Video Article

In Situ Characterization of Boehmite Particles in Water Using Liquid SEM

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Abstract

In situ imaging and elemental analysis of boehmite (AlOOH) particles in water is realized using the System for Analysis at the Liquid Vacuum Interface (SALVI) and Scanning Electron Microscopy (SEM). This paper describes the method and key steps in integrating the vacuum compatible SAVLI to SEM and obtaining secondary electron (SE) images of particles in liquid in high vacuum. Energy dispersive x-ray spectroscopy (EDX) is used to obtain elemental analysis of particles in liquid and control samples including deionized (DI) water only and an empty channel as well. Synthesized boehmite (AlOOH) particles suspended in liquid are used as a model in the liquid SEM illustration. The results demonstrate that the particles can be imaged in the SE mode with good resolution (*i.e.*, 400 nm). The AlOOH EDX spectrum shows significant signal from the aluminum (Al) when compared with the DI water and the empty channel control. *In situ* liquid SEM is a powerful technique to study particles in liquid with many exciting applications. This procedure aims to provide technical know-how in order to conduct liquid SEM imaging and EDX analysis using SALVI and to reduce potential pitfalls when using this approach.

Video Link

The video component of this article can be found at https://www.jove.com/video/56058/

Introduction

Scanning Electron Microscope (SEM) has been widely applied to investigate a variety of specimens by producing high resolution imaging[1]. The energy dispersive x-ray spectroscopy (EDX) associated with the SEM enables the determination of elemental composition[1]. Traditionally, SEM is applied for imaging only dry and solid samples. In the last 30 years, Environmental SEM (ESEM) was developed for analyzing the partial hydrated samples in a vapor environment[2,3,4,5]. However, ESEM is unable to image the wet, fully fluid samples with desired high resolution[6]. Wet SEM cells were also developed to image wet specimens using SEM[7,8]; nevertheless, these cells were developed mainly for biological specimens and backscattered electron imaging, and are more accessible for applications with those designs[9,10].

To address the challenges in analyzing various samples in their native liquid environment using SEM, we invented a vacuum compatible microfluidic device, System for Analysis at the Liquid Vacuum Interface (SALVI), to enable high spatial resolution secondary electron (SE) imaging and elemental analysis of liquid samples using the high vacuum mode in SEM. This novel technique includes the following unique features: 1) liquid is directly probed in a small aperture of 1 - 2 µm in diameter; 2) liquid is held within the hole by surface tension; and 3) SALVI is portable and can be adapted to more than one analytical platform[11,12,13,14,15,16,17,18].

SALVI consists of a 100 nm thick silicon nitride (SiN) membrane and a 200 µm wide microchannel made of polydimethylsiloxane (PDMS) block. The SiN membrane window is applied to seal the microchannel. The fabrication details and key design considerations were detailed in previous papers and patents[11,19,20]. Currently, a leading manufacturer and distributor of consumable supply for microscopy has purchased the license to sell SALVI devices commercially for liquid SEM applications[21,22].

The applications of SALVI in vacuum-based analytical instruments have been demonstrated using a variety of aqueous solutions and complex liquid mixtures including biofilms, mammalian cells, nanoparticles, and electrode materials[12,14,17,20,23,24]. However, most of the aforementioned work utilized time-of-flight secondary ion mass spectrometry (ToF-SIMS) as the key analysis tool, thus the application of liquid SEM with SALVI has not been explored fully. In this work, SALVI has been used to study larger non-spherical colloidal particles in liquid using liquid SEM imaging and EDX elemental analysis. The sample consists of AlOOH particles synthesized at our laboratory. Submicrometer-sized boehmite particles are known to exist in high-level radioactive waste at the Hanford site. They are slow to dissolve and may cause rheological problems in waste treatment. Therefore, it is important to have the capability to characterize boehmite particles in liquid[25]. This technical approach can be used to study boehmite in various physicochemical conditions for improved understanding of these particles and related rheological properties. These particles were utilized to demonstrate step-by-step how to apply SALVI to high vacuum SEM in order to study particles suspended in liquid. Key technical points for SALVI and SEM integration and SEM data acquisition are highlighted within the paper.

The protocol provides demonstration of the liquid sample analysis using SALVI and liquid SEM imaging, for those who are interested in utilizing this novel technique in diverse applications of liquid SEM in the future.

Protocol

1. Prepare AlOOH Liquid Sample

NOTE: Do not touch the specimen or anything inside the SEM chamber with bare hands. Powder free gloves should be worn at all times when handling the SALVI device and mounting it onto the SEM stage in order to avoid potential contamination during surface analysis.

1. **Make an AlOOH stock solution (1 mg/mL)**
    1. Dissolve 10 mg of AlOOH powder in 10 mL DI water to make the 1 mg/mL AlOOH stock solution.
    2. Ultrasonicate the stock solution for 5 min.
       NOTE: The pH of the stock solution is approximately 4.6 measured by a pH meter. The solution of pH is not adjusted and used as it is in this work.

2. **Make a diluted solution of 10 µg/mL**
    1. Dilute the 1 mg/mL AlOOH stock solution to 10 µg/mL by dispensing 1 mL into 99 mL DI water via pipette.
    2. Ultrasonicate the solution for 5 min.
       NOTE: The pH of the stock solution is approximately 5.8 measured by a pH meter after dilution.

2. Sputter Coat the SALVI SiN Membrane Window with Carbon

1. Insert the carbon rod into the rod holder.
   NOTE: The rod holder can be identified as the piece that is attached to the hinged lid.
2. Use a pair of tweezers and carefully remove the tape on the SALVI's SiN membrane window frame.
   NOTE: The tape is used to protect the SiN membrane prior to surface analysis.
3. Secure the SALVI device upright inside the carbon coater chamber using carbon tape to fix the polytetrafluoroethylene tubing of the SALVI device on the stage of the coater. Close the lid.
4. Press the "POWER" button to initiate the vacuum pump.
5. Press the "VOLTAGE" button at the front panel of the carbon coater and set the value to 4.6 V by adjusting the up (▲) and down (▼) buttons for this operation.
   NOTE: The voltage setting may vary due to various carbon coaters.
6. Turn on the coating thickness monitor by switching on its "POWER" button. Clear the reading displayed in the screen of "THICKNESS (nm)" to zero by pressing the button "ZERO" if the reading is not zero. Press the "TIMER" at the carbon coater's front panel to set the deposition time to 30 s by adjusting the up (▲) and down (▼) buttons.
7. Keep the carbon coater's operation mode to auto by switching the button "AUTO ◄►MANUAL" to "AUTO". Switch the "START/STOP" button to "START" when the vacuum reaches about 4×10^{-4} mbar as measured by the vacuum gauge at the carbon coater's front panel.
8. Once the thickness monitor indicates that the carbon coating has reached 20 nm, press the "stop" button to terminate the coating process and to vent the vacuum seal.
9. Open the lid and take out the carbon coated SALVI device using vinyl gloves when handling the device.
   NOTE: Coating the sample with carbon creates a conductive layer on the sample to inhibit the charging effect and improve the SE signal required for the SEM imaging. Securely store the coated device in a clean Petri dish with a cover until the device is ready to be installed in the SEM stage. In order to ensure the SiN membrane is sufficiently coated, it is recommended to check the device visually after the coating. If the coating is not thick enough, a second time of sputter coating can be applied with the thickness measured until 10 nm.

3. Mount the Device and Use SEM/Focused Ion Beam (FIB) to Make Apertures on the SALVI SiN Membrane Using FIB

1. **Open the SEM specimen chamber**
    1. Open the associated microscope control software on the SEM instrument control computer.
       NOTE: The control software may vary due to various SEMs.
    2. Vent the specimen chamber by clicking "Vent" on the graphic user interface (GUI) of the associated microscope control software under "Beam Control" tab in order to open the chamber door.
    3. Open the chamber door carefully (once venting has completed).

2. **Mount the SALVI device onto the SEM stage**
   NOTE: Check the surface of the SiN membrane to see if it is intact either visually or using a light microscope before mounting. The SALVI device mounted on the SEM stage must not touch the Everhart Thornley Detector (ETD) detector inside the specimen chamber.
    1. Select the standard SEM sample holder stub. Fix the stub onto the center of the stage using the appropriate bolt and hex wrench.
    2. Place two strips of double-sided carbon tape on the stub.
    3. Stick the SALVI device onto the carbon tape placed on the stub with the SiN membrane side facing up.
    4. Securely immobilize the SALVI on the stub using an additional two strips of single-sided copper tape to bind the SALVI PDMS block to the SEM metal stub. In addition, use the copper tapes to connect the SiN frame and the metal stub. Make sure that the tape does not completely cover the SiN membrane.

NOTE: The use of carbon and copper tapes help ensure a continuous grounding path for the removal of charge from the SiN membrane during the SEM measurement. The position of the tape on the edge of the SiN frame is quite important, because it ensures grounding and reduces charging during analysis. The bottom of the device must also have full contact with the SEM stub via double-sided carbon tape. The tape must not cover the SiN membrane to avoid possible damage in handling.

3. **Pump down the specimen chamber**
    1. Close the specimen chamber door. Select the "High Vacuum" mode on the SEM software GUI under the "Beam Control" page.
    2. Click the "Pump" button on the "Beam Control" page to start vacuuming and apply pressure by hand to the chamber door until the desired vacuum is established.
    NOTE: The chamber pressure must reach at least 1.0×10^{-5} Torr and must steadily remain at or below this value before imaging. This is an important step to enable the highly-resolved resolution for imaging. The pressure setting can be monitored from the right corner of the GUI.

4. **Make apertures in the SiN membrane using FIB**
    1. Activate the electron beam imaging area by clicking the "Pause" icon on the toolbar. Turn on the electron beam by clicking the "Beam On" button on the "Beam Control" page. Select the ETD detector and the SE mode for imaging from the "Detectors" drop down menu.
    NOTE: The detector may vary due to different configuration of the SEMs. The in-lens detector is also applicable for liquid SEM analysis.
    2. Link the Z coordinate value to the actual Free Working Distance (FWD) value by clicking the "Link" icon on the tool bar. Set the working distance (WD) as 10 mm by typing the number 10 into the text box of coordinate "Z" on the "Navigation" page when the "Actual" distance is selected.
    NOTE: The WD may vary due to various SEMs.
    3. Set the electron beam current to 0.47 nA, the accelerating voltage to 8 keV, and the resolution to 1,024 × 884 from the corresponding list boxes displayed on the toolbar at the electron beam imaging area.
    NOTE: The current and voltage setting may vary due to different SEMs.
    4. Locate the microchannel (0.2 mm x 1.5 mm) by twisting the "X" and "Y" shift knobs on the Manual User Interface (MUI) board to observe the live image on the control monitor. Draw a line paralleled to the microchannel from one end to the other using the mouse. Click "xT Align Feature" from the drop down menu of "Stage" tab on the toolbar and select "horizontal" to align the microchannel.
    5. Set the stage tilt to 0 ° by selecting the value from the "T" list box on the "Navigation" page. Locate a distinct particle feature near the microchannel and center it under the yellow cross by moving the stage using the "X" and "Y" shift knobs. Magnify the feature to 1,000X and twist the "Contrast", "Brightness", "Coarse", and "Fine" knobs on the MUI to optimize the image of the particle feature.
    6. Tilt the stage to 15 ° by selecting the value from the "T" list box on the "Navigation" page. Use "Z-control" by pressing down the wheel on the mouse and drag the feature back under the yellow cross on the screen of the electron beam imaging area after the stage is tilted.
        1. Tilt the stage again to 30 ° and bring the feature back under the cross using the "Z-control". Tilt the stage back to 0 ° and observe the location of the feature; the eucentric height is confirmed if the feature does not shift significantly.
        NOTE: Locating the eucentric height is performed to keep the ion beam and electron beam focused at the same position to achieve good FIB milling precision. Repeat the processes in step 3.4.6 if the feature shifts significantly after tilting the stage back to 0 °. The eucentric height should be adjusted for each new mounted sample for the greatest accuracy.

    7. Tilt the stage to 52° by selecting the value from the "T" list box on the "Navigation" page.
    NOTE: The tilting degree may vary due to various SEMs.
    8. Deactivate the "Pause" icon on the toolbar by clicking the button to ensure that the Ion beam imaging area is on. Turn on the gallium source ion beam by clicking on the "Beam On" button under the "Beam Control" page.
        1. Set the accelerating voltage of the ion beam to 30 keV and beam current to 0.3 nA by selecting these values from the corresponding voltage and current list boxes located at the toolbar. Bring the microchannel to the center of this imaging area.

    9. Choose the circle as the pattern by selecting this feature from the list box of "Pattern" on the "Patterning" page. Set the "Outer Diameter" to 1 µm, the "Inner diameter" to 0 µm, the "Z size" to 500 nm, and the "Dwell Time" to 1 µs in the corresponding text box.
        1. Type "Si" in the "Application" text box because the main component of the about-to-be-milled detection window is silicon nitride. Then click the "Patterning menu/Start patterning" button to begin milling the holes on the detection window that covered the microchannel. Repeat the milling process multiple times to obtain a series of round holes. In an experiment, several holes may be made.
        NOTE: The holes are 100 µm apart, from one side of the microchannel to the other. Move quickly to minimize the beam damage on the SiN membrane. The SEM FIB milling process usually starts from either the very left or right side of the microchannel in order to track and number the apertures easily. The operator may choose to go from the bottom or top depending on the orientation of the channel and personal preference. Ensure that the SEM FIB milling is completed and adequate so the specimen can be probed within the apertures.

5. **Vent the chamber after the SEM/FIB**
    1. Tilt the stage back to 0 ° by selecting 0 from the "T" list box on the "Navigation" page. Turn off both the electron beam and ion beam by clicking "Beam On" when the corresponding beam imaging area is activated. Click "Vent" on the "Beam Control" page to vent the specimen chamber.

4. Load SALVI with Liquid Samples

1. **Clean the SALVI using DI water**
    1. Carefully open the SEM chamber door after it is fully vented, and leave the SALVI device as it is on the stage.

NOTE: To save time on mounting the device and focusing, it is strongly recommended to keep the device on the stage when loading the sample.

2. Draw 1 mL DI water into a sterile syringe, connect the syringe with the inlet of the microfluidic device using a polytetrafluoroethylene tubing adaptor fitting, and slowly inject the liquid for 3 - 5 min.
   NOTE: A syringe pump is recommended for all steps where injecting solutions into the SALVI is required. This can be done in this step by setting a 1 mL sterile syringe containing the solution to a flow-rate of 100 - 250 µL/min. Utilizing a syringe pump at a constant flow-rate can decrease the likelihood of damage to the SiN membrane.
3. Repeat step 4.1.2 three times using 1 mL of 10 µg/mL AlOOH, prepared in step 1, to ensure the concentration of the sample is not diluted by the preloaded DI water.
4. After the injection, remove the syringe. Connect the inlet and outlet of SALVI using the polyether ether ketone union. Dry any liquid outside the SALVI with lab wipes. If there are any bubbles within the polytetrafluoroethylene tubing or microchannel, redo the AlOOH sample injection until no bubbles are seen within the polytetrafluoroethylene tubing.
   NOTE: Finger-tighten the polyether ether ketone union. Do not use too much strength when tightening the union to avoid creating a significant internal pressure increase inside the SALVI device, which could result in damage to the SiN membrane.
   NOTE: The bubbles inside the microchannel may affect the scanning and cause image shift. Any liquid outside of the device will affect the vacuum status, therefore the outside of the SALVI and polytetrafluoroethylene tubing should be thoroughly dried prior to inserting it to the vacuum chamber. In addition, the device should not have physical damage (*e.g.*, cut on the tubing, broken SiN membrane window) that leads to leaking. Otherwise, the chamber pressure may not reach the desired high vacuum, bubbles may form in the tubing, and the liquid sample will be lost during vacuuming.

5. Conduct Liquid SEM Imaging and Elemental Analysis

1. **Take images using the ETD detector and SE mode**
   1. Close the specimen chamber door. Select the "High Vacuum" mode on the SEM software GUI under the "Beam Control" page. Click the "Pump" button on the "Beam Control" page to start vacuuming and apply hand pressure to the chamber door until the desired vacuum is established.
   2. Activate the electron beam imaging area by clicking the "Pause" icon on the toolbar. Turn on the electron beam by clicking the "Beam On" button on the "Beam Control" page. Select the ETD detector and the SE mode for imaging from the "Detectors" drop down menu.
      1. Set the accelerating voltage to 8 keV and beam current to 0.47 nA from the corresponding list boxes displayed on the GUI toolbar at the electron beam imaging area. Set the WD as 7 mm by typing the number "7" into the text box of the coordinate "Z" on the "Navigation" page when the "Actual" distance is selected.
         NOTE: The parameters of beam voltage, current and working distance may vary due to different SEMs.
   3. Magnify the feature to 1,000 × and twist the "Contrast", "Brightness", "Coarse", and "Fine" knobs on the MUI to optimize the image of the particle feature.
   4. Center the first hole in the live image of the electron beam imaging area by twisting the "X" and "Y" shift knobs on the MUI board. Enlarge the images with particles to magnification 200,000× by twisting the "Magnification" knob on the MUI board. Select the screen resolution "1,024 × 884" from the list box on the toolbar.
   5. Set the scan rate as 30 µs from the list box on the toolbar. Press the F4 key to take the snapshot of the current image shown in the electron beam imaging area.
   6. Press Ctrl + S keys to save the image as.tif file to the desired location with the defined filename including an incremental number.
   7. Zoom out by twisting the "Magnification" knob to locate the next adjacent hole. Repeat the operations in steps 5.1.4 - 5.1.6 to image the AlOOH particles in the rest of the holes.

2. **Conduct elemental analysis using EDX**
   1. Insert the Energy Dispersive Spectroscopy (EDS) detectors into the chamber.
   2. Select the ETD detector on the microscope control monitor and SE mode for viewing the sample on the electron beam imaging area. Set the accelerating voltage to 8 keV, the current to 0.47 nA and the WD to 7 mm as described in step 5.1.2.
   3. Enlarge the AlOOH particles in each hole with magnification 200,000X by twisting the "Magnification" knob on the MUI board.
      NOTE: Keep the electron beam focused on the same spot so as to provide more localized elemental information. An image of AlOOH is provided in **Figure 1a**.
   4. Open the associated EDAX software.
      NOTE: The associated software may vary due to different instruments' configurations.
   5. Click "start recording new spectra" in the user interface (UI) to collect the EDX spectrum. Select "Peak ID" to choose the probable elements of the spectrum. Type in observed elements, *e.g.*, oxygen in this case, into the "Element" field. Click "Add" to apply the element to the spectrum.
   6. Click on "File" and then click "Save As". Save the spectral data in.csv format using the desired file name for further plotting using a graphing software.
   7. Repeat the operations in steps 5.3.3 - 5.3.6 to record the EDX spectrum from each hole.
   8. After finishing the imaging and spectrum recording for each of the holes, turn off the electron beam by clicking "Beam On" button on the "Beam Control" page when the electron beam imaging area is on. Vent the SEM chamber by clicking "Vent" on the same page. Carefully take the sample off the stage by removing all the tapes after the chamber door is open.
   9. Repeat the procedure to conduct the control experiments using the DI water and an empty microchannel.

6. Plot the EDX Spectrum

1. Import the.csv spectrum file into a graphing software.

2. Plot the spectrum using the energy level as the x-axis and the intensity received and processed by the EDX as the y-axis to show the reconstructed spectra, as shown in **Figures 2a, 2b and 2c**.

Representative Results

The representative results are presented to show how the particles are imaged and analyzed using *in situ* liquid SEM imaging coupled with EDX. The results include SE images and EDX spectra. The SE images were obtained at 100,000X and 200,000X magnification levels in **Figure 1**. **Figure 1a** depicts the SE image of the AlOOH, **Figure 1b** DI water, and **Figure 1c** the hole in an empty channel. The images were obtained by applying SE with 8 keV accelerating voltage and 0.47 nA beam current. The screen resolution utilized was 1,024 × 884 with a scan rate of 30 µs. Correspondingly, **Figure 2** shows the EDX spectra detected from the AlOOH particles in water (**Figure 2a**), DI water sample (**Figure 2b**) and the hole in an empty channel (**Figure 2c**), based on the measured elemental composition. The EDX spectra were obtained using the same current and voltage setting as that for SE images. The information depth is from the shallow region at the sample surface due to the choice of low voltage. The raw data of the elemental spectra is outputted as.csv file and plotted using a graphing software for presentation.

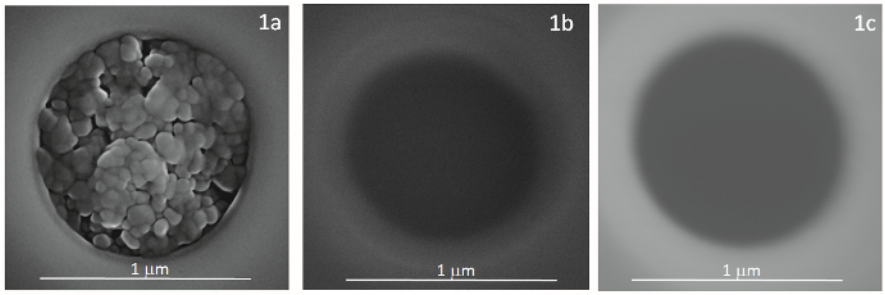

Figure 1: SE Images. These images were obtained by applying SE with 8 keV accelerating voltage and 0.47 nA beam current. The screen resolution utilized was 1,024 × 884 with a scan rate of 30 µs. **(1a)** AlOOH at 200,000X, (**1b**) DI water at 100, 000X (**1c**) and an empty channel at 200,000X. Please click here to view a larger version of this figure.

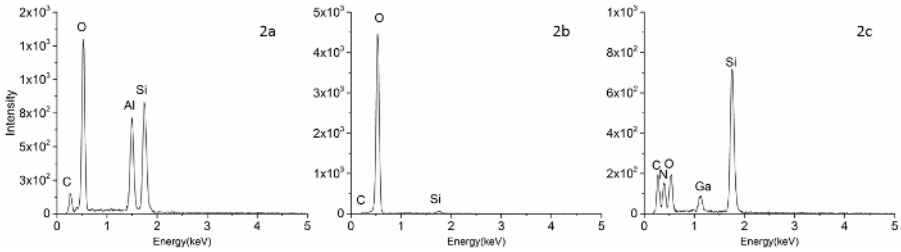

Figure 2: EDX Spectra. EDX spectra were acquired in the SE mode with 8 kV accelerating voltage and 0.47 nA beam current. (**2a**) Spectrum of AlOOH in water. (**2b**) Spectrum of DI water sample. (**c**) Spectrum of the hole in an empty channel. Please click here to view a larger version of this figure.

Discussion

SEM is a powerful technique in surface characterization of organic and inorganic materials on a nanoscale (nm) level with high resolution[1]. For example, it is widely used for analyzing the solid and dry samples such as geological materials[26] and semiconductor[27]. However, it has limitations in characterizing the wet and liquid samples due to the incompatibility of liquid within the highly vacuumed environment required for electron microscopy[1]. SEM sample preparation often requires dehydration or freeze-drying for a hydrated specimen, and particularly for biological specimens[2]. As a result, it is challenging to accurately capture the information of naturally hydrated or liquid samples, as their intrinsic information may be lost during the sample preparation process[28,29]. This may include but is not limited to, biological activity in cells, synthesis of nanoparticles in solution, aggregation of particles in complex liquid, and electrochemical reactions. Even though the ESEM can image hydrated samples in a controlled vapor environment, the resolution of the images could not reach as high as the SEM images of the solid samples in the high vacuum mode[30,31,32,33]. Recently, wet samples were covered by an electron transparent thin film[6] or sealed by a specimen capsule[30] when SEM was employed, and backscattered electrons were collected for images using this approach.

SALVI is a versatile microfluidic interface that has enabled surface analysis of liquids using vacuum-based instruments such as TEM and ToF-SIMS.[11,12,13,14] Our technique using SALVI and optimized SEM conditions can provide SE images and EDX compositional information. **Figure 1a** presents the SE image of boehmite particles in DI water with a submicron scale (400 nm) and high magnification of 200,000. The SEM image shows the morphology and distribution of the boehmite particles in liquid, which validates that the particles in liquid can be seen and held safely within the SiN membrane by surface tension[20]. In contrast, **Figures 1b** depicts the SE images of the DI water within the hole at 100,000× magnification level. It provides direct evidence that water can be hold by its surface tension without leaking outside. In addition, the chamber pressure was kept constant at 1.0×10^{-5} Torr during measurement. **Figure 1c** presents a hole in an empty channel with 200,000× magnification; nothing is observed inside the hole under the same current and voltage settings. The SE liquid imaging capability via this approach provides high resolution SE images compared to the micrometer resolution of backscattered electron images acquired using the reported wet SEM technique[30].

EDX elemental mapping is conducted using AlOOH particles in DI water, DI water only, and the empty channel, respectively. The latter two are used as reference controls. As shown in **Figure 2a**, the aluminum peak occurs at around 1.5 keV with significant signal, while there is no prominent peak appearing at the same energy in the DI water and the empty channel EDX spectra. The oxygen signal is dominant in both AlOOH and DI water, which confirms that this signal comes from water. This further validates that particles are immersed in water during imaging. The C and Si peaks in **Figures 2a, 2b and 2c** are from the carbon coating on the detection window and SiN membrane forming the detection area, respectively. The N peak is also from the SiN membrane. The EDX comparison shows the detection of the aluminum composition of AlOOH in water, indicating that the boehmite particles are indeed observed.

In previous papers, we have demonstrated the feasibility of employing a microfluidic cell and high vacuum SEM to image and characterize the liquid sample, using DI water and immunoglobulin G (IgG) gold nanoparticles[12,20]. In these earlier works, gold nanoparticles were smaller than 10 nm. In this work, we show that boehmite particles with much larger sizes (< 100 nm) can also be studied through liquid SEM. The hole size was calculated to ensure sufficient imaging area yet enough surface tension to hold the liquid within. Originally, the hole was fabricated using the gallium ion beam to make round apertures of 2 µm in diameter prior to device assembly in the initial invention[12,20]. In this update, we show that the detection apertures can be made after the device is assembled, making the entire process more streamlined. One can also open up as many detection windows as needed in an analysis, and is not limited by the holes made before an experiment. The 2 µm diameter detection windows are suitable for techniques such as ToF-SIMS, and it is also feasible in liquid SEM. Because of the high magnification capability of SEM, the new result shows that smaller aperture (*e.g.*, 1 2 µm) works well in SEM (**Figure 1a**).

Several technical details are worth mentioning in order to make *in situ* liquid SEM measurements successful. First, the device needs to be coated with carbon or gold in order to reduce charging during the measurements. Second, the device mounting is quite critical in this procedure. Loose contact of the device with the mounting stage will result in significant charging, difficulty in focusing, and poor images. Third, if one wants to analyze more than one sample using the same device, the sample sequence needs some thought. Although the device is disposable, it is likely a device can be used more than once. For example, one can use water or the solvent for obtaining data of the control sample followed by the analysis of a sample with particles or other species of interest using the same solvent. It is recommended to introduce the sample introduction after the SALVI device is secured and detection holes are made using SEM/FIB. The FIB is used solely for milling the holes on the detection window membrane. If the membrane is prepared by another instrument or the membrane is made with holes available from the suppliers, it is not necessary to use FIB to make holes prior to the SEM analysis. Moving the device away from the sample stage for sample introduction and remounting it again wastes a lot of time, while also adding the risk of poor connections between the device and the sample stage and resulting in a different working distance. The SEM operator may also have to refocus and find the channel and micrometer sized round detection windows again.

With submicron resolution and precise elemental information showcased in this study, we envision that the integration of the vacuum-compatible microfluidic cell (*i.e.*, SALVI) with the high vacuum mode SEM can be widely utilized in identifying and observing various naturally hydrated specimens, geological specimens, biological samples, and nanomaterial synthesized in liquid. With the technological improvements made to the liquid SEM approach are discussed previously, we demonstrate that a larger variety of submicron particles of different sizes may be investigated using this new approach. Ultimately, *in situ* liquid SEM opens more opportunities to study specimens in liquid using high vacuum SEM.

Disclosures

The authors have nothing to disclose.

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