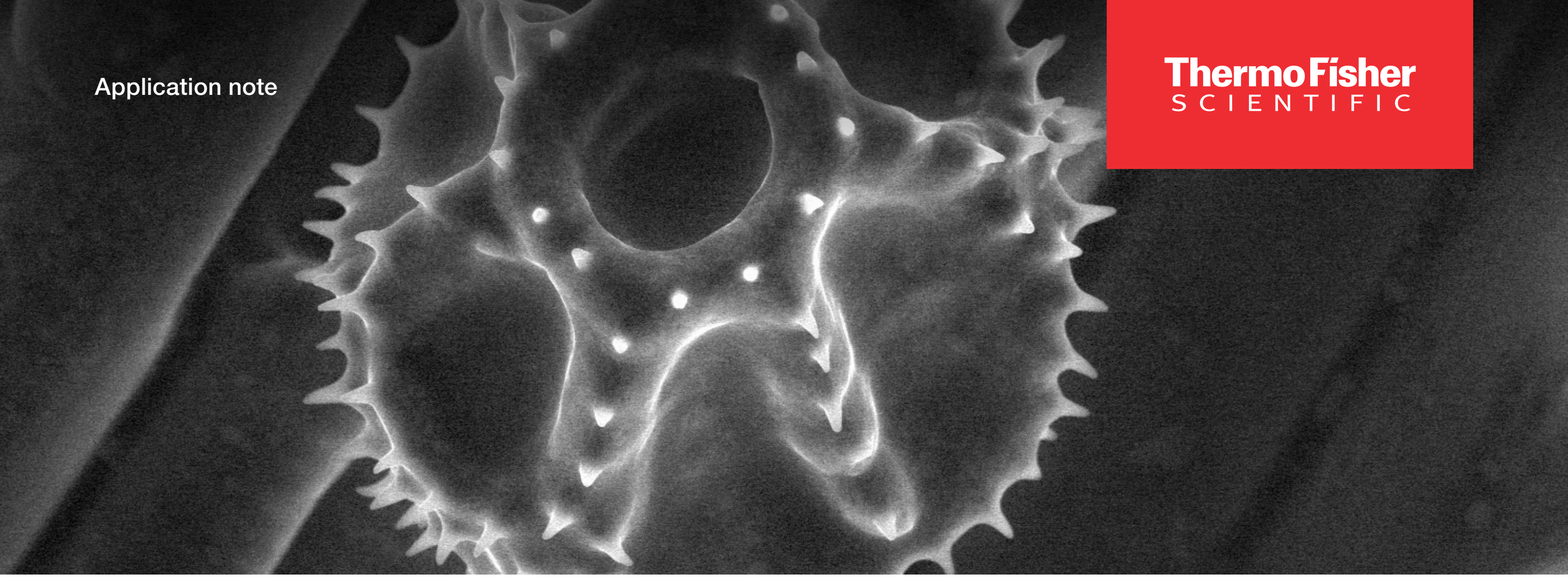
Application note



WetSTEM Technology: a flexible system for SEM's *in situ* cooling experiments

Introduction

Traditional scanning electron microscopy (SEM) characterization has been generally linked to the imaging of dry materials due to the conditions imposed using high vacuum in the chamber. This has always prevented direct studies of wet samples and dynamic imaging in a changing sample environment.

However, industries in fields such as chemicals, pharmaceuticals, polymers, cotton, and wood produce products heavily subjected to environmental conditions that can affect their features, shapes, or even prevent them from functioning properly when not in the correct temperature-humidity conditions. The use of high-vacuum imaging in a traditional SEM is not enough for a complete understanding of a material, which has led to a tremendous increase in the use of environmental SEMs (ESEMs). Observation of wet organic and inorganic materials as a straightforward analysis technique allows an increasing variety of users, including those inexperienced with SEM, to run complicated experiments and study their materials in wet environments.

The ever-growing demand for additional functionality and options on SEMs has led to the development of systems that allow such wet experiments in combination with various types of imaging, of which scanning transmission mode has become predominant. With Thermo Scientific™ WetSTEM Technology, STEM is available in all Thermo Scientific SEMs, with a wide variety of functions, options, and parameters to be tuned.

WetSTEM Technology

ESEM is known as a top-down technique; however, Thermo Fisher Scientific has also developed *in situ* STEM that allows the unique observation of wet samples and the imaging of suspensions in transmission mode through thin membranes of water; for example, objects from a few nanometers to several millimeters stabilized in a liquid phase. This enables the study of nanoparticles and fluids, which is not possible with other means.

Compared to the STEM imaging in TEMs, the main advantage of the WetSTEM System is that, in an SEM, lower acceleration voltages are used and that results in higher contrast. Additionally, EDS analysis is also possible and will provide high spatial resolution results.

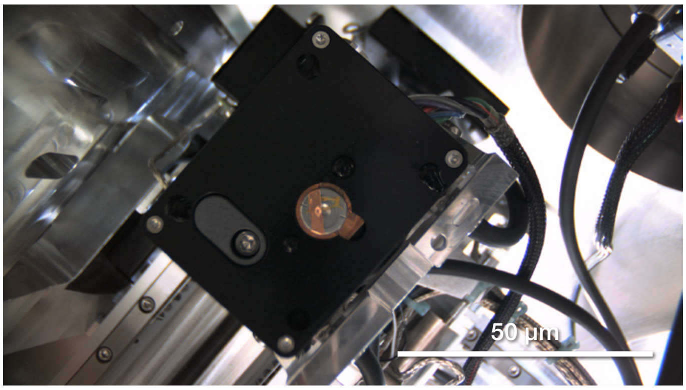


Figure 1. Navigation camera image showing the WetSTEM stage mounted in the SEM chamber.

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Due to the increased versatility of the different options and features on the Thermo Scientific Quattro ESEM, the WetSTEM stage allows for STEM *in situ* experiments by loading TEM grids and allowing for more conventional *in situ* cooling experiments and running top-down imaging via SEM stubs. The stage is designed to use both TEM grids and to employ the same accessories of the Peltier cooling stage (same stubs on which the samples are mounted).

In this application note, use cases are presented to show two ways of using the WetSTEM System: in transmission mode and for top-down imaging in cooling conditions. The ability to run different types of experiments and imaging with a single piece of hardware, and with no need to purchase an additional cooling stage, provides greatly increased flexibility.

STEM imaging of liquid silver nanoparticles

The material under study is a sample of silver nanoparticles in water. A 1.5 µl drop of the solution was drop-casted onto a carbon-coated holey copper TEM grid. The characterization was carried out on a Quattro ESEM using the WetSTEM stage.

Water vapor was added to back-fill the chamber, which is essential to accurately control the relative humidity (RH) level of the sample. An initial purging cycle was employed to switch from the room pressure (from the venting cycle) to the fully wet and hydrated condition (RH 100%) without drying the sample. The chamber pressure was then decreased with the aim of removing excess water while keeping the sample fully hydrated. The temperature of the stage was set at 1°C and maintained at this temperature by the Peltier stage for the entire experiment.

The imaging was conducted with the use of the STEM3/3+ solid state detector, which, thanks to its configuration with several segments, allows the acquisition of bright field (BF), dark field (DF), and high-angle annular dark-field (HAADF) contrast.

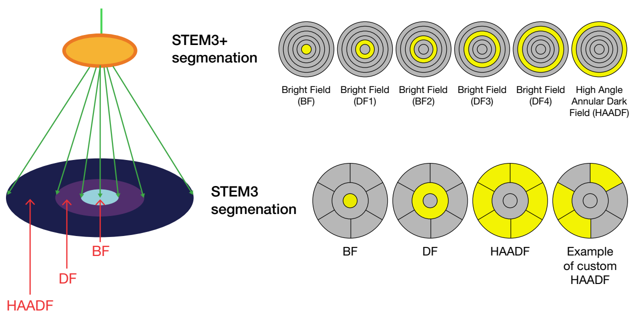

Figure 2. Schematic of the STEM3/3+ detector with two different segmentation systems.

This possibility, together with the SEM user interface design that offers simultaneous detection of up to four signals/detector segments, provided a complete and optimal characterization of the experiment.

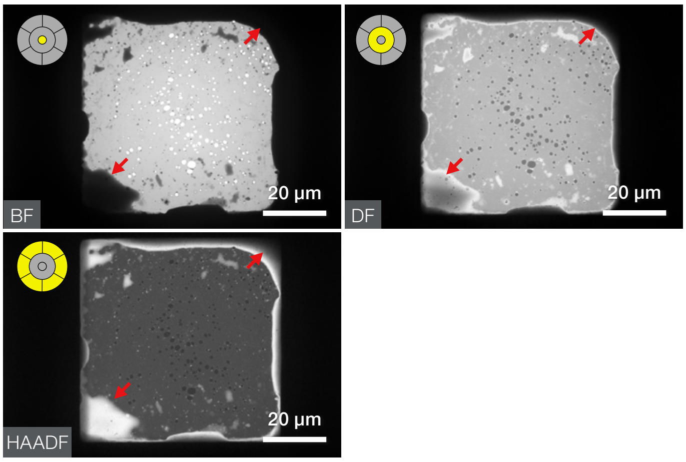

Figure 3. BF, DF, and HAADF images of a large-scale overview of one of the meshes. The images were acquired in wet mode. Liquid water is visible at the edges of the copper mesh.

A first overview of one of the grid's meshes is shown in Figure 3. Liquid water is visible at the edges of the copper mesh, and it appears as either darker or brighter than the sample, depending on the different contrast obtained from the different detectors' segments.

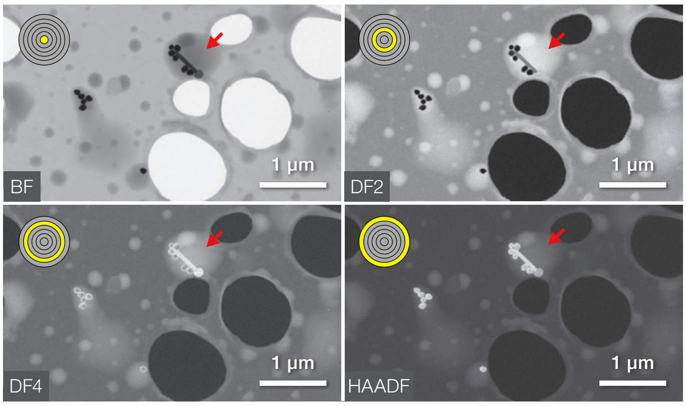

Figure 4. BF, DF2/4, and HAADF images of the silver nanoparticles in the water droplets.

Higher magnification images (Figure 4) were acquired to characterize the nanoparticles' shape. In this case, the STEM3+ segmentation has been used and, as can be seen, different dark field images have different contrasts due to the signal coming from more inner or outer rings of the STEM detector. Additionally, with an RH of 100%, the WetSTEM stage allowed the inspection of the nanoparticles' arrangement in liquid and how they interacted with each other. (Examples are shown in Figure 5.)

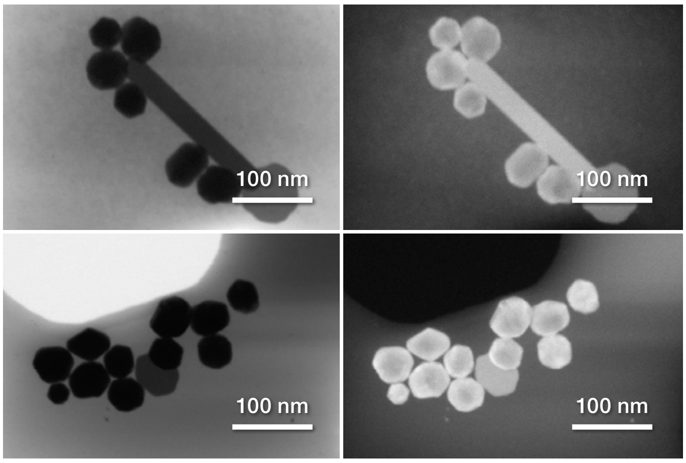

Figure 5. High-magnification BF and HAADF images of different nanoparticles' shapes.

Top-down imaging of pollen

The material of interest for this application example consists of small pieces obtained from a flower and its pollen. Thanks to the WetSTEM stage's flexibility, the system allows you to image bulk samples in hydrated conditions with the same stage used to mount TEM grids and run STEM imaging. As mentioned above, bulk samples can be mounted onto the Peltier stub as it's shown for this specific application, for which copper tape has been also used to improve conductivity (Figure 6).

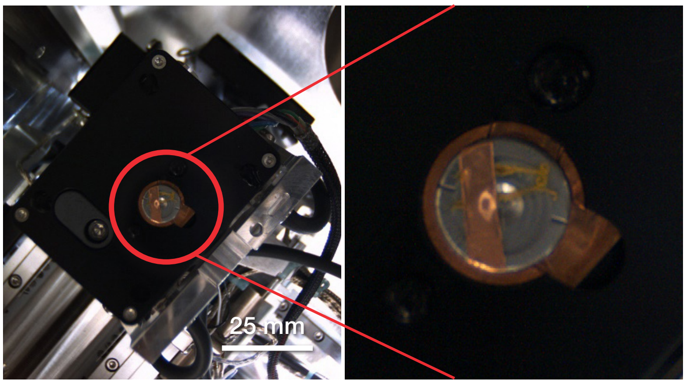

Figure 6. Navigation camera image showing the WetSTEM stage used for the top-down investigation. Two flower sepals are fixed to the Peltier stub using a small piece of copper tape.

For these specific materials, the purging cycle at the beginning of the experiment was optimized to avoid drying the samples while saturating the chamber with water, which would have caused the presence of artifacts on the surface of the sepals.

The imaging has been conducted with an acceleration voltage of 8 kV to show enough surface details while keeping the sample fully hydrated. The relative humidity, in fact, has been kept at 100% for the entire duration of the imaging, which corresponds to a pressure of around 800 Pa.

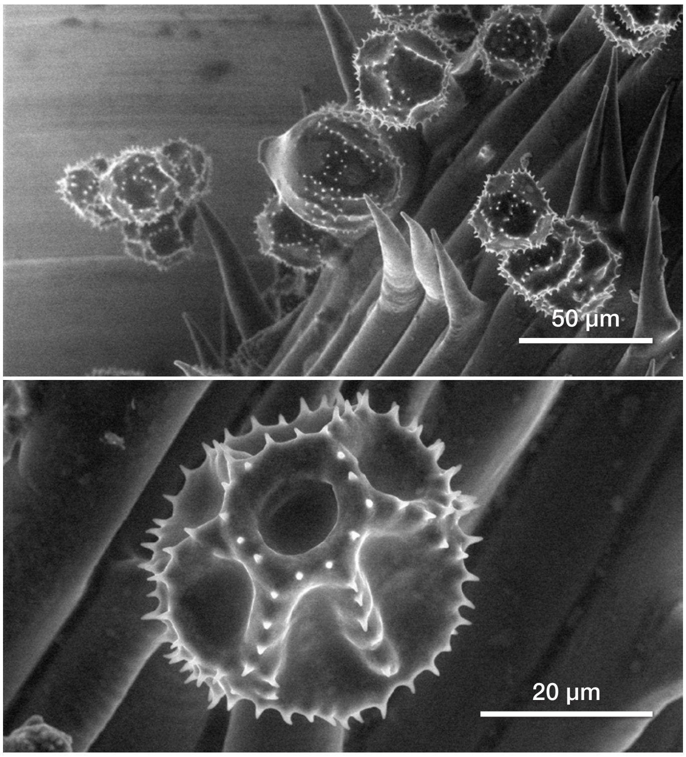

Figure 7. Secondary electron images obtained at 8 kV acceleration voltage in ESEM mode with a temperature of 2°C and a pressure of 800 Pa.

Figure 7 shows two different images obtained using the WetSTEM stage for top-down imaging. The pollen was kept hydrated and swollen as shown in both images, thanks to the high relative humidity. A few water droplets on the aluminum stub are visible in the top left of the upper image.

Conclusion

This application note illustrated how nanoparticles that are in solution can be imaged *in situ* with the WetSTEM holder. Typically, it is also useful to observe bulk samples that are wet, which is likewise enabled by the WetSTEM holder. The experiments in this application note were carried out on a Quattro ESEM, which has the WetSTEM holder integrated in the software.

With the WetSTEM holder on the Quattro ESEM, researchers are no longer limited to imaging only the surface of a water droplet. With the WetSTEM System, suspensions and their inner content can be characterized easily in STEM mode.

Learn more at **thermofisher.com/quattro-esem**