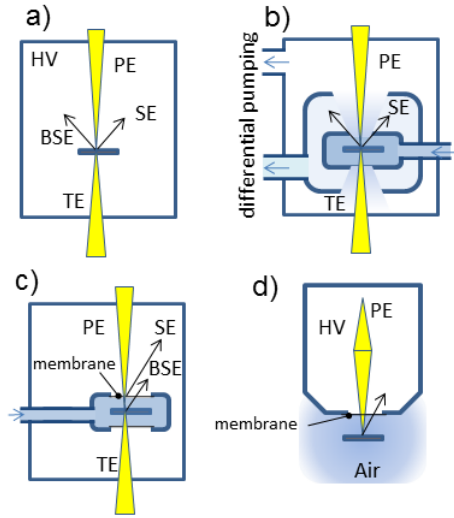


Figure 1. a) Standard high vacuum SEM/TEM setup. HV, PE, SE and TE stand for high vacuum, primary electrons, secondary electrons, backscattered electrons and transmitted electrons correspondingly; b) open cell environmental SEM/TEM; c) closed cell environmental SEM/TEM; d) atmospheric pressure SEM

The great majority of the electron microscopy research in liquids and gases is based upon use of (S)TEM thanks to its great spatial resolution and analytical power or ESEM.

In spite of somewhat lower resolution, the membrane based liquid SEM has its specific niche thanks to:

a) High surface and near surface sensitivity enabling high resolution analysis of interfacial processes.

b) Wider pressure and temperature ranges that can be achieved independently inside closed cells. The latter are only limited by the mechanical stability of the membranes and pressure differential of few Bars can routinely be achieved.

b) Closed E-cells enable research of chemically reactive, toxic or even radioactive media without contamination of the main chamber of the microscope.

c) Unique or precious samples can be studied without losing them due to sublimation or evaporation process.

d) As opposed to TEM techniques, there are fewer limitations of the imaged sample size. Membrane based SEM techniques enable both large lateral fields-of-view (FOV) of up to a few mm² as well as facile incorporation of optical, electrical, micro-mechanical, fluidic and other connectors. As a result, SEM

based membrane techniques meet the requirements for correlative optical microscopy and spectroscopy.

f) Finally, this methodology can be adapted by a large number of scientists using standard SEM instruments.

Part 4.2 Basics of SEM through the membranes

4.2.1 Image formation mechanism. Detectors.

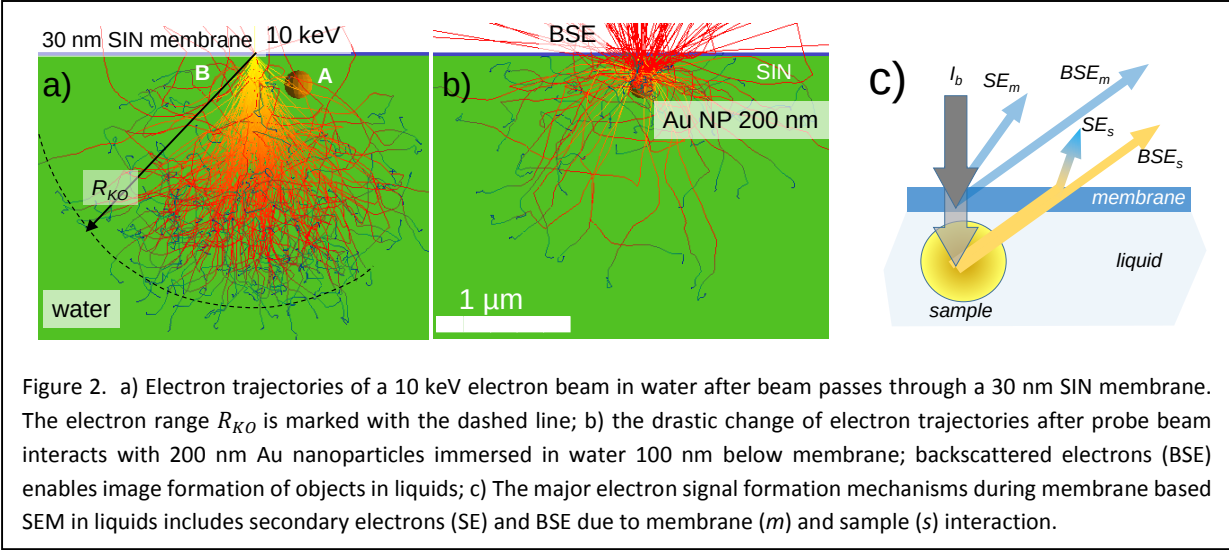
The processes occurring during electron beam interactions with objects inside liquid cell can be best illustrated with Monte Carlo simulations of electron trajectories¹⁰ (Fig. 2 a, b). When a high energy electron probe beam with energy E_b impinges upon the membrane and enclosed liquid specimen, the primary electrons dissipate their energy and change the directions via multiple inelastic and elastic processes. As a result, the electrons, ions, radicals and electronic excitations become distributed within the interaction volume (defined by colored trajectories in the Figure 2a) whose dimensions depend on primary energy of the beam, properties of the membrane and the media behind the membrane, as well as probe/surface interaction angle. One of the commonly used parameter to estimate the dimensions of the interaction volume is the electron range:

$$R_{KO} \approx \frac{28 \cdot W}{Z^{0.89} \cdot \rho} E_b^{1.64} [nm] \quad (4.1)$$

parameterized by Kanaya and Okayama¹¹ (here W [g/mol] is the atomic weight, Z is atomic number and ρ [g/cm³] is the density of the media). For example, liquid water enclosed behind a 30 nm SiN membrane yields R_{KO} that ranges from ca 50 nm to ca 2000 nm for 1 keV and 10 keV primary electrons correspondingly (see Fig. 2 a). While secondary electrons (SE) originate from the first few nanometers of the membrane, it is important to note that ca 90% of backscattered electrons (BSE) originate within ca 0.1 R_{KO} (for high Z materials) to 0.3 R_{KO} (for light elements).¹² Thus for liquid SEM imaging the R_{KO} parameter (and its energy

dependence) has crucial importance since it determines the ultimate “probing depth” in liquid and therefore the contrast and resolution of the resultant images.

Consider high Z object A (e.g., Au nanoparticle) being immersed into low Z liquid background B which is separated from high vacuum of electron beam and detectors space by thin membrane m (as in



the figure 2 a, b). Assuming the standard SIN membrane thickness being few tens of nanometers, low energy secondary electrons (SE) from the object behind the membrane will be completely attenuated. The signal (S) detected by standard cumulative SE/BSE electron detector from such an object as the following contributions:

$$S = SE_m + BSE_m + BSE_s + SE_s \quad (4.2)$$

(Fig 2 c) which includes SE and BSE electrons from the membrane (subscript m) and sample (subscript s) behind the membrane. Note that SE_s component (often called SE type 2 electrons or SE_2) originates from backscattered electrons BSE_s scattered from sample in the direction of the incident of the probe beam. These BSE_s excite detectable SE_s from the membrane. The total yield of backscattered electrons η (also called backscattered electron coefficient) will be:

$$\eta = \eta_m + \eta_s \quad (4.3)$$

Similarly, the secondary electron coefficient is:

$$\delta = \delta_m + \Delta \cdot \eta_s \quad (4.4)$$

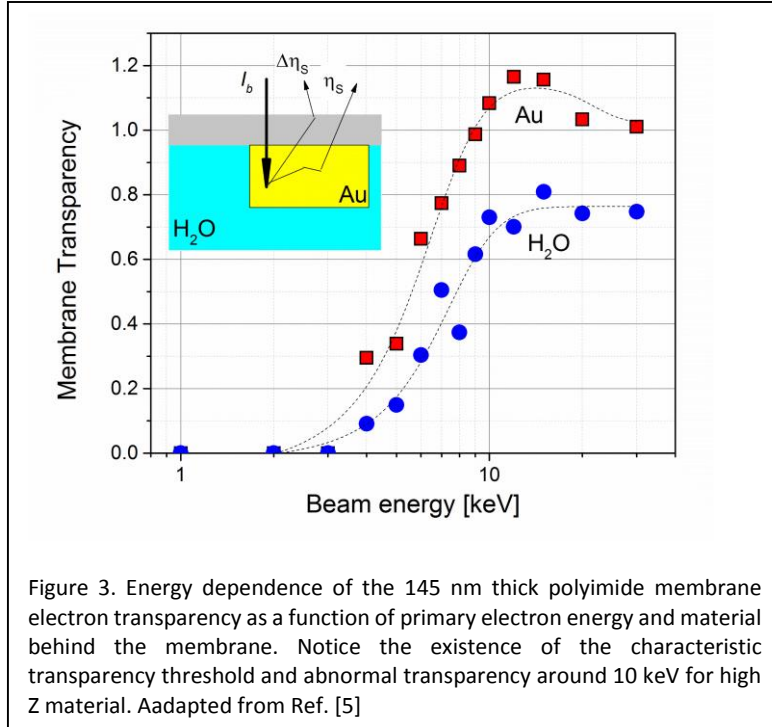
(here sub-indexes s, m correspond to membrane and sample respectively and Δ coefficient is an efficiency of secondary electron emission from the membrane by outgoing BS electrons emitted from the sample). Electron detector efficiencies ϵ_{SE} , ϵ_{BSE} enables the cumulative electron signal formula to be written:

$$S = \epsilon_{SE} (\delta_m + \Delta \cdot \eta_s) + \epsilon_{BSE} \cdot (\eta_m + \eta_s) \quad (4.5)$$

The contrast C between two points A and B in SEM image Figure 2 is defined⁵ as

$$C = (S_A - S_B) / S_A \text{ and can be reduced to } C = (\eta_{sA} - \eta_{sB}) / \eta_{sA} \quad (4.6 \text{ a, b})$$

assuming both sufficiently high energy (few keV) of primary electron beam penetrating few tens of nanometers thick membrane and featureless signals from the membrane and from liquid backgrounds. Under these conditions, SEM contrast is predominantly determined by the ratio of the backscattered electron coefficients of the object and the liquid, emphasizing the importance of backscattered detectors



for membrane based SEM. The validity of these considerations has been verified by Thiberge *et.al.* experimentally through use of liquid cells equipped with 145 nm thick polyimide membranes⁵. One of their revealing experimental results describes the energy dependence of the ratio:

$$T = (\eta_s \cdot (1 + \Delta)) / \eta_{s, theor}, \quad (4.7)$$

as shown in Fig. 3. The numerator of this equation denotes the sample signal (without featureless membrane's terms) experimentally recorded through 145 nm polyimide membrane while the denominator describes the theoretical values of $\eta_{s, theor}$ for the membrane material. Since this is a ratio of the amount of BSE transmitted through the membrane to the theoretically

expected total, this dimensionless parameter has minimal material dependence and defines the electron transparency of the particular membrane. Several important conclusions can be deduced from the analysis of these experimental dependences:

- The experimental electron transmittance curves of the membranes have a characteristic *transparency threshold* indicating, for example, that 145 nm thick polyimide membranes are practically opaque for electrons with energies below 4 keV, as shown in Fig. 3. This transparency threshold is not a constant value but can be reduced for low probe beam energies by decreasing thickness and/or density of the membrane material.
- The transmittance curve shape also depends on the media behind the membrane. For a water sample (e.g., low Z material) the electron transmittance of the polyimide membrane is systematically lower compared to gold (e.g., high Z) one (Figure 3). The latter can be a consequence of so-called *Z-filtering* effect of the membranes which are actually more transparent for samples with higher Z number⁵. This is a feasible explanation since for any given energy of the primary electron beam E_b the maximum of energy spectra of the backscattered electrons from high Z targets is centered closer to E_b compared to one for light materials which peaks at ca $E_b/2$. Thus electrons from the low Z targets become more attenuated by the membrane on their way out to the detector.
- The presence of the apparent maximum in transmittance curve for Au and its value in excess of 1 can be attributed to the significant contribution of SE_2 electrons to the total detectable signal from the sample. Further increase of energy of the BSE_s reduces the cross section for SE_2 generation, implying that an optimal condition exists for signal generation and contrast that enables both BSE_s and SE_2 detection of objects from beneath the membrane.

4.2.2 Signal quality and spatial resolution

The attenuation of the primary beam electrons and BSE signal by the membranes raises the question of probe beam currents required to generate sufficient contrast from objects immersed in liquid behind the membrane. This is also important question from the sample's stability point of view since elevated beam currents generate image artifacts, degrade samples, and complicate interpretation of

experimental results (see section 3.2.3 as an example). A measure of image contrast is based upon the Rosen criteria, stating that the signal difference between points (subscript A and B) should be $(S_A - S_B) > 5n^{1/2}$ to yield reliable imaging of the object A behind the membrane over the background B (Fig. 2a) where n is the quantity of electrons reaching the detector¹². The latter can be translated⁵ in to so-called *threshold equation*:

$$I_B > \frac{16}{\eta_s T C^2} [pA], \quad (4.8) \text{ where } \eta_s \text{ is the}$$

backscattering coefficient of the sample under the membrane, T is the time to complete image scan (in seconds) and $C = (\eta_{sA} - \eta_{sB}) / \eta_{sA}$ is a contrast between the sample and background. Using (4.8) equation Thiberge *et.al.*⁵ calculated the minimal SEM beam current needed to image characteristic objects immersed in water (Table 1). The data shows that reliable imaging of samples like living cells with effective Z numbers and back scattered coefficients close to water require more than four orders of magnitude

<i>Material</i>	<i>Z</i>	<i>η</i>	<i>Contrast to water</i>	<i>I_B (pA)</i>
Water	7.22	0.075		
Cells	7.07	0.073	0.027	2900
Oil	5.8	0.055	0.267	30
Gold	79	0.78	0.9	0.25

Table 1. Calculated minimal SEM beam current needed to image few characteristic objects immersed in water using threshold equation. Scanning time T=100 s and η values for 20 keV were used; adapted from Ref [5]

larger beam current compared to materials with larger Z as gold. Potentially, this is a limiting factors of the liquid SEM of unstained living cells since such this radiation doses would lead to direct radiation damage of the cells or their indirect degradation due to series of chemical reactions induced in surrounding water (see also sections 3.2.3 and chapters 7, 16, 18, 24).

The diameter of the primary electron beam broadens due to scattering by the membrane and enclosed liquid media (Fig. 4a) thus probe beam diameter increases with increased sample depth. Probe beam diameter increase in liquid cell SEM was analyzed both theoretically and experimentally by Thiberge et al.⁵ Effective electron beam diameter in the liquid is described as:

$$d_{eff} = \sqrt{d_b^2 + d_m^2 + d_{water}^2(t)} \quad (4.9)$$

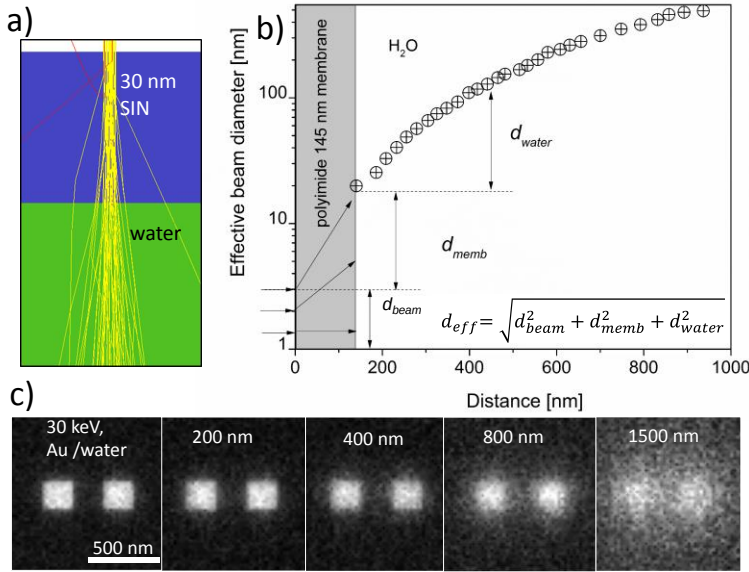


Figure 4 a) Monte Carlo simulations beam broadening while passing 30 nm SiN membrane and impinging the water layer; b) Effective electron beam diameter d_{eff} in water as a function of the probing depth (the data points were adapted from Ref. [5]); c) the simulated SEM images of the two separated 200 nm x 200 nm square and one micron long Au rods as a function of the water layer thickness between 30 nm SiN membrane and the sample

(here d_b is an electron beam diameter, d_m is beam broadening due to scattering inside the membrane and $d_{water}(t)$ – beam broadening while passing the water layer of thickness t). From Monte Carlo electron trajectory simulations for $d_{water}(t)$, the authors determined the beam diameter as a function of depth for the object in water as shown as Fig. 4b. This equation implies that the beam diameter is expected to be as large as 100 nm at ca. 400 nm below the surface of standard 145 nm polyimide membrane and two objects separated by 200 nm would barely be resolved at these conditions.

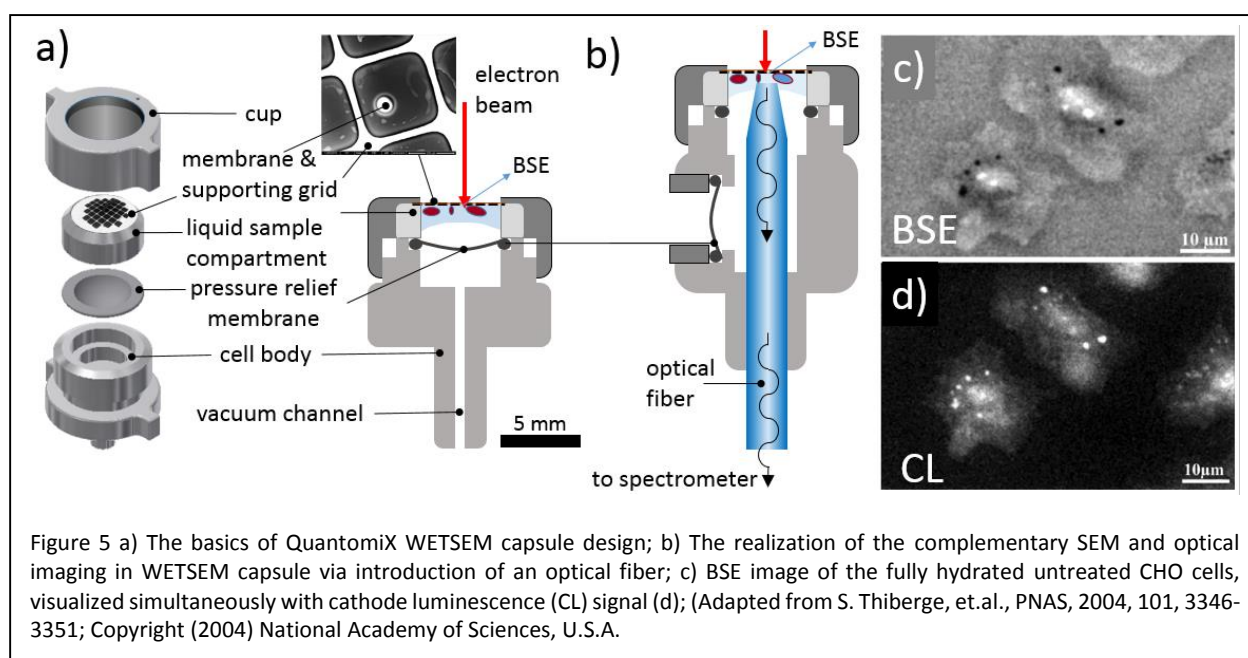
In practice, the experimentally observed resolution for objects with $Z > Z_{water}$ was found to be much better compared to simulated one and the latter one can be seen as a lower limit. This improvement in resolution is attributed to much higher

electron scattering from high Z objects immersed in water compared to water background. In this case, resolution is not defined solely by incident probe beam diameter in water (as it would be the case for the object with comparable Z) but mainly by the size of the object and by dramatic increase of the BSE signal when beam hits the object (compare BSE yield in Figures 2a and 2b). To illustrate this point, the simulated SEM images of the two separated 200 x 200 nm², 1 μm long Au rods are depicted in the Figure 4c as a function of the water layer thickness between 30 nm SiN membrane and the sample. The simulation demonstrates that two strongly absorbing high Z objects can be resolved up to two microns from the membrane using 30 keV primary electrons. In general, the broadening of the electron beam in liquid leads to the rule of thumb (see Ref.⁵) that high Z objects can be detected if their size is comparable to the local size of the beam in liquid. The contrast from the object whose size is two-three times smaller than the broadened beam diameter reduces the SNR of the imaged object. This effect is illustrated by simulating image conditions of the particles. When beam diameter exceeds the size of the nanoparticle (at the depth above 600 nm) both the resolution and contrast deteriorate quickly, as shown in Fig. 4c. This result implies a limitation on maximal depth where the particles of certain size can be detected and resolved. It is important to note that increasing the probe beam energy improves the depth and resolving power of

liquid cell microscopy but does not necessarily leads to optimal imaging, since electron scattering cross section becomes reduced with energy and small nanoparticles even being close to the membrane would not have enough material to provide a measurable BSE signal.

Part 4.3 Few examples of environmental cell designs and liquid SEM applications

Recent progress in liquid electron microscopy is a direct result of success in high yield microfabrication technology of ultrathin membranes developed for MEMS devices, ultrafiltration and X-rays (electron) transparent windows and development of commercial available tools for research application. A number of vendors offer an array of Si, SiO₂, Si₃N₄ (SiN), SiC, polyimide and other membranes with the thickness ranging from 5 nm to 200 nm which can be used for assembling custom made liquid cells. To date the majority of the liquid cell SEM results have been obtained using Quantomix wet cells, though alternative commercial membrane based liquid cells have become available recently.¹³



¹⁴ In this section we describe the commercially available Quantomix wet cells and related research that extends this instrumentation to new applications.

4.3.1 Quantomix WETSEM capsule design

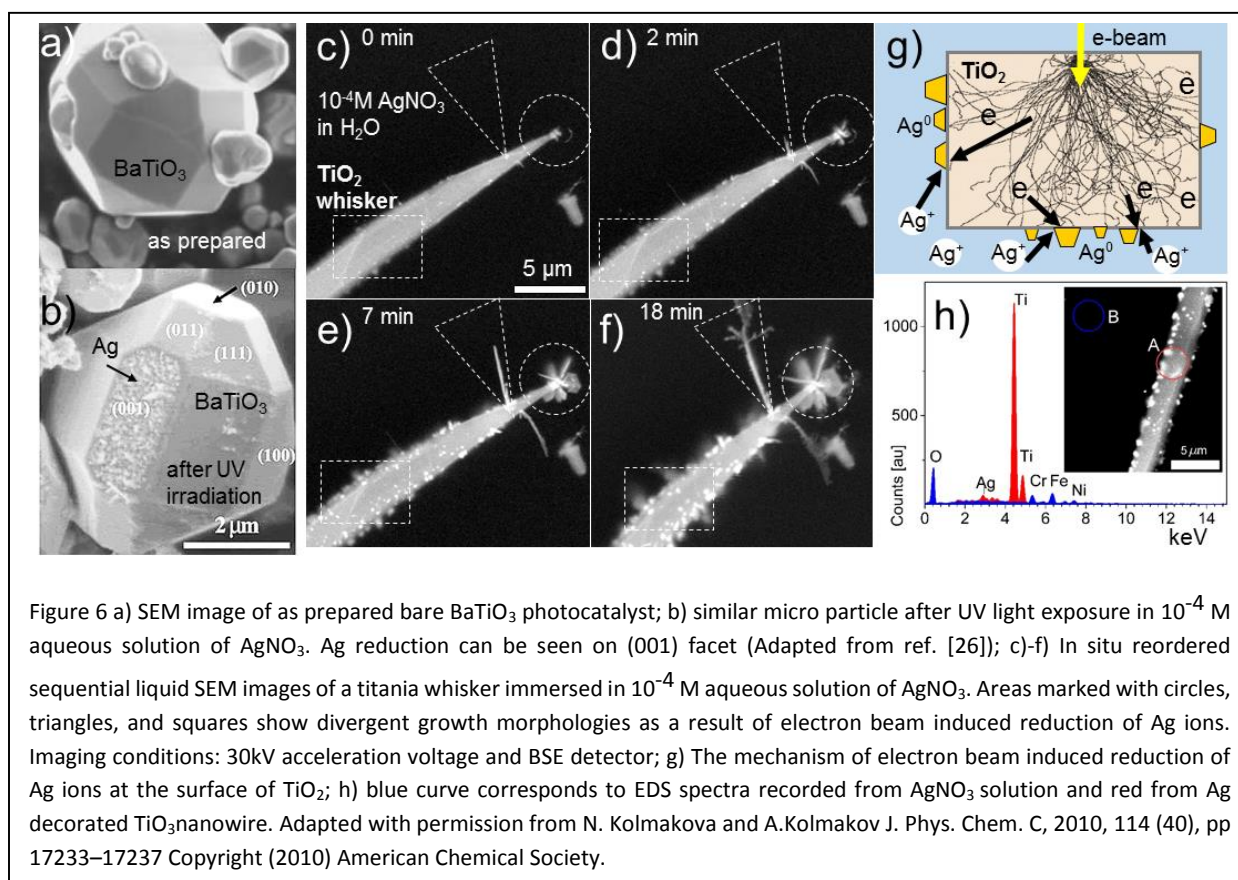
The development of the WETSEM technology by Quantomix¹⁵ in early 2000s was a response to growing demand for *in vivo* imaging of biological tissues and live cells with spatial resolution better compared to conventional light microscopy. Detailed designs, capabilities, and applications of this approach can be found in the literature^{5, 15, 16}. The cell principal design is depicted in the Fig. 5 and it consists of two parts: the top sample dish with electron transparent membrane covering the sample compartment and the stub. The cell can be vacuum sealed, with a rubber membrane used to joining the two elements. The sample compartment can be filled with ca. 15 μ L of liquid sample. Several modifications to the conventional cell body design were proposed.¹⁵ Proposed modifications include insertion of fiber optics for correlative SEM and cathodoluminescence imaging of hydrated samples¹⁶ (see Figure 5 b-d). The Quantomix WETSEM is enabled with a robust 145 nm thick polyimide electron transparent membrane supported by metal grid. The grid provides sufficient mechanical stability for the membrane to withstand atmospheric pressure

differential and yet offers a large segmented ca. 4 mm² FOV. The rubber membrane that separates the two elements of the liquid cell is a thin elastomer material that expands in vacuum and functions as a pressure relief element that reduces the risk of polyimide membrane rupture during cell closing and SEM vacuum pumping.

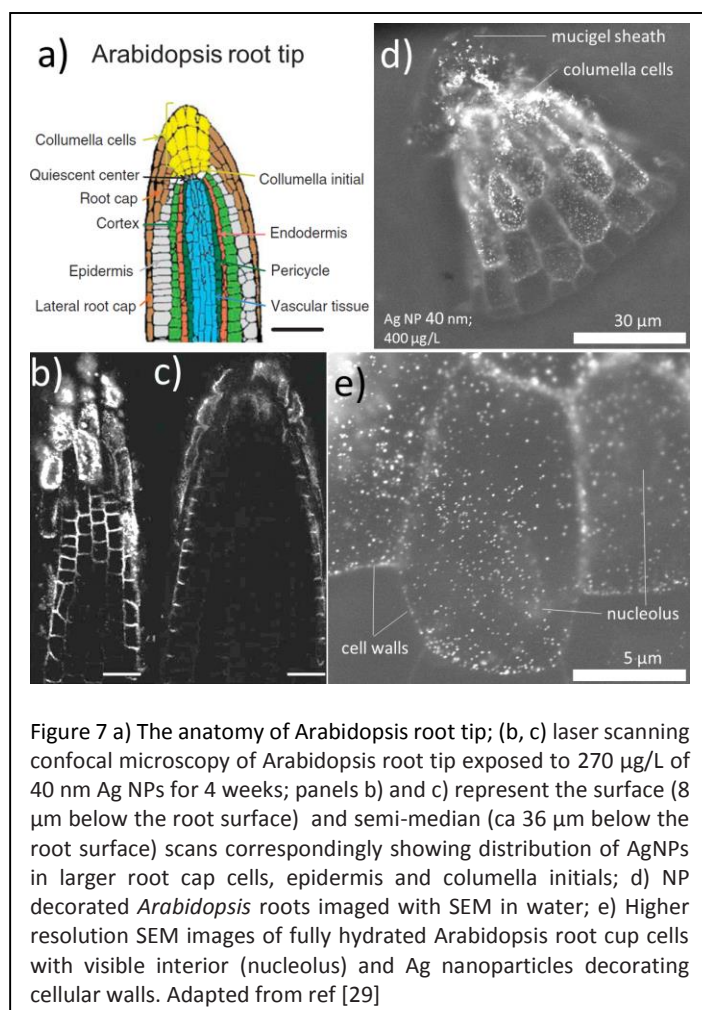
The 145 nm thickness of the polyimide membrane implies usage of *BSE* with energies in excess of 10 keV for SEM imaging of wet samples (see Figure 3). Due to strong attenuation of the *BSE* signal by the membrane and interfacial water layers, it is crucial requirement to have a sample in a close proximity or adhered, to the inner surface of the membrane. Thus a number of protocols that functionalize the membrane surface to promote nanoparticles adhesion as well as cells culture growth have been developed. To work with wet biological tissue, biopsies, plants sections and other soft samples an upgraded capsule was designed¹⁵. The latter was equipped with the special spring loaded piston which gently pushes the tissue against the membrane.

4.3.2 Applications examples

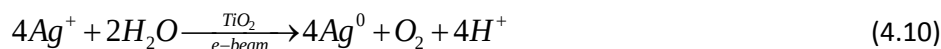
4.3.2.1 Water remediation



Numerous studies in variety of fields have been performed using QuantomiX cells.¹⁷⁻²³ The selection of research described in the Fig. 6 demonstrates use of the wet SEM cells for *in situ* observation of the metal remediation in water by TiO₂ based photo catalysis²⁴. Electron-hole pairs generated in catalysis particles by the UV radiation from the Sun can diffuse to the surface and reduce/oxidize ionic and molecular pollutants in water such as heavy metal ions²⁵. An example of such a photo catalytic reaction is UV photon induced reduction of ionic Ag to metallic nanoparticles at the surface of BaTiO₃ catalysis in AgNO₃ water



decorated with metal particles, as depicted in Fig. 6 c-f. The origin of this phenomenon is similar to photocatalysis and is due to electron induced reduction of the Ag ions from the solution at the surface of the TiO_2 nanostructure. The difference between these two experiments is that electron-hole pairs are created in TiO_2 by the primary electron beam in the liquid cell system. Assuming that the stoichiometry of the electron induced reaction taking place at the surface of TiO_2 nanowire submerged in AgNO_3 solution is analogous to the classical photocatalytic reaction (photoreduction) ²⁷ one can write:



where the first $\frac{1}{2}$ reaction is a transfer of an excited electron to the Ag^+ bound to the TiO_2 surface, leading to the growth of Ag^0 domains with high quantum yield. The hole transfer to the surface-bound water or hydroxyl groups completes the second $\frac{1}{2}$ reaction. The diagram of the electron induced silver deposition process is depicted in the Fig. 6g. Evolution of the EDS spectra before (blue curve) and after (red) nanoparticle growth demonstrate the accumulation of Ag depositions on the nanowire and the feasibility of recording of EDS spectra on objects in liquid cells.

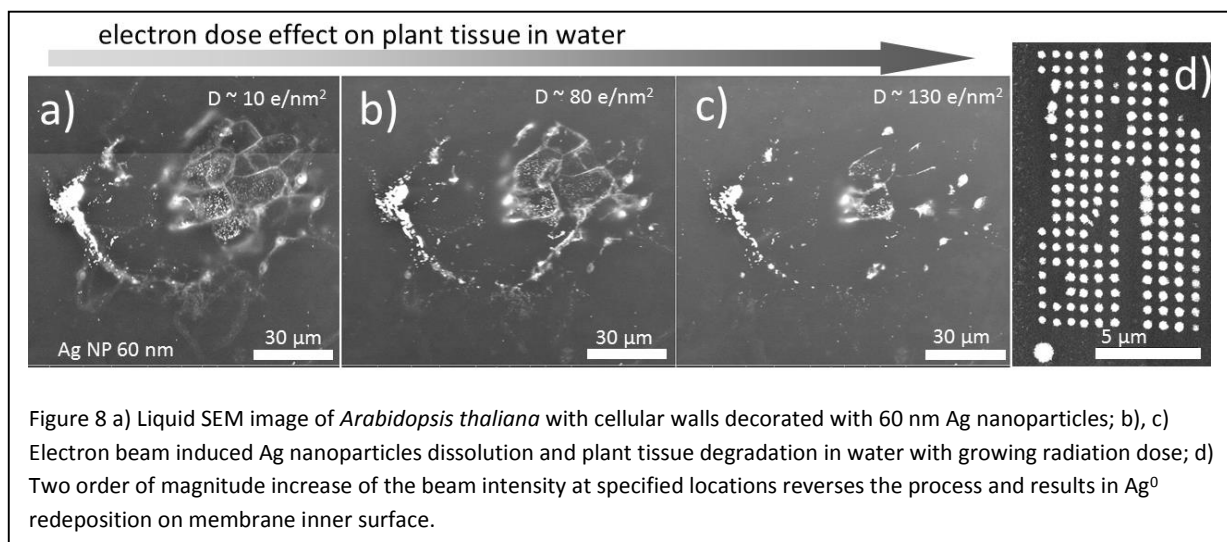
4.3.2.2 Phytotoxicity

The growing production of nano-enabled materials such as engineered nanoparticles (ENP) raises concerns regarding waste byproduct and potentially negative ENP impacts on human health and

solution²⁶. Fig. 6 a, b demonstrates an *ex situ* SEM comparison of BaTiO_3 photo catalyst before and after illumination with UV light. Since BaTiO_3 is ferroelectric, it has a spontaneous polarization which affects the chemical reactivity of different crystal facets. As can be seen in the Fig. 6b, Ag becomes deposited only on (100) facet implying that the internal polarization promotes the photo catalytic action to occur predominantly at this interface²⁶.

Observation of such a process *in situ* is possible using QuantomiX WETCELL instrumentation. In this case, TiO_2 single crystal whiskers are adhered to the liquid cell inner membrane and in either a pure water or ca 10 μL of 10^{-4} M AgNO_3 aqueous solution was dropped into the E-cell compartment. No changes occur in morphology and shape of the titania nanostructure as a result of exposure to a 30 keV electron beam in pure water. Conversely, imaging TiO_2 single crystal whiskers in an AgNO_3 solution shows the TiO_2 nanostructure becoming progressively

ecosystems. Plants are direct or indirectly consumed element in the human food chain motivating the investigation of the uptake and accumulation of ENP by plant roots and leafs. Therefore, an application of liquid SEM is ENP phytotoxicity on living plants. We test the capabilities and limitations of this imaging modality to probe the *in vivo* uptake and accumulation of nanoparticles and metal ions by plants at the cellular level. A recent example of such an approach is a study of cellular accumulation and subcellular transport of Ag nanoparticles in *Arabidopsis thaliana*²⁸. *Arabidopsis* root anatomy is well understood (Figure 7a) and is often used as a model plant system. In this experiment, a diluted Ag nanoparticles-water colloid was added to a hydroponic nutrient solution (Hoagland solution) where seeds of *Arabidopsis thaliana* were germinated and different sizes and concentrations of Ag ENPs were tested. After controlled growth and uptake, the tips of the plant roots were sectioned in a wet state and adhered to the back side of the electron transparent membrane of the QuantomiX WETSEM and cell was filled with water. Figs. 7 b, c shows two laser scanning confocal microscopy optical sections of the root tip exposed to 40 nm Ag ENP and recorded at near surface and at the median part of the root correspondingly. Correlative liquid cell SEM images are depicted in the Figs. 7 d, e. Significantly improved resolving power and signal to noise (SNR) ratio from the WETSEM instrumentation reveal that after two week of roots exposure to ENP, the accumulation predominantly takes place at root cap, at columella cells, and border cells²⁸. At low concentrations and exposure times the cell walls of those are coated with individual nanoparticles at surface densities sufficient to impede incoming solute transport. The detailed SEM image analysis of the intercellular regions (supported with ex situ TEM studies) indicated the presence of Ag NPs aggregation at plasmodesmata connecting adjacent cells. Ag ENP aggregation at plasmodesmata may lead to blockade of the symplastic transport between cells and thus degrade system viability.



4.3.2.3 High radiation dose effects

In spite of the demonstrated advantages of liquid cell SEM for high resolution analysis of ENP fate in plants, limitations exist for application of this technique to biological objects. Inelastic interaction of the probe beam with liquid water and biological material results in the generation of a variety of ionized and excited molecular species in the interaction volume (see chapters 7, 9 and references therein²⁹). As a result, steady state concentrations of strongly reducing and oxidizing species such as hydrated electrons (e_h^-), hydroxyl radicals ($OH^\bullet$), hydrogen peroxide H_2O_2 , hydrogen radicals ($H^\bullet$), H_3O^+ as well as molecular hydrogen and oxygen (H_2 , O_2) are formed in and around the interaction volume. Assuming that the radiation dose rate in standard SEM measurements is somewhat larger (ca 10^8 to 10^9 Gy/s) compared to

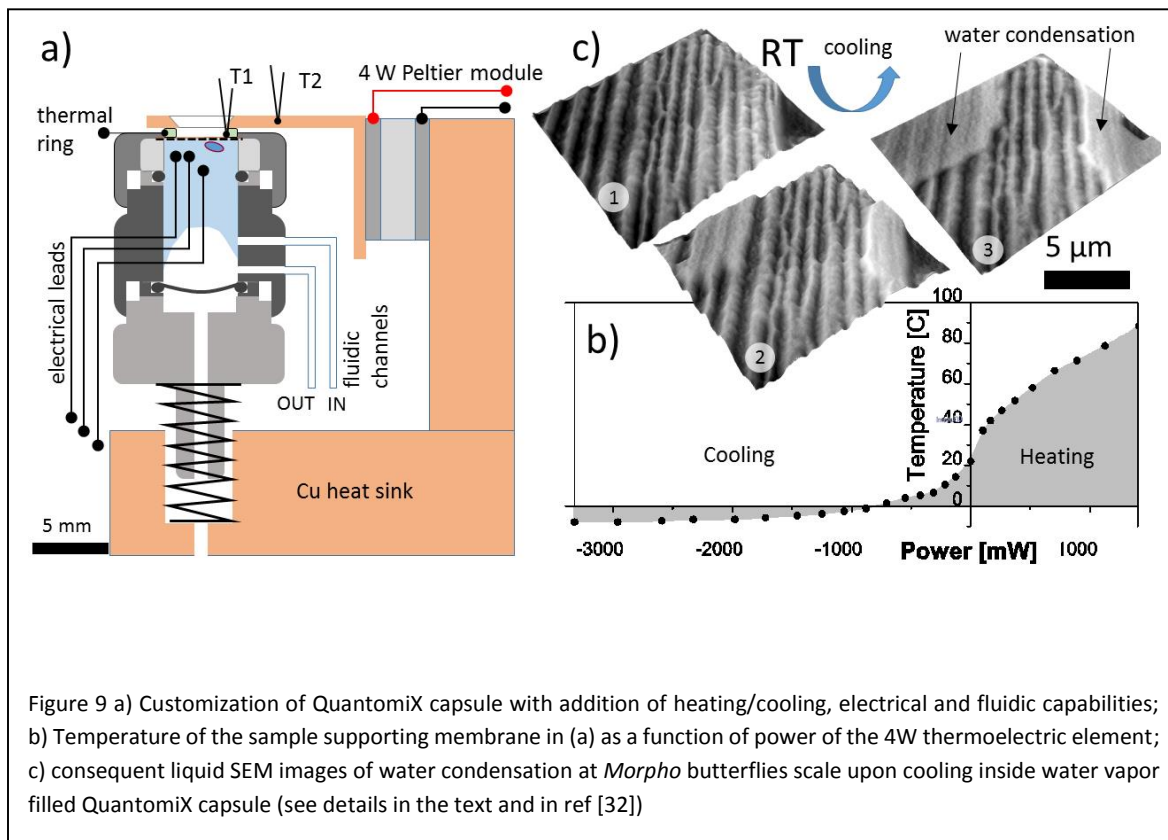
TEM case (ca 10^8 Gy/s), and following the scaling laws deduced in ref. ²⁹, one can expect the steady state molar concentrations of $\text{OH}^\bullet$ and H_2O_2 species to be in excess of 10^{-4}M . The radiolysis byproducts from probe beam illumination contribute significantly to chemical stability of nanoscopic objects in solution and degradation of biological samples. An example of the probe beam induced degradation of fully hydrated biological tissue is shown in the Fig. 8. Here panels a, b, and c show a selection of sequential liquid SEM images of the root cup of *Arabidopsis thaliana* immersed in water following incubation in Ag ENP. Similar to Fig. 7, nanoparticles accumulate at root cup cell walls and are immobilized. Significant degradation of the cell walls can be observed following low dose SEM imaging (i.e., 10 e/nm^2), while continued exposure leads to complete decay of the cell walls (Fig. 8 c) evidenced by reduced image contrast as well as release and dissolution of decorating 60 nm Ag nanoparticles.

Noticeably, increasing the dose by two orders of magnitude for the same solution by increasing scanned probe beam dwell times results in aggregation of Ag on to the back side of the membrane (Fig. 8 d). This interplay between ENP dissolution and growth phenomena as function of electron dose rate can be explained by the radiolysis conditions driven by the incident probe beam. Local redox reactions with participation of metal ions are controlled by relative concentrations of primary reducing (e_h^-) and oxidizing ($\text{OH}^\bullet$) agents (see chapter 7 of this book). The relative concentration of solvated electrons increases with incident probe beam electron dose driving reduction of $\text{Ag}^+ + \text{e}_h^- \rightarrow \text{Ag}^0$ and growth of Ag depositions. At lower beam intensities, the opposite process occurs where metal dissolution prevails. The probe beam dose borderline between deposition and dissolution was determined to be between 10^{-9} and 10^{-10} Gy/s for TEM experiments, a value close to SEM dose imaging conditions.

These recent findings will help better control of the probe beam induced nucleation and growth processes in liquids as well as promote the development of SEM electron beam induced deposition (EBID) process in liquids, reviewed in Chapter 14.³⁰ Coupled with electrochemistry, the latter process will have drastically increased selectivity and deposition yield compared to its vacuum analog. Furthermore, a much greater moiety of chemical and bio species can be used for EBID in liquids. Flushing of gas or liquid media inside E-cell can be easily done without braking SEM vacuum enabling nanoscopic sequential deposition of different chemical species in the system.

4.3.3 Prospective upgrades of QuntomiX WETSEM capsules

Currently the QuantomiX WETSEM capsules are mainly used by the bio-medical research



community and the usage of these E-cells for materials research is limited. Adoption of this instrumentation by the materials community is restricted by the limited capabilities of these capsules to vary the temperature of the imaged sample as well as altering *in situ* its chemical and electrical (electrochemical) environment. Development of these capabilities would broaden the utility of WETSEM systems for martial science application. Our Initial efforts to develop these instrumentation capabilities is depicted in the schematics of Fig. 9, where central stub of QuantomiX QX-302 capsule has been modified to be used for connection of multiple electrical leads and tubing for gas or fluid delivery. Use or removal of the metal coating of the standard capsule stub assembly enables control of electrical connections to the capsule and fluid solution, while vacuum sealing of electrical leads and fluidic tubing is enabled by application of UV curable glue.

Thermal control over the sample environment was enabled through development of a simple heating and cooling system based upon a miniature 4 W Peltier element and copper heat sink (Fig. 9a)³¹. In this system, the heat from the thermoelectric module is delivered to the electron transparent membrane using a copper bar connecting the membrane supporting metal grid of the capsule to the thermoelectric module. The bar is spring loaded against the membrane supporting mesh and maintains thermal contact with the grid while enabling capsule assembly and disassembly. Using this setup, temperatures as low as -10 °C and as high as 100 °C can be achieved at the membrane (Fig. 9b.). The use of more powerful thermoelectric elements enables a larger range of system temperatures.

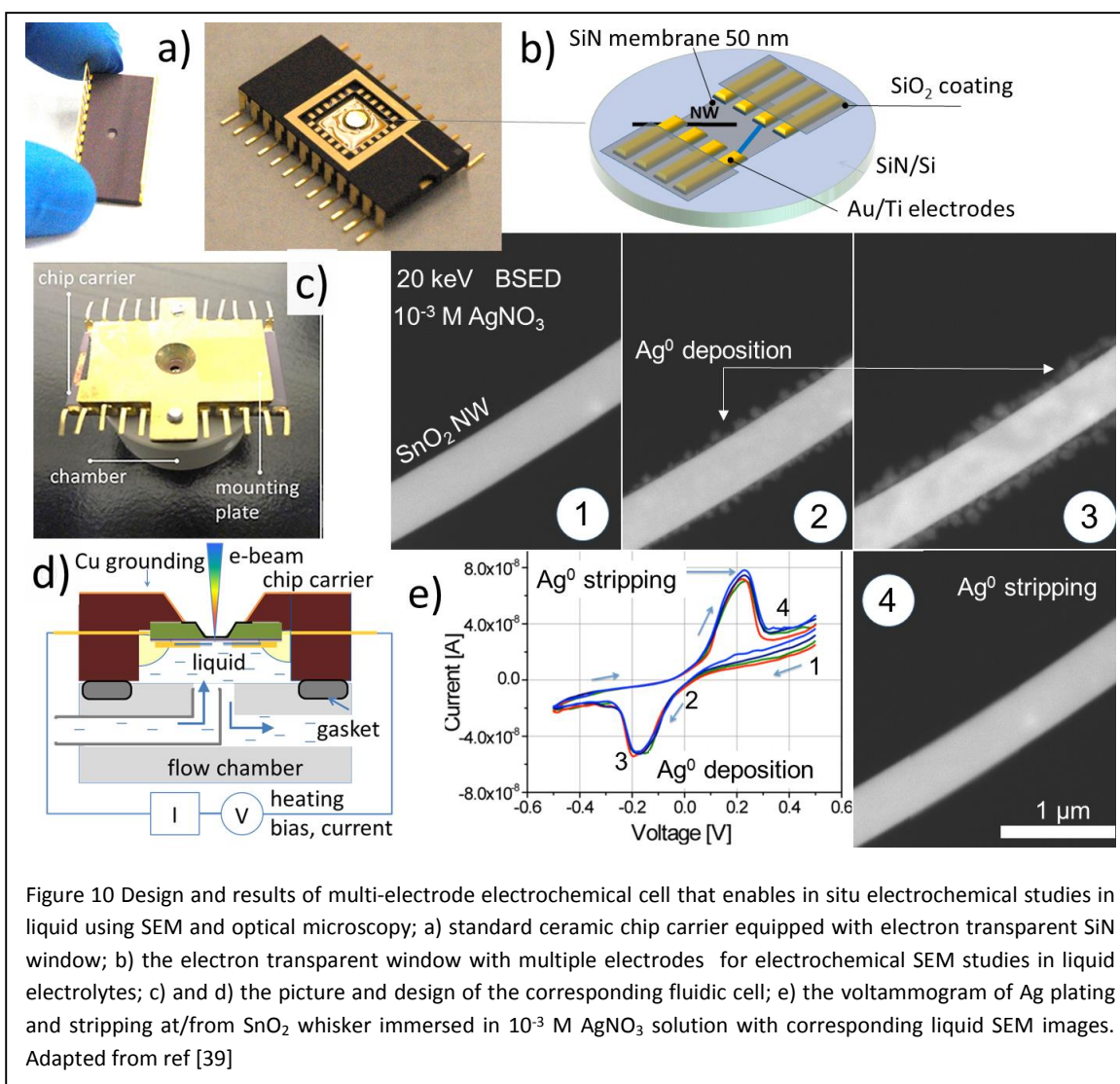
The ability to cool or to heat samples in their native gaseous or liquid environments at a variety of pressures opens new possibilities in SEM which cannot be achieved using commercially available cryo- or heating stages. For example, the particularities of water condensation, wetting and flow in 3D photonic structures enable understanding the fundamental processes controlling optofluidics^{32, 33} and sensorics³⁴.

Fig. 9 c shows mesoscopic details of initial stages of water condensation at the surface of natural photonic structure: butterfly wings. The butterfly scales were adhered to the back side of electron transparent membrane which can be heated or cooled inside the QuantomiX capsule. The capsule contained the water droplet separated from the membrane thus dew can be formed or evaporated via cooling or heating the membrane loaded with butterfly scales. Panel 1 of Fig. 9 c shows a SEM image of the characteristic mesoscopic ridge structures of a stacked periodic layers of cuticle separated by the air gaps of a dry *Morpho* butterflies scale. For comparison, panels 2 and 3 of Fig. 9 show sequential images of the same region upon cooling in the presence of water vapor. In this figure water first condenses at the top of the ridges after which the condensation front spreads along the ridges. As recently discovered, this particular condensation pattern is due to existing gradient of surface polarity along the height of the ridge: from the polar ridge tops to the non-polar bottoms³⁵. The water therefore preferably condenses and spreads along the top part of the ridges leaving air pockets deeper inside the scales resulting in a hydrophobic wing structure that allows the butterfly to shed water droplets. Note that the improved lateral resolution of this process is achieved using conventional ESEM vs. a liquid cell system. However the former approach does not allow decoupling of thermal and pressure induced processes and has limited pressure range.

4.3.4. Example of a custom design of the E-cells for electrochemical studies in liquids

Increased functionality of the environmental cells used for electron microscopy is a current trend in TEM and STEM studies. Several types of fluidic, heating and electrochemical cells have become available recently^{36, 37}. And many of the commercially available or custom developed E-cells for (S)TEM can be directly adapted to SEM research as well^{14, 38}. The latter approach, however is rarely adopted by SEM community since the core advantage of the SEM: large field of view, fast exchange and analysis of arbitrary samples cannot be easily implemented in thin TEM flow cells with characteristic submicron fluidic flow channels. As a result, several custom made E-cells specifically designed for complex SEM studies have been reported³⁹⁻⁴¹. One of these simple and cost effective instruments is described below.

Recent energy and environmental initiatives resulted in growing application of SEM in liquids and dense gases including research into morphological and compositional alterations of nanoscopic devices



and interfaces during operation in realistic environmental conditions. In many research settings there often require variety of objects within the imaged FOV for comparative or combinatorial analysis. The latter experiment requires development of the complex circuitry on electron transparent membrane and parallel indexing of multiple electrodes. A practical solution to this experimental challenge is to employ SiN membrane windows on SiN/Si chip with lithographically defined electrodes array. Such an electronic chip-with-a window can be wire bonded to standard vacuum compatible ceramic chip carrier (Figure 10 a,b), requiring only slight modification such as a millimeter size hole through the center of the device³⁹. For electrochemical studies, the surface of the electrical leads and other metal surfaces are coated with SiO₂ insulating layer, as shown in Fig. 10 b. The assembly can be used in two different setups. For correlative optical and electron microscopy the 70 μL cavity was filled with a 1 mM solution of AgNO₃ to act as a model electrolyte and covered with a glass lid sealed to the device with UV curable adhesive. The glass lid acts as a viewing port making the cell usable with both optical and SEM, thus enabling correlative microscopy analysis. Alternatively, the fluidic chamber can be attached to the chip carrier (Figure 10 c, d) to enable exchange of the solution within the device. Fig. 10 e shows a cyclic voltammogram of Ag plating (stripping) from AgNO₃ water solution on to (from) SnO₂ nanowire wired as a working electrode. The

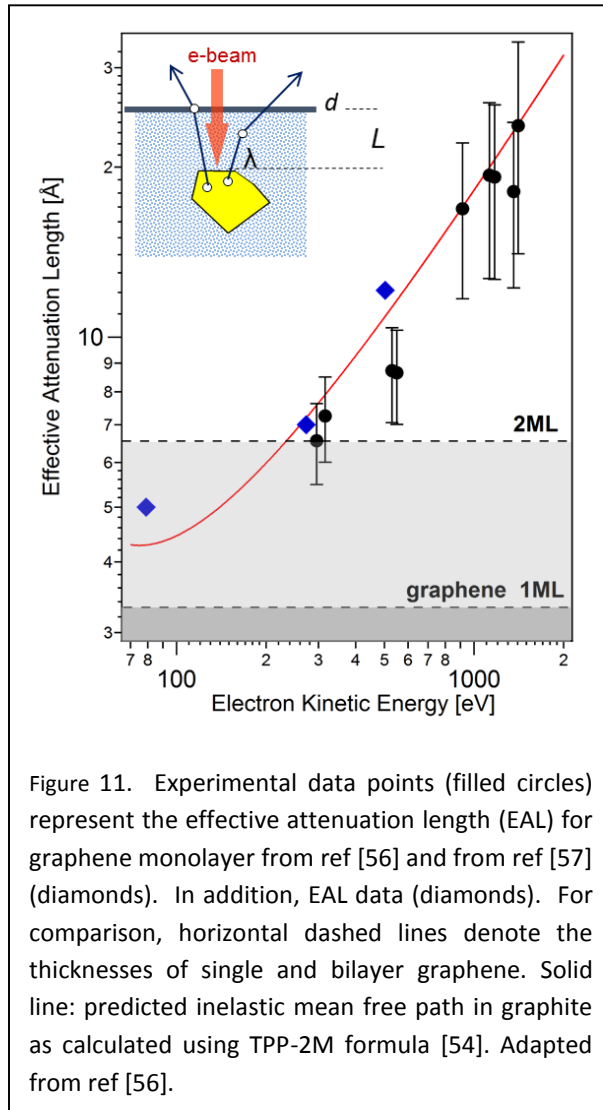
corresponding in situ liquid SEM images demonstrate the morphology of Ag^0 deposit from the solution as well as its complete stripping at a reverse potential. An in-situ comparative energy dispersive X-ray (EDS) analysis has been recorded (not shown here) before and after the deposition of Ag supporting the deposition of pure silver on to the surface of the SnO_2 nanowire.

Part 4.4 Novel 2D materials as electron transparent membranes for liquid SEM cells

4.4.1 Why do we need them?

In spite of great progress in implementation of SiN, SiO_2 and polyimide membranes in liquid SEM (STEM) cells, reduction of beam scattering and signal attenuation by the membrane itself would improve SEM image quality, analytical capability, and reduce electron dose into the sample. The strategy to improve the scattering and transparency of the membranes are based upon the reduction of their thickness and atomic Z number of the membrane materials. Commercially available SiN and SiO_2 -based membranes fabricated via SiN (SiO_2)/Si wafer back KOH etching process are as thin as 5 nm. However, due to fluctuations in Si etching speed, such membranes can be produced reliably with minimal window sizes of ca 10 microns or larger. With large effective diameters such thin windows are prone to mechanical collapse under the one atmosphere pressure differential in vacuum systems and are thus not ideal for liquid SEM E-cells. Prospective technological approaches based upon fabrication of large-area perforated-supporting membranes with micron size orifices enable much thinner membranes. Such electron transparent windows are the base of modern E-chips of ambient pressure TEM and can in principle be adapted for ambient pressure SEM studies.

An alternative approach to reducing atomic number and membrane thickness includes novel free standing membranes^{42, 43} made of single atomic layer two dimensional (2D) materials such as graphene, graphene oxide and *etc.* These membranes are impermeable to liquids and gasses and enable new capabilities for TEM and SEM in liquids. The two major differences with respect to standard SiN or SiO₂ membranes are that graphene based membranes have extremely high breaking strength⁴⁴ that facilitates fabrication of atomically thin electron transparent windows such that inelastic electron mean free path



the conducting liquid media.

e) Longer stability of the graphene membrane with respect to knock-on process since SEM electron beam energy is well below the energy threshold for graphene (~80 keV)⁴⁶.

The electron transparency of the 2D free standing graphene, boron nitride or graphene oxide membranes or coatings was evaluated in multiple reports using TEM^{43, 47, 48} for high energy electrons as well as point projection microscopy^{49, 50} and SEM⁵¹ for slow ones. Complementing surface science studies, the electron attenuation length (EAL) parameter was used as an equivalent to inelastic mean free path assuming that elastic scattering is negligible as signal returns to the detector⁵². EAL relates the initial electron flux of secondary and back scattered electrons (I_0) from objects under the membrane to the one (I) measured by the detector:

lengths in single layer graphene, for an example, are larger than its thickness at SEM range electron acceleration voltages. We infer from this property, that incoming and outgoing electrons can pass such a membrane without significant attenuation. Opportunities are even more intriguing for very low energy electrons (<5 eV)⁴⁵. For such electrons, the electron-electron scattering is further hampered and electron – phonon scattering is inefficient. Therefore for low energy secondary electrons these membranes can be nearly totally transparent. Thicker multilayer membranes (e.g., 1 to 2 nm) have higher strength and more robust performance. These membranes are thus practical for liquid SEM measurements. As a result of these properties new opportunities for SEM emerge:

a) True SEs can be used for imaging the surfaces of objects covered with such a membrane. Therefore data can be acquired from specimens at ambient pressure, in fully hydrated conditions, with SEM resolution and surface sensitivity.

b) An array of powerful surface sensitive analytical techniques such as XPS, AES, NEXAFS etc. can be applied to such objects. Additional advantages include:

c) Smaller primary beam energies can be used enabling smaller excitation volumes and reduced beam induced damage

d) Negligible charging effects due to fast neutralization of the accumulated charge either by conducting electrons (in G, GO) or in case of dielectric membranes (e.g. BN) via tunneling from

$$I/I_0 = \exp\left(-\left(\frac{L}{\lambda_M} + \frac{d}{\lambda_G}\right)\right); \quad (4.11)$$

here λ_G , λ_M are EALs for graphene membrane and the media between the object and the membrane while d and L stand to thickness of corresponding layers, as shown in the inset of Fig. 11). This method is widely used to determine the thickness of overlayers for materials with known EALs⁵³. Using this calculation, EALs for graphene and graphene oxide were measured via collecting the substrate's XPS^{54, 55}, AES⁵⁶ and secondary electrons⁵⁷ signals attenuated by the known amount of graphene or GO layers. Fig. 11 compiles some of this experimental data and compares them with theoretical predictions for carbon⁵³. The reasonable agreement can be observed for the electrons acceleration voltages exceeding 70 eV. EALs for energy ranges below this value have yet to be explored.

4.4.2 Preparation of the graphene and graphene oxide membranes

Graphene oxide (GO) was the first 2D material tested as an electron transparent window for SEM E-cells^{54, 58}. The attractiveness of GO membranes stems from the well-developed, high-yield GO production protocols of this material as discussed by Park and Rouff⁵⁹ and references therein. Conveniently, chemically exfoliated GO flakes have hydrophilic edges due to terminating COOH groups and hydrophobic basal plane due to the presence of domains of intact graphene. Being amphiphilic, GO flakes in water solutions segregate at air-water and water-solid interfaces to form membranes^{60, 61}, thus enabling Langmuir-Blodgett or simple drop casting methods to be applied to fabricate suspended membrane. When dried, such membranes (often called GO-paper when thicker than few microns) have excellent 100 MPa⁶² tensile strength that can be further enhanced by GO functionalization with divalent ions like Mg²⁺ and Ca²⁺⁶³.

Many different approaches to fabricate GO suspended membranes have been reported^{43, 54, 58, 64}. We have used diluted GO water or methanol solutions to cover micron wide orifices with a single flake of GO using Langmuir-Blodgett deposition method⁵⁴. This procedure can be repeated multiple times to fabricate multi-layer membranes. Such membranes are molecular impermeable and mechanically stable under pressure differential if their diameter is below 3-5 microns. Larger and therefore thicker membranes can be easily fabricated simply by drying of the GO solution over the small orifices⁵⁸. Alternatively, GO solution can be dried on the support which then can be suspended with a selective backside chemical or electrochemical etch^{58, 64}.

As shown in recent reports⁶⁴⁻⁶⁶, GO membranes made of interlocked flakes have selective permeation properties that favor the capillary uptake and diffusion of water between stacked GO flakes. GO permeation property results from GO flake separation distance d which is between 7 Å and 11 Å, depending on degree of GO reduction. The flake separation distance is sufficient to accommodate one to two layers of water. Interestingly, such GO membranes are vacuum tight for many other gases and liquids such as atmospheric gases and alcohols⁶⁴. This property makes GO excellent prospective material for selective filtering of media but a poor system for liquid water SEM experimental research. To perform liquid SEM studies with GO membranes one can adopt several strategies: (i) completely cover the orifice with an *individual* single layer or multilayer GO flake using the Langmuir-Blodgett approach; (ii) adopt thicker GO membranes covering larger orifices that have less than one percolation channel inside the orifice. The latter approach can be achieved by accounting for total length l and surface density s of the percolation channels in GO membrane of thickness h scaling as $l \sim hL/d$ and L^{-2} correspondingly, where L is the average size of the GO flake⁶⁴. Thus the number N of the water conducting channels in an orifice of size D is $N \sim (D/L)^2$. Assuming the average size L of the GO flake in solution ~ 10 microns, fabrication of GO water impermeable multilayered electron transparent membranes can be done providing the size of the orifice D does not exceed $L \sim 10$ microns⁵⁸. Furthermore mildly annealed GO in the range between 120 and 150

°C eliminates the percolation water layers, reducing d of the membrane without causing irreversible thermal reduction.

Graphene, on the other hand, can be fabricated as a single layer carbon sheet and therefore is a nearly ideal material for e-cells since it is low atomic number, has a small scattering cross section, chemically inert, ultrathin, mechanically robust and lacks the aforementioned GO drawbacks. The bulge and indentation tests demonstrated that suspended membranes are gas impermeable and yet have unprecedented robustness with stiffness in excess of 1 TPa⁶⁷, what allows to sustain pressure differential of few Bars with diameters of the window below ca 5 μm .

High quality, large scale single and multi-layer graphene is routinely grown by CVD on Cu or Ni substrate and can be easily transferred to the substrate of interest by chemical etching of the substrate and via using PMMA film as sacrificial supporting layers. Many other wet and dry transfer protocols were developed in recent years (see reviews^{68, 69} and references therein). As in the case of GO, graphene membrane can be transferred on to the micron size apertures made by FIB, micro patterning or laser drilling in the SiN, SiO₂ or metal supporting frame. Graphene transfer processes involve removal of the interfacial liquid layer and residual contaminants from the graphene growth and release protocol. This can be best done via slow annealing either in vacuum or under reducing (Ar/H₂ mixture) ambient (see protocols in⁷⁰). The annealing process also improves adhesion of Graphene to the substrate.

4.4.3 Graphene and GO based E-cell design

Two different graphene based closed cell designs have been tested, as shown in Fig. 12. The single use Si

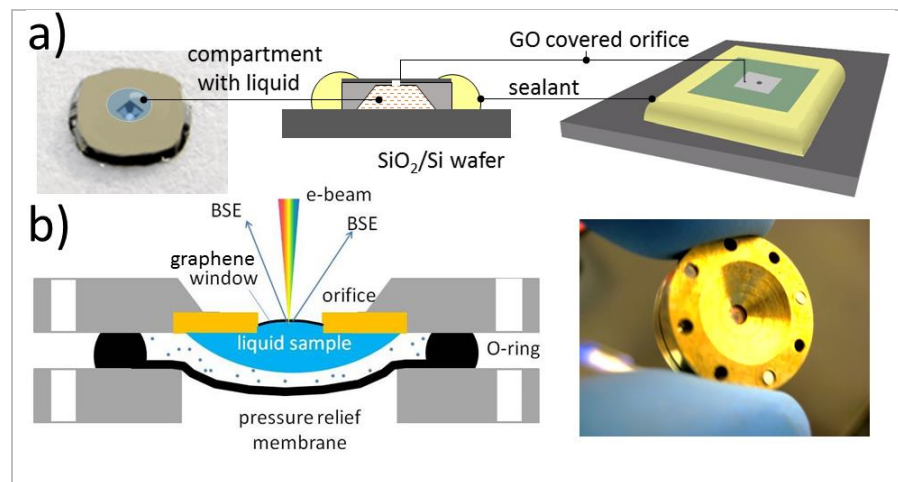


Figure 12

Principle design of closed graphene/graphene oxide E-cell; a) single use cell; b) refillable cell with exchangeable graphene (GO) membranes. Adapted from refs [55] (a), and with permission from M. Krueger et al ACS Nano, 2011, 5, 10047-10054 Copyright (2011) American Chemical Society (b).

based cell consisted of SiN primary membrane with FIB drilled micro-orifice LB covered with one or more graphene or GO layers. The chip was filled with liquid sample and placed on a supporting Si plate (Fig. 12a). Two part device are sealed with UV curable glue. A silver paste patch is used to make a conductive ground for the device. The principle design of the liquid cell with exchangeable graphene or GO windows is depicted in Fig. 12 b. The core of this E-

cell is a metal plate or SiN/Si membrane with a laser or focused ion beam drilled hole for an aperture. The standard PMMA based graphene transfer protocol was used to cover these 1-3 micron orifices. Following deposition of graphene on the surface the system was annealed in air and PMMA was dissolved using warm acetone⁷¹. In the case of GO membranes, the aforementioned Langmuir-Blodgett or drop casting protocols can be used to construct a membrane. An exchangeable disk with a droplet of liquid sample is placed between two metal plates and vacuum sealed using pressure relief rubber membrane gasket. The design of the cell allows isolation of a ca 20 μL sample and reduction of the pressure differential on to the graphene (GO) membrane when the liquid contains an excess of trapped gas and in vacuum.

4.4.4 SEM imaging with GO and Graphene membranes

The performance of the GO liquid cell filled with 50 nm Au colloid water solution can be evaluated from the images in the Figure 13 a, b. Both images were obtained under the same SEM settings but using different detectors: backscattered electrons detector (BSED, Fig. 13 panel (a)) and Everhart-Thornley detector (ETD, Fig. 13 panel (b)) which have larger and lower probing depth correspondingly. Since ETD is sensitive to low energy secondary electrons, greater detail of the membrane surface morphology (such as multiple GO wrinkles) can be seen. The profile analysis of the gray scale values of the individual 50 nm Au

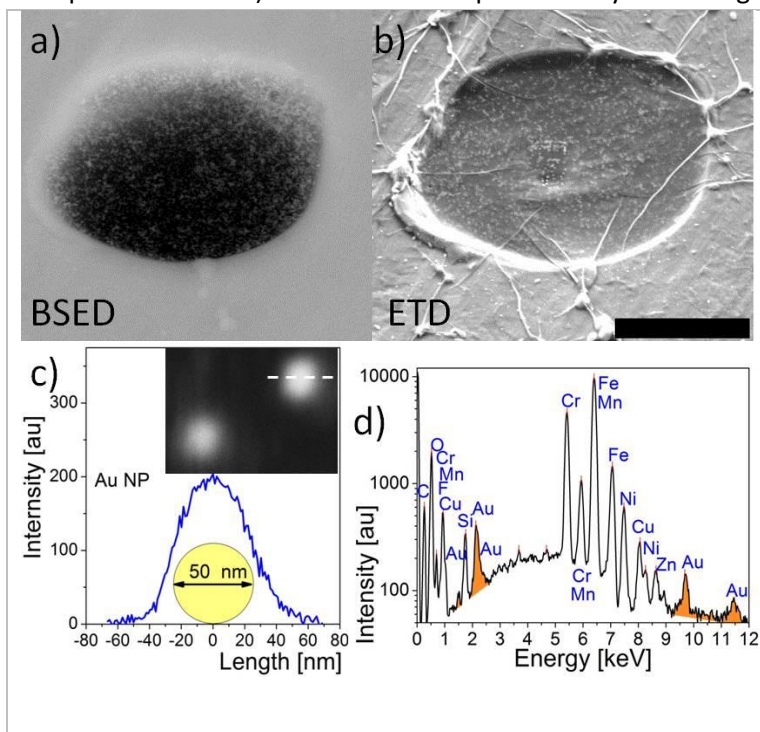


Figure 13

a) SEM image of the Au NP solution through ca 40 nm thick GO membrane. Image conditions: beam energy 30 keV, detector BSE, tilt 39°; b) SEM taken under the same conditions but using Everhart-Thornley detector. The scale bar corresponds to 5 μm ; c) Inset: high resolution SEM image of two 50 nm Au nanoparticles immersed in water and adhered to the back side of the membrane. Gray scale value line profile over the individual 50 nm Au nanoparticle d) EDX spectrum taken from Au NP aqueous solution through the GO membrane. The red peaks correspond to Au lines and unmarked peaks originate from the surrounding stainless steel frame. Adapted with permission from M. Krueger et al ACS Nano, 2011, 5, 10047-10054 Copyright (2011) American Chemical Society.

nanoparticles adhered to the back side of the ca 30 nm thick GO membrane (Figure 13 c) indicates that electron scattering in this low Z membrane does not deteriorate the resolution of the NP image significantly. In addition to imaging, EDX analysis can routinely be performed on a particles through the membrane, as shown in the Fig. 13 d.

The examination of the gray scale variations taken with the ET detector as a function of the beam energy (not shown here) reveals the possibility for imaging high Z Au nanoparticles through the GO membrane using very low electron beam energies (starting from 1 keV). Since the thickness of the membrane (ca 30 nm) is an order of magnitude larger compared to inelastic mean free path for secondary electrons in GO⁵⁴, detected electrons are backscattered and related secondary electrons of type S_2 and S_3 that compose SEM signal¹².

4.4.5. Radiation and chemical stability of the graphene based membranes

Electron beam induced water radiolysis and radical generation is a significant effect in liquid electron microscopy, and usually discussed in terms of impact on to the imaged objects. Different from thick SiN membranes, the direct defect formation or indirect beam induced chemical damage to thin 2D membranes are important issues in liquid SEM measurements since membrane damage limits the life time of the electron transparent windows of E-cells. A selection of the available results are discussed below.

The direct defect formation in the membranes can be due to electron induced atomic displacement ("knock-on" process) or as a result of relaxation of the electronic excitation inside the membrane. The former mechanism has an energy threshold estimated to be 80 keV⁴⁶, and is predicted to be inefficient at probe acceleration voltage energies used in SEM. On the other hand, the cross section for the inelastic electron excitation processes increases with lowered electron beam energies, and is

significant at low keV acceleration voltages. This damage mechanism can result in chemical bonds breakage and local heating. The thermal conductivity of the graphene at room temperature is high (ca 5000 W/m·K)⁷² and no noticeable damage due to electron beam heating effect is expected. This damage mechanism is even less probable when gaseous or liquid media is in contact with 2D membrane.

For practical SEM imaging through 2D membranes it is important to know these threshold doses of noticeable electron induced defect formation and to minimize all aforementioned effects via reducing the electron beam exposure. Beam exposure effects is difficult to define as there is significant variation in published experimental data, experimental conditions, and often dose measurements are not recorded in SEM experiments. This issue is further complicated by a variety of energy dissipation mechanisms during e-beam irradiation of hydrated samples. GO membranes were found to be prone for photo-thermal reduction with a flash light^{73, 74} and laser light illumination. A characteristic threshold was measured to be ca 10² mJ/ cm² for a micron thick GO membrane⁷³ corresponding to an electron beam dose of ca 1 e/nm² for 10 keV electrons, assuming full absorption of probe beam energy by the film. Alternative results indicate that GO films did not suffer reduction from a 20 keV electron probe beam until reaching doses as high as 10³ e/nm².⁷⁵ Data is also available for electron beam induced damage of graphene layers from SEM imaging and electron beam lithography. It has been observed for graphene field effect transistors (GFETs) that the appearance and growth of the disorder D peak in Raman spectrum of the supported graphene as well as concomitant increase of its resistivity (mobility drop) occur after irradiation with doses as low as 20 e/nm².⁷⁶ Bi-layer graphene was found to be significantly more stable to radiation damage. Similar measurements on the suspended graphene FET demonstrated considerably smaller change of the electronic properties upon even larger (10² e/nm²) irradiation doses, while the growth of the D/G Raman peaks ratio remained nearly the same as in the case of supported graphene⁷⁷. The latter observation implies that the observed changes in Raman signatures or electron mobility can be due to beam induced modification (e.g. local charging) of the supporting substrate or electron beam induced carbonization of the graphene layer⁷⁸ but not due to massive defect formation in the graphene itself.

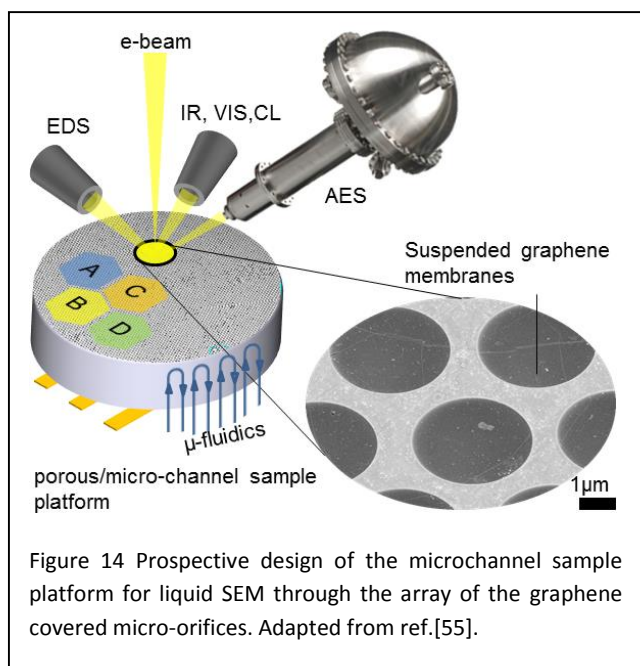
The presence of the dense gaseous or liquid media in contact with graphene (or GO) can catalyze the formation of new or develop pre-existing defects indirectly via generation of ionic or radical species that attack 2D membrane chemically. As it has been shown recently in STM studies of water exposed to graphene, this is particularly relevant to linear defects and grain boundaries in this material⁷⁹. Another example is reduction of the GO by electron beam generation of plasmas produced in inert (Ar), reducing gases (CH₄), or their mixtures⁸⁰. Similar processes occur in water during beam induced water radiolysis which has been extensively studied (e.g. ref.⁸¹ and references therein). Chemically reactive products such as molecular hydrogen (H₂), hydrogen peroxide (H₂O₂) and hydroxyl radicals (·OH) produced in water by radiolysis can accumulate⁸¹ and reduce or oxidize the 2D membrane weakening or dissociating C-C bonds at defects sites. In addition, under prolonged exposure volatile products of radiolysis can eventually segregate as a separate phase forming microbubble under 2D membrane potentially leading to a pressure buildup inside the E-cell and capillary induced collapse of the membrane. We estimated that 20 keV electron beam irradiation of liquid water under the graphene membrane leads to bubble formation following accumulation of critical dose of ~10⁴ e/nm².^{55, 82} SEM operation below bubble forming dose levels enables good image acquisition conditions as data maybe be acquired at dose levels of (~10² e/nm²) from illumination areas ca ~10² μm². This problem can be further reduced in fluidic cells where replenishable solution is used thus enabling removal of radiolysis byproduct from the probe beam scan area.

Outlook

Nanoscale imaging in liquids and at solid- gas -liquid interfaces enables research in the fields of materials science, biomedical, forensics, chemistry and environmental research. We expect membrane based liquid scanning electron microscopy be a growing field of research in years to come. Based on the current trends

one can envision three different lines of near future activities and developments of this microscopy modality:

1. **Theory, modelling and fundamental research** on chemistry and physical aspects of the electron beam interaction with liquid matter and immersed objects. This research includes the understanding of beam induced local chemistry in liquids and dense gases, spatial and temporal distributions of reactive species and their possible influence on the immersed objects. The probing of the different systems such as operational electrochemical and fuel cells as well as living biological objects will continue along with the efforts to minimize beam induced artifacts via improving the sensitivity of the detectors and implementation of modern image processing and data mining algorithms. There is also clear trend to combine different imaging modalities.
2. **Liquid cells designs.** The electron transparent windows with the thickness of 20-50 nm based on modern SiN, SiO₂ or SiC microfabrication processes will be a dominating technological platform for near future liquid SEM. The variety of experimental tasks and objects makes it almost impossible to create a universal liquid SEM cell, therefore, similar to modern liquid TEM trends, some degree of specialization in the cell will take place. For example, fabrication of SiN electron transparent windows dedicated to electrochemical, variable temperature studies, *in situ* micromanipulation and electrical measurements can be envisioned. The overall design of the future liquid cells will be single use monolithic chip with so-called on board fluidics where the source liquids and electrophoretic, electroosmotic or micromechanical pumps are integral parts of the lab-on-chip liquid SEM technology.



micromanipulation and electrical measurements can be envisioned. The overall design of the future liquid cells will be single use monolithic chip with so-called on board fluidics where the source liquids and electrophoretic, electroosmotic or micromechanical pumps are integral parts of the lab-on-chip liquid SEM technology.

3. **Membranes development.** Novel 2D materials with ultimately high electron transparency and mechanical strength will remain the object of active research and technical developments. The driving force behind this line of the research is the unique capability to use powerful surface characterization techniques for liquid interfaces such as XPS⁵⁵. The combination of the requirement to have large FOV with micron size graphene covered orifices will result in implementation of a new environmental cell platform where the membrane is composed of multiple orifices. One of the possible designs of

the graphene based liquid cell is proposed in the Fig. 14. The core of the cell is multichannel or microporous matrix which contains high density isolated or interconnected microfluidic channels. The channels have openings which are covered with graphene membrane. Such a cell can be equipped with heaters and electrodes as well channels can be impregnated with different liquids (A-D, Figure 14). Such a design is favorable for the combinatorial SEM studies with correlative electron (e.g. XPS, AES) or optical (cathodoluminescence (CL), IR, Vis) spectroscopies.

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