Application Note

Poseidon Select™

EDS and EELS analysis in the TEM using the Poseidon Liquid Cell System

Introduction

In situ liquid systems for the transmission electron microscope (TEM) have become increasingly popular over the past several years. This increase arises from the availability of complete, off the shelf *in situ* solutions such as the Protochips Poseidon liquid cell system. *In situ* liquid imaging with the Poseidon system allows users to view samples inside the TEM in native liquid environments, and unlocks a compelling opportunity to analyze materials at the nanoscale in a fundamentally new way.

Although liquid cell imaging provides a platform to obtain new, meaningful information about samples, the technique itself is still in its early stages. Recently, a small community of researchers has dedicated significant resources toward optimizing *in situ* liquid techniques, studying optimal imaging conditions, sample preparation, identification of compatible and incompatible samples, and possibly the most critical factor: the influence of the electron beam. Notable progress has been made, and high resolution images of biological and materials samples, including lattice resolved images, are commonly published. Material dynamics such as real time video of nanomaterial nucleation and growth, sample-sample or sample-liquid interactions, kinetics and the movements of materials in the small environment of the liquid cell have been demonstrated. In several cases, imaging of biological materials in their native environments supersedes traditional analysis with less cumbersome sample prep techniques because there is no need to freeze, fix, stain or dry samples. While these results are remarkable examples of what is possible using the liquid cell, they only demonstrate imaging with conventional TEM and scanning TEM (STEM), and significantly underutilize the microscope capabilities for materials analysis.

Many TEMs are analytical tools, meaning they have qualitative and quantitative elemental analysis capabilities such as electron energy loss and energy dispersive x-ray spectroscopy. Unfortunately, these techniques have not found common use in liquid cell experiments. Electron energy loss spectroscopy (EELS) can be a complicated technique, and is only compatible with thin liquid layers, as will be discussed below. Energy dispersive x-ray spectroscopy (EDS) requires direct line-of-sight from the sample to the detector, something not yet widely available on liquid cell holders. Thus, reports showing sample analysis with EELS and EDS to date are limited. Moreover, a comprehensive understanding of how EELS and EDS work with a liquid cell has, until recently, not been explored. This demands careful work if these techniques are to be widely adopted. EDS and EELS analysis augments conventional TEM and STEM imaging, provides critical information about sample behavior, and significantly extends the benefits of *in situ* experimentation in the TEM. Before one can determine the analysis possibilities with the liquid cell in the TEM, it is necessary to understand how the Poseidon system holder itself functions.

The Poseidon system uses a pair of semiconductor devices, called E-chips™, which each contain a small, thin, amorphous silicon nitride (SiN) window. The window is thin enough to provide good electron transparency, but strong enough to prevent liquid from escaping into the high vacuum of the TEM column. Protochips uses semiconductor device fabrication processes to create E-chips, which enables large-scale reliable fabrication of windowed cells, the foundation

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of *in situ* liquid systems. Starting with a silicon wafer, a 50 nm amorphous SiN layer is deposited. A defined area of silicon is chemically etched leaving a free-standing SiN window. Each window is rectangular in shape, with an aspect ratio of approximately 10 to 20. Each process step is shown in detail and described in figure 1, along with two finished Poseidon system E-chips. A pair of E-chips are placed membrane-to-membrane in the Poseidon system holder, and a rubber o-ring compresses against each E-chip to create a hermetic seal, preventing liquid from escaping into the column of the TEM.

A primary consideration in liquid cell analysis relates to sample thickness. As the sample thickness increases, the signal to noise (S/N) ratio decreases as a result of increased electron beam scattering. The liquid present inside the cell also scatters electrons, and as the liquid becomes thicker the loss in S/N is compounded. To preserve resolution and maximize S/N, microscopists prefer samples and liquid layers that are as thin as possible. However, even with thin spacers of 150 nm or thinner, such as those available through Protochips, an important caveat persists. Due to the enormous pressure differential between the inside of the liquid cell, at 1 atm, and in TEM column, which is in the microTorr range, the thin SiN windows bow, as shown in figure 2. Bowing leads to thicker liquid layers, which increases multiple scattering and degrades resolution.

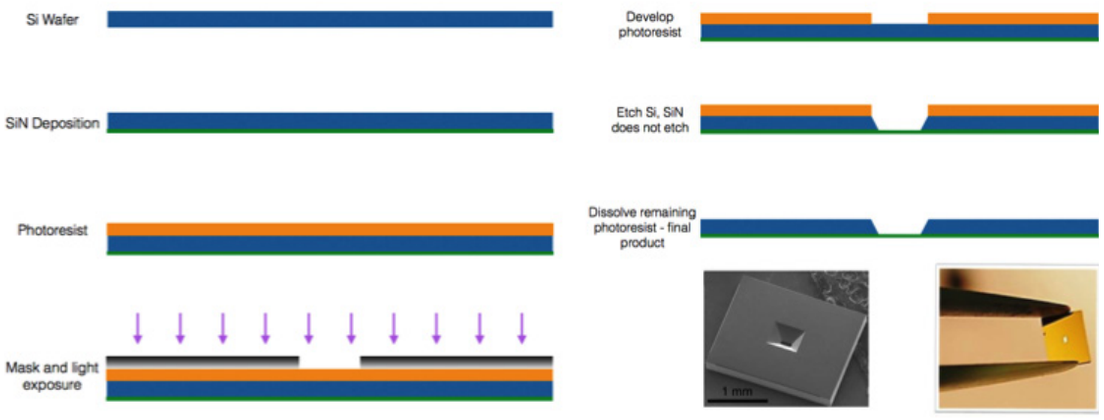

Figure 1: Poseidon E-chip fabrication. Starting with a silicon wafer, a silicon nitride membrane is deposited. A layer of photoresist is deposed then exposed to UV light though a photomask. The exposed photoresist is dissolved leaving a small silicon window. The wafer is immersed in an etchant that preferentially dissolves silicon leaving a thin SiN layer. An SEM image and photograph of completed E-chips are shown in the bottom right.

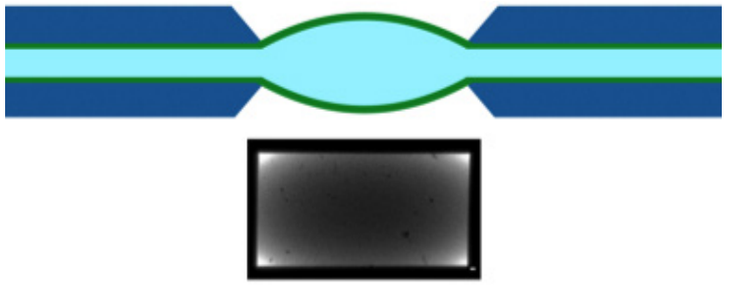

Figure 2: The top figure illustrates the bowing effect. When two windows are placed back to back the pressure differential causes the thin windows to bow, or bulge out. The bottom figure shows a TEM image of a window in the crossed configuration. The light and dark contrast is a direct result of bowing. The thinnest regions are at the corners, resulting in bright contrast.

To address bowing, and provide strong and durable SiN windows, Protochips has designed long rectangular windows. The smallest window dimension determines its strength, and resistance to rupture and

bowing. Creating a long window in the second dimension provides a larger field of view. When performing liquid cell experiments using the Poseidon system, users can choose between a parallel or crossed window orientation, as shown in figure 3. Although the parallel orientation yields the largest field of view, it may not yield the thinnest liquid layers. If slightly misaligned, thick liquid areas dominate as a result of window bowing. Figure 3 shows this effect. Alternatively, if the crossed configuration is used, each corner provides a thin liquid layer, with a smaller field of view as a trade-off. The thinnest liquid layers result in the best EEL spectra, so the crossed window configuration is preferred. A large field of view and geometry prevails when performing EDS analysis, so the parallel configuration is preferred in that case. Thus, multiple parameters such as sample thickness, window bowing, and window orientation must be accounted for during the design of the experimental setup for EELS and EDS analysis as discussed in more detail below.

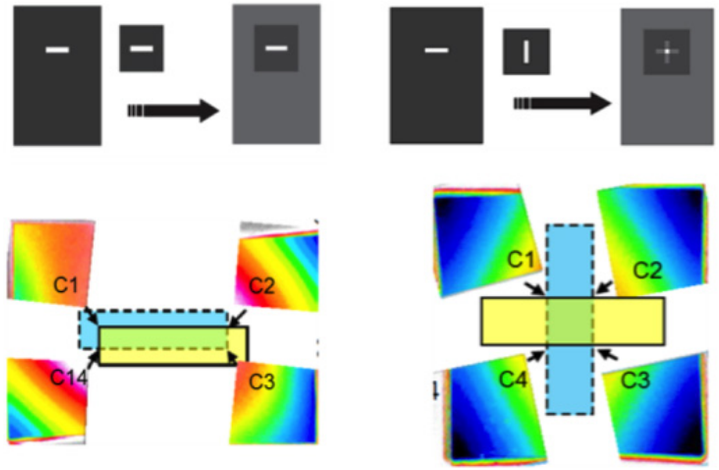

Figure 3: The top figure shows the parallel (left) and crossed (right) window configurations. The bottom left figure illustrates how a slightly misaligned windows in the parallel configuration leads to thick liquid layers. The bottom right figure illustrates window bowing in the crossed configuration. In this configuration the corners always provide the thinnest liquid layers. The designations C1-C4 refer liquid thickness maps taken at each of the four corners of the window.

EELS

EELS, including energy filtered TEM (EFTEM), is used by microscopists for element identification and to obtain electronic structure information. An EEL spectrum is generated when fast electrons interact with the atomic electrons surrounding a nucleus via inelastic scattering. They can excite the atomic electrons either by exciting a single atomic electron to a higher energy level, or by a collective excitation. The fast electron beam will lose as much energy as it takes for a given excitation. The excitation of the tightly bound core electrons near the nucleus of the atom requires more energy and forms the core-loss EEL spectrum, and the collective or single excitation of electrons in the valence states requires less energy (<50 eV) and forms the low-loss or valence spectrum. After exiting the sample, electrons pass through an energy filter that contains electromagnets strong enough to deflect the beam. Inelastically scattered electrons that have lost more energy deflect to higher angles than electrons that have lost less energy. The EEL spectrometer precisely detects the amount of deflection, and creates a spectrum of electron energy loss. Since each element in the periodic table has well defined electron energy levels, core-less EELS can identify elements present in the sample, and through careful analysis one can obtain electronic structure information, such as the oxidation state, of a particular element. EELS can also identify light elements, such as Li, more effectively than EDS. EFTEM uses an energy selecting slit located in the energy filter to select a specific energy range

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part to generate an image and spatially map elements. EEL spectra contain the zero-loss peak (ZLP), which are unscattered and elastically scattered electrons from the primary beam. The ZLP provides useful information when quantifying the amount of inelastic scattering in the sample, and can be used to measure sample thickness.

The EEL spectrum is sensitive to liquid thickness. The fast electrons can scatter multiple times, quickly obscuring the single scattered spectrum with increasing thickness. The thickness is not limited to the sample itself, but includes the liquid and the SiN windows. The SiN membranes, are each 50 nm thick for a total of 100 nm, this thickness contributes to the EEL spectrum and must be accounted for.

In order to quantify the limitations for EELS analysis, the liquid thickness must first be accurately measured. EELS provides a useful method to measure sample thickness in terms of the inelastic mean free path or t/λ ratio. This is done by measuring the ratio of the integrated area of the ZLP and the total number of electrons in the primary beam. The actual thickness of the cell can also be extracted from this ratio. More details can be found in Egerton[1], Holtz[2], Klein[3] and deJonge[4]. Figure 4 shows a thickness map of a liquid cell with a nominal spacer thickness of 150 nm. Window bowing is readily apparent in this crossed window orientation, where the thickness varies from about 300 nm at the corners to over 650 nm at the center. A simulation also shows the magnitude of bowing in the crossed configuration.

Using these thickness measurements, researchers in David Muller's group at Cornell University sought to quantify the effects of liquid thickness in EELS

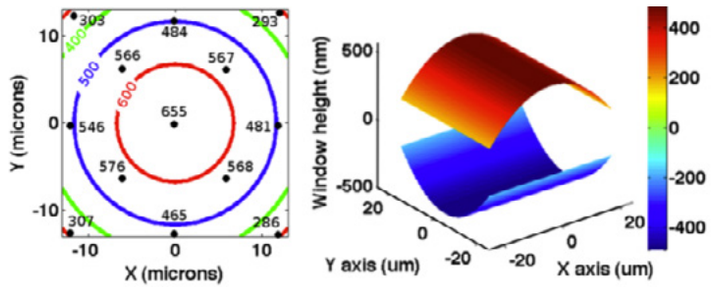

Figure 4: The left figure shows a thickness map measured using EELS. The right image is a simulation showing the effects of window bowing in the crossed configuration.

measurements using the Poseidon system in order to understand the possibilities and limitations.

Namely:

1. At what point does the liquid become too thick for meaningful spectra?
2. Is core-loss or valence EELS more sensitive to liquid thickness? If so, why?
3. Are particular liquids more sensitive than others?

In a cell filled with pure water, the researchers took EEL spectra at several places across the window. At each place they measured the thickness and compared the core-loss and the low-loss spectra. They found that both meaningful low and core-loss signals can be obtained if the t/λ ratio falls below a threshold value, and that the core-loss spectrum is significantly more sensitive to sample thickness. The core-loss spectrum degrades more quickly as the thickness increases, because electrons that undergo scattering off of outer shell electrons (low-loss spectrum) often scatter again losing more energy and show up in the core-loss spectrum smearing out the entire core-loss component. This occurs when the thickness is more

than about 300 nm or t/λ ~ 2.7. Below this threshold value meaningful data can be extracted from the core-loss EELS component. Figure 5 shows EEL spectra from this experiment, demonstrating how the core-loss spectra smears out as a function of thickness. The oxygen K-edge appears in only the thinnest liquid layers (< 300 nm), and subsequently smears with increasing thickness. The inset shows a zoomed view of the lowloss spectrum from the same sample. Meaningful information in the low-loss spectrum exists through thicknesses of up to about 650 nm or t/λ ~ 6.5. A Poisson distribution, right figure, shows single scattering events (fundamental assumption for meaningful EEL spectra) up to a t/λ ratio of 6.5, agreeing well with the experiment.

Muller's group also performed measurements on a variety of liquids, including ethylene glycol, propylene carbonate and 10 mM copper sulfate in water, and found similar results.[2] When falling below the threshold t/λ ratios described above, core- and low-loss EEL spectra prove meaningful.

With a quantitative understanding of EELS analysis with the liquid cell, one can perform meaningful measurements within this spectral parameter space. One such example done by Muller's group used low-loss (valence) EELS to track the movement of Li ions,

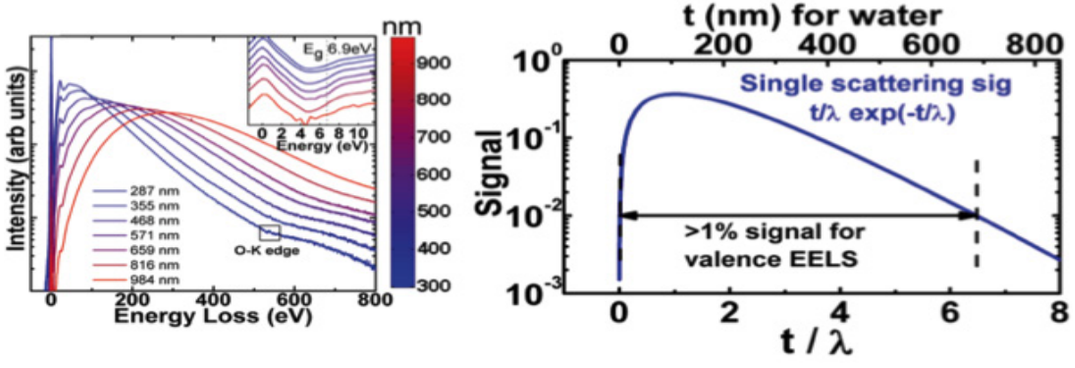

Figure 5: The left image shows the core- and low-loss (inset) components of the EELS spectra of water. The right image shows a simulation confirming the experimental findings. With a t/λ value of up to 6.5 low-loss EELS is generally meaningful.

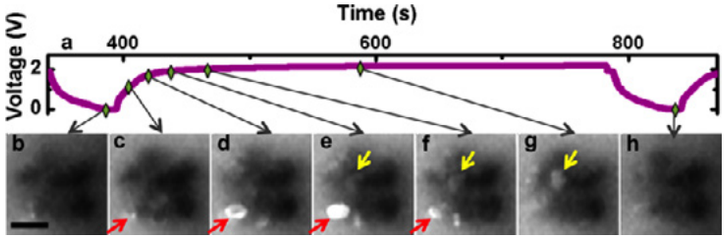

Figure 6: The TEM images show a charge/discharge cycle of a single group of FePO4 nanoparticles. The EFTEM images (b-h) indicate where lithium is located spatially during the cycle.

and the lithiation state in a battery material. Li ions in $LiFePO_4$ and electrolyte were tracked as a function of charging and discharging using the Poseidon system electrochemical cell.[5] $LiFePO_4$ is an important cathode material in Li ion batteries, and possible alternative to other lithium transition metal oxides as it is cheaper and generally safer that current materials commonly used today such as $LiCoO_2$. Delithiated $FePO_4$ exhibits a peak in the EEL spectrum at 5 eV, which is not present after lithiation, enabling a method for visualizing the Li movement spatially using EELS. Note that analysis of the Li core-loss ionization edge is not practical in the liquid cell, and analysis is limited to the lowloss region of the spectrum. As the material was charged and

discharged using the electrochemistry function of the Poseidon system, the researchers used EFTEM to track how individual nanoparticles of $FePO_4$ changed state, as shown in figure 5, and determined spatially where Li intercalated and where it did not. They observed inhomogenous intercalation at the nanoscale, including core-shell structures and stronger delithiation on the edges of agglomerates.

EELS depends strongly on thickness, and is generally limited to thin liquid layers. If samples are kept thin, and the liquid layers also kept to a similar thickness, EELS provides a powerful means to analyze samples. With a better understanding of the limitations in terms of the t/λ ratio, researchers can now use EELS more easily and frequently. EELS compliments EDS analysis as well. However, EDS may prove a more versatile tool for the liquid cell technique.

EDS

EDS, commonly used for element identification in the TEM, operates via a fundamentally different physical effect than EELS. An EDS system detects x-rays generated by the primary electron beam when it interacts with the sample. When the primary beam ionizes an atom, an electron in a higher energy state can relax and fill the vacant lower energy state, emitting an x-ray in the process. The difference in energy between these states equals the energy of the x-ray. Each element has well-defined electron energy levels, thus each element emits defined characteristic x-rays that can be measured. While EEL spectra are generated after the electron beam has passed through sample, x-rays scatter in all directions. EDS detectors are placed at angles between 10 and 20º relative to the sample, and require direct line-of-sight to the area of interest. This requirement has to date precluded EDS analysis using the liquid cell holder, and required modification to the geometry of the Poseidon system tip to enable EDS compatibility.

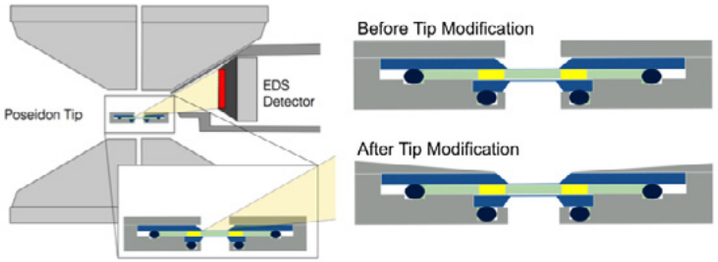

Figure 7: The left image shows the upper and lower pole pieces of a TEM objective lens. The EDS detector is situation just above and to the side of the sample holder. The yellow cone indicates the path the x-ray need to take to be detected. With the current design, there is no line-of-sight from the sample to the EDS detector. The right show the before and after modification to the holder tip. The holder lid has been modified to allow for line-of-sight.

Strategic modifications to the tip, and primarily the lid, of the Poseidon system holder enables line-of-sight directly from the sample to the EDS detector, see figure 7.[6] EDS also works best when the holder material is composed of element that generates x-rays with energies below the detection limit of the system. Normally, the Poseidon system holder tip is made of titanium owing to its machinability, stiffness and good chemical compatibility. However, x-rays generated from the holder tip, even with a tip that is machined for EDS compatibility, can appear in an EDS spectrum. Beryllium is commonly used for low background holders, and x-rays generated by beryllium are below the detection limits of EDS systems. Beryllium, like titanium, can be machined, and is stiff enough to reliably maintain the hermetic seal required for

operation in the microscope. Ti lids come standard on the Poseidon system, and Be is a lid option available through Protochips.

When performing liquid cell experiments, it is often necessary to prove that liquid is present in the cell. EELS t/λ measurements as described above are useful, but take time and effort to setup, obtain and interpret. EDS on the other hand can quickly detect one or several elements that make up the liquid. For example, water contains oxygen, which can be used to map the location of water in the cell, along with the relative amount determined by comparing peak heights.[7] Figure 8 shows an EDS spectrum image (EDS SI) of a small area with a bubble. The bubble has liquid present, but less than the neighboring area. The SI clearly shows where oxygen is concentrated spatially, and where the areas remain hydrated. The accompanying plot shows the spectra from the boxed areas, and where water is present oxygen dominates the spectrum.

The ratio of silicon (from the SiN window) to that of the element making up the solution, can be used to determine the amount of liquid present. EDS spectra of other liquids, such as ethanol and dichloroethane, are shown in figure 9. In each case the primary element in the liquid was identified. Two controls were taken with air and another with water. In the case of ethanol a strong carbon peak was identified, and in the case of dichloroethane, chlorine was identified.

EDS can also be used to detect elements present in solid materials inside the liquid cell. For example, figure 10 shows several EDS SIs of a suspension containing Au and Pd nanoparticles, Ag nanowires, Cu that was reduced from solution by the electron beam, and

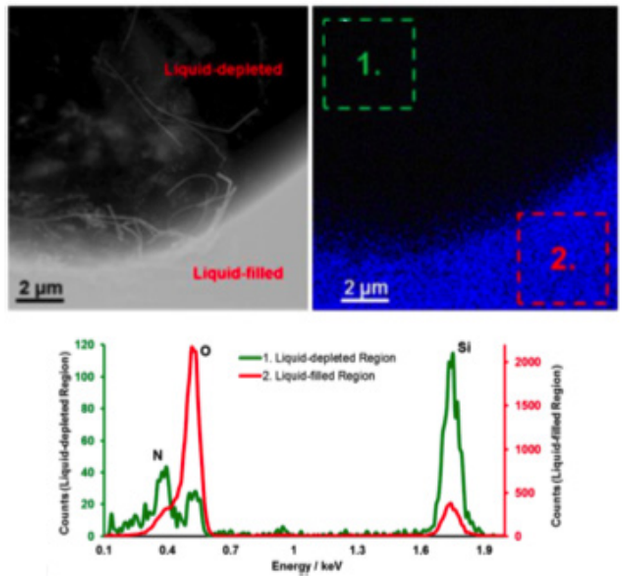

Figure 8: The top images are a STEM dark field image (left), and an EDS spectrum image (right) of the same area. The blue area on the right shows a larger volume of water present. The bottom image shows EDS spectra from region 1 and region 2 indicated in the spectrum image. In region 2 where water is present, oxygen dominates the spectrum.

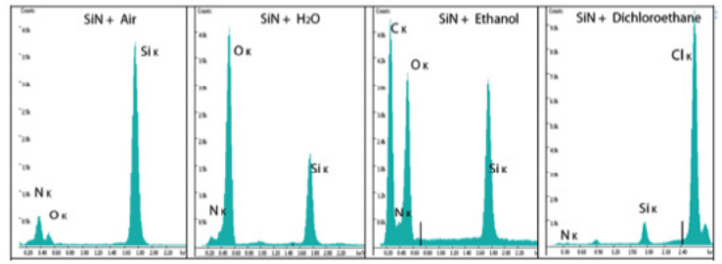

Figure 9: EDS spectra of different liquids. The left spectra shows the cell with 1 atm of air as a control. The Si peak dominates emanating from the E-chip membrane. N is also present. The middle-left spectra shows a cell filled with water. O from the water dominates, and the ratio of Si to O can be used to determine the amount of water present in the cell. The middle right spectrum shows a cell filled with ethanol. In this case C from the ethanol dominates. The right spectrum shows a cell filled with dichloroethane, where Cl dominates.

a multi walled carbon nanotube with an iron catalyst particle left over from the growth process in water, which is the source of O. Each element was identified and mapped spatially at the nanometer scale.

The ability to directly detect elements that compose a liquid or are in solution is a fast and powerful way to analyze materials present in the cell. It can be used to determine when a compound enters the tip after it is introduced from outside of the holder, and track the progress of a chemical reaction. However, special considerations must be taken to obtain the best quality spectra, and are specific to the liquid cell. Often samples in the liquid cell are beam sensitive. The beam can reduce materials, induce nucleation and growth from material dissolved in the liquid and cause beam damage. The electron beam dose, as with normal imaging, should always be known and accounted for, and can be adjusted through the condenser aperture spot size and by avoiding a focused beam when operating in bright field TEM mode. Precautions should also be taken when operating in STEM mode. The probe dwell time should be short, and beam current density reduced to the lowest acceptable value. This holds true when performing EDS analysis. The results described above were taken in STEM mode with the probe continually scanned while acquiring the spectra, just long enough so the S/N ratio was high enough to obtain meaningful spectra. It should be noted that spectrum images can also be taken by placing the STEM probe at a spot, taking a full EDS spectrum, moving it to an adjacent spot, taking a subsequent full EDSspectrum, and so on until the area of interest has been mapped. This is not an optimal method, because the intense STEM probe exposes a small area to a high electron beam dose and may cause damage to the sample or induce other spurious effects. When performing EDS analysis in bright field mode, the beam should not be focused, or too intense, as it could damage the sample.

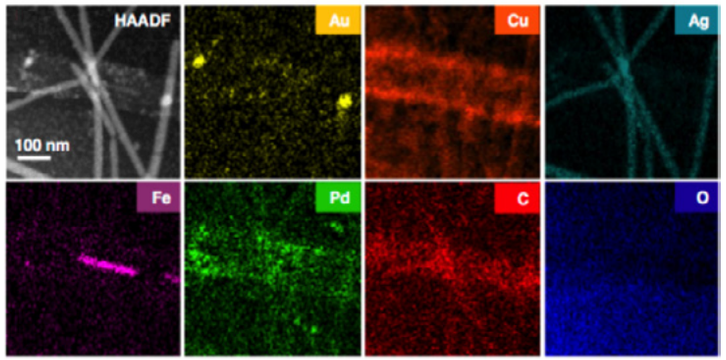

Figure 10: EDS spectrum image of a nanomaterial suspension consisting of Au and Pd nanoparticles, Cu ions in solution reduced by the electron beam, Ag nanowires, Fe catalyst and a multi walled carbon nanotube all in water.

EDS is a powerful tool used to identify elements present in a sample, and is indispensable in the modern TEM. Now compatible with the Poseidon liquid cell system, the composition of materials can be explicitly and unambiguously determined quickly and easily. Before and after images and EDS spectra of reactions and other material processes can be compared, opening the possibility of a new understanding of phenomena at the nanoscale.

Conclusion

The analytical techniques used in TEM, such as EELS and EDS, are critical when analyzing samples. This is especially important for *in situ* microscopy, as users often induce and watch dynamic reactions and material evolution. Understanding the products of reactions and other sample changes is now easier with the

ability to do analysis with EELS and EDS. The recent work described here showing EELS and EDS capability in the liquid cell represents an important leap forward for the technique. Now that users can quickly and reliably understand the composition of their samples, they can better understand the behavior.

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