

# Characterisation of Native-State Matter using ‘Multi-Mode’ Electron Microscopy

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## ABSTRACT

Soft condensed matter and complex fluids are of increasing technological significance. It is therefore crucial that we extend the capabilities of conventional solid state imaging and analytical techniques to include the observation of materials such as polymers, gels, colloidal dispersions and emulsions in their soft and/or liquid states. SEMs that permit the presence of water vapor and other gases in the specimen chamber make it possible to tackle these otherwise challenging specimens, with no need for freezing/drying or an electrically conductive coating. More recently, this technology has been further expanded to allow characterisation of nanostructured soft materials, again in their native state, using a specialised STEM detector, allowing the collection of low voltage transmitted electron signals. This means that we now have the capability to collect high resolution information from the bulk material, in addition to the simultaneous collection of surface-sensitive secondary and backscattered electron signals, whilst also carrying out x-ray microanalysis. These approaches offer tremendous potential for static and *in situ* dynamic studies of numerous soft nanostructured systems.

**Keywords:** Soft matter, STEM-in-SEM, variable pressure SEM, native-state, complex fluids

## 1 INTRODUCTION

One of the ways to image insulating materials without an electrically conductive coating is to employ a partial pressure of gas in the specimen chamber of an SEM. Upon accelerating the electrons emitted from the specimen, ionizing collision in the gas result in positive species that can help to compensate for negative charge build-up due to the electron beam.

Gases may include water vapor, nitrogen, nitrous oxide, argon, helium, carbon dioxide and air, typically at pressures of around a few tens to hundreds of Pa. At present, several units of pressure are in common use in the literature, so some useful conversion factors are given in Table 1.

Water vapor is particularly useful as it can be used, in conjunction with specimen cooling and higher pressures (up to a few thousand Pa) to maintain thermodynamic stability for moist or liquid specimens (see Section 1.2).

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Atmospheric pressure = 760 torr (or mmHg)  
=  $101 \times 10^3$  Pa = 1 bar

1 torr ~ 133 Pa ~ 1.3 mbar  
1 Pa ~  $7.5 \times 10^{-3}$  torr ~ 0.01 mbar  
1 mbar ~ 0.75 torr ~ 101 Pa

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Table 1 : Common units of pressure and their conversion factors

### 1.1 Uncoated electrically insulating materials

Figure 1 shows an example of imaging an uncoated electrically insulating material, using water vapor as the imaging gas (40 Pa). The secondary electron signal is obtained via a cascade process, in which daughter electrons are generated during ionizing collisions of secondary electrons with gas molecules (see, for example, Thiel *et al* [1]).

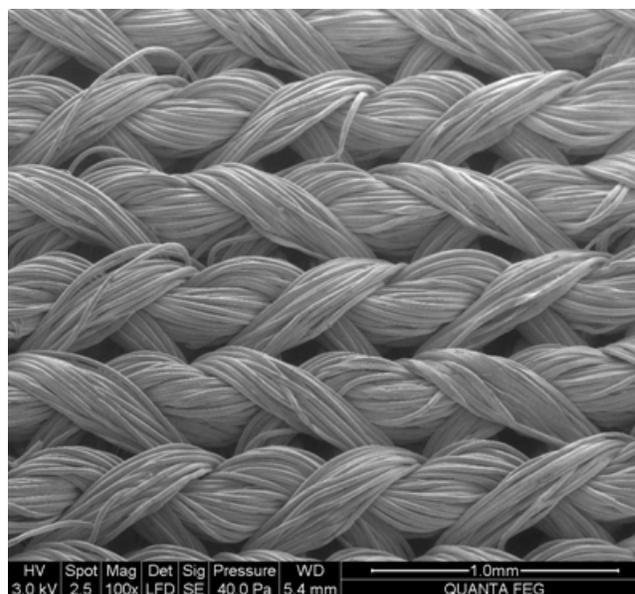


Figure 1 : PET fibers imaged without a conductive coating

## 1.2 Moist or liquid specimens

As already mentioned, the presence of water vapor is excellent not only for providing charge stability but also for thermodynamic stability. If the specimen is cooled slightly, then water vapor pressures are such that high quality imaging can still take place. Figure 2 gives an indication of the temperatures, pressures and humidities involved.

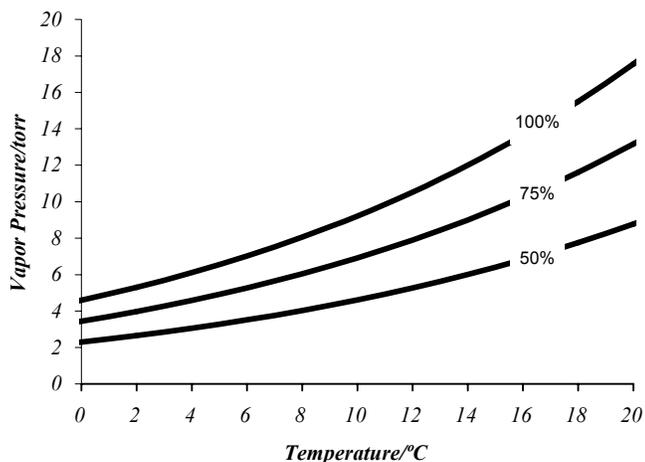


Figure 2 : Humidity curves, based on the phase diagram for water

Meanwhile, Figures 3 to 5 demonstrate how this capability can be put to practical use.

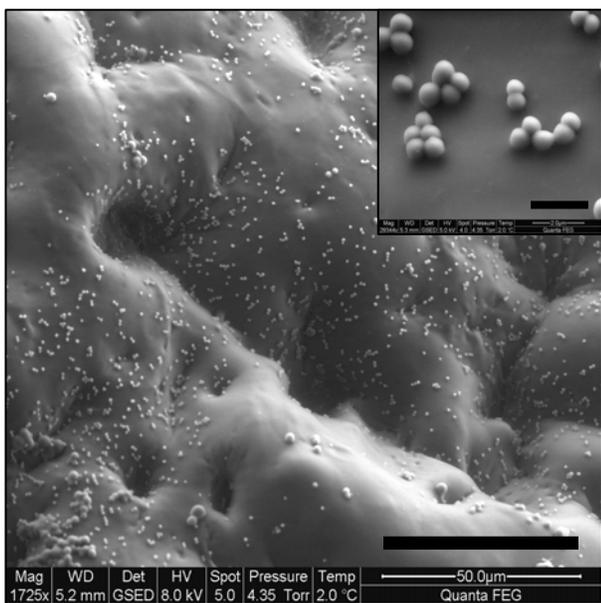


Figure 3 : A hydrated biofilm with  $\sim 750\text{nm}$ -diameter particles. Scale bar =  $50\ \mu\text{m}$ . Inset: higher magnification image, scale bar =  $2\ \mu\text{m}$

Figure 3 shows a hydrated, uncoated biofilm with particles on the surface. The advantage of this approach is that there is little or no specimen preparation involved, and so the film is observed at or near its 'native-state'.

If conditions are shifted to more than 100% relative humidity, then water condenses onto the specimen surface (see Figure 4). Similarly, water can be driven off the specimen to deliberately cause dehydration.

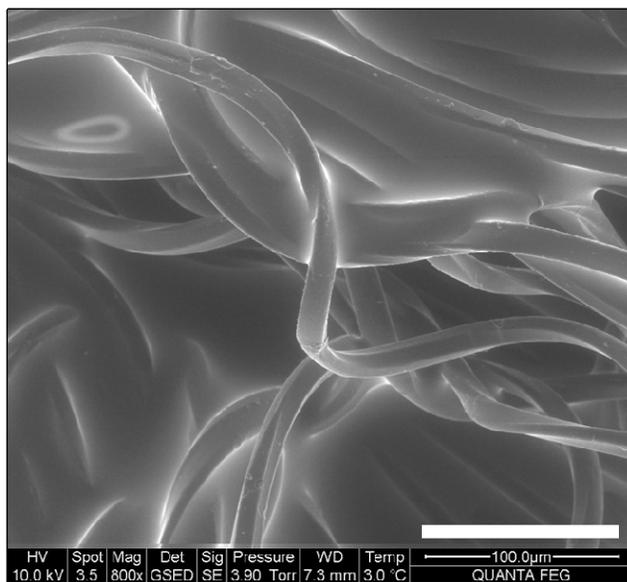


Figure 4 : Water can be condensed onto the specimen, as seen on these fibers, for *in situ* experiments. Scale bar =  $100\ \mu\text{m}$

Finally, in Figure 5, we see how an entirely liquid specimen, such as this oil-in-water emulsion, can be observed.

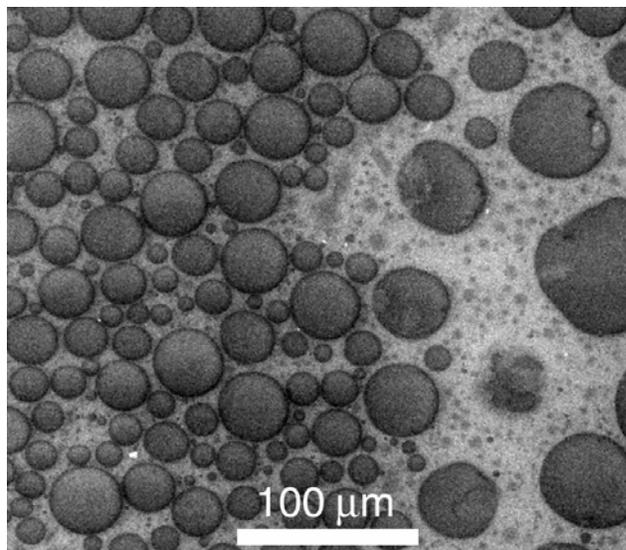


Figure 5 : Entirely liquid specimens can be imaged (reproduced, with permission, from ref [2])

## 2 LOW VOLTAGE SCANNING TRANSMISSION ELECTRON MICROSCOPY (STEM)

### 2.1 STEM-in-SEM (high vacuum)

Positioning of a solid-state detector beneath a thin specimen in the SEM permits collection of transmitted electrons at 'low' voltages (relative to a conventional S/TEM instrument). We can thus form high-contrast STEM images of organic materials. An example is shown in Figure 6 where the membrane stacks are separated by just a few nanometers.

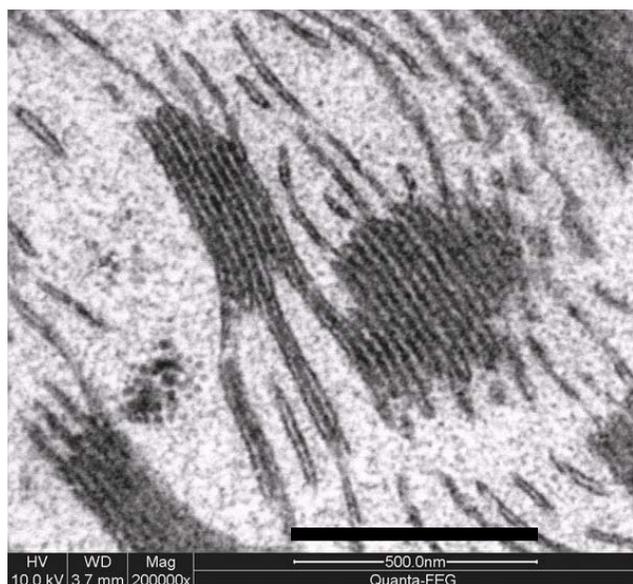


Figure 6 : High-resolution bright field STEM-in-SEM image of a TEM-prepared leaf showing membrane stacks inside a chloroplast. Scale bar = 500 nm

### 2.2 Wet STEM

An extension to the STEM-in-SEM idea is to combine the concepts of imaging in water vapor and specimen temperature control to enable wet specimens to be viewed in STEM mode. This can be achieved by Peltier-cooling the TEM grid on which the specimen is held, and utilizing the water vapor capabilities of the microscope. This was recently shown for a range of specimens by Bogner *et al* [3].

To demonstrate the concept, Figure 7 shows a suspension of latex spheres placed on a holey carbon film, imaged in water vapor at a pressure of approx. 730 Pa and a temperature of 2°C.

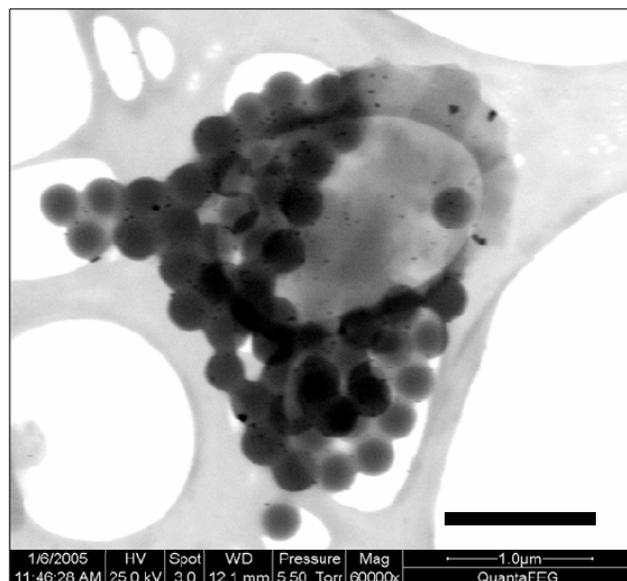


Figure 7 : Bright field STEM image of hydrated latex spheres. Scale bar = 1 µm

## 3 MULTI-MODE IMAGING

Finally, we can choose to combine a number of different signal detectors, with or without the thermodynamic approach for stabilizing a moist or liquid specimen, in order to get 'multi-mode' information. Several features of such a multi-mode imaging system are shown in Figure 8.

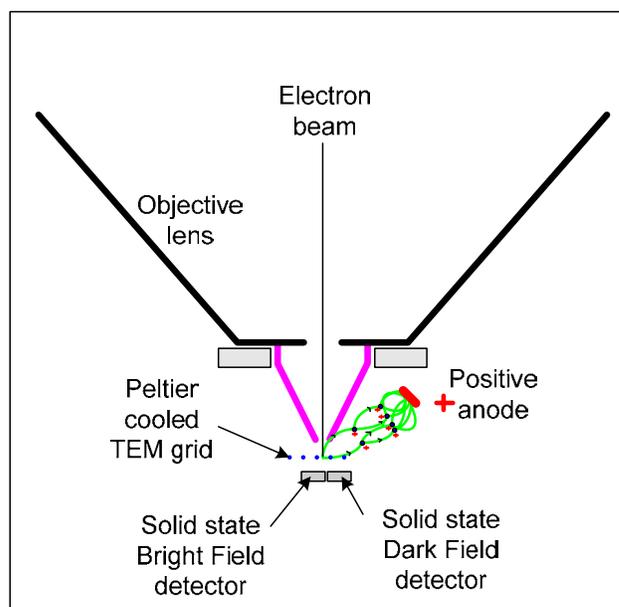


Figure 8 : Schematic diagram showing part of the arrangement of detectors for multi-mode imaging

The main features of this system can be summarized as follows:

- Peltier-cooled stages, including TEM grid holder
  - Permits thermodynamic control
- Solid-state crystals beneath specimen collect transmitted signals
  - STEM-in-SEM
  - High resolution, ‘low kV’ imaging
  - Good contrast for low Z materials
- On-axis backscattered electron detector
  - For visualising labels/stains, for example
  - Can be used in both high vacuum and in the presence of a gas
  - Added cone minimizes primary electron gas path length (to reduce scattering of primary electrons in gas)
- Off-axis gaseous secondary electron detector
  - For collection of surface-sensitive information in a gas
- Everhardt-Thornley detector
  - For SE imaging in high vacuum
- Plus x-ray detection in all modes

Figure 9a shows a ruthenium-stained polymer section, viewed with STEM-in-SEM and figure 9b is the corresponding x-ray elemental ruthenium map.

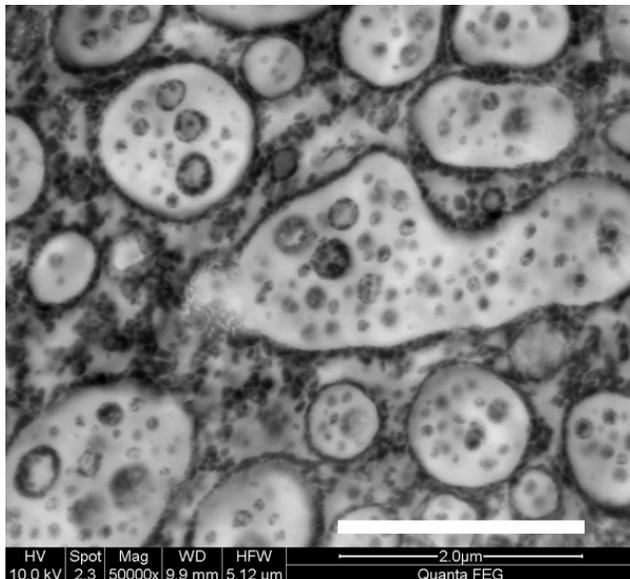


Figure 9a : Bright field STEM image of a ruthenium-stained, microtomed polymer section showing high-resolution detail. Scale bar = 2  $\mu\text{m}$

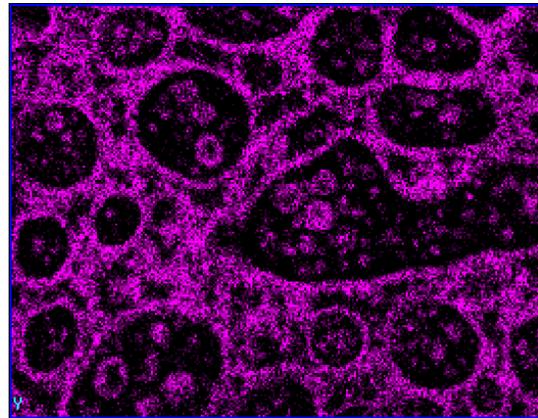


Figure 9b : x-ray elemental map of ruthenium in the region shown in Figure 9a.

## CONCLUSIONS

SEMs that operate in both high vacuum and in the presence of a gas have helped to open up new possibilities for imaging soft matter in the native state. In particular, the development of STEM-in-SEM, to include moist or liquid specimens, enables high-resolution imaging and analysis of native-state soft matter at the nanoscale. Further, the combination of several signal detection modes potentially makes this a very powerful combination of techniques.

## REFERENCES

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