QUICK USE GUIDE FOR IMAGING

IMPORTANT NOTES

- **WETSEM™ images can be obtained with various detectors. With most SEM configurations, best images are obtained with a BSE detector positioned at the end of the SEM column (located under the pole piece).**
- **High probe currents, especially when scanning at high magnifications, may damage the QX capsule membrane and sample. To prevent damage, avoid scanning the same area at high magnifications for prolonged periods. See Table I for the maximum recommended probe current for your SEM configuration.**

TABLE 1: SUITABLE PARAMETER RANGE FOR WETSEM™ IMAGING

PARAMETER	RECOMMENDED RANGE	COMMENTS
Acceleration Voltage	15 - 30 kV	Not lower than 10 kV
Probe Current (based on source type)		
Tungsten filament	0.4 - 1.0 nA	Not higher than 1.0 nA
FEG	0.1 - 0.5 nA	Not higher than 0.5 nA
Working Distance (based on detector type)		
Semiconductor (BSE)	6 - 10 mm	Acceptable: 4 - 15 mm
Robinson (BSE)	10 - 20 mm	Better efficiency at high keV
Scintillator (BSE)	6 - 10 mm	Acceptable: 6 - 10 mm
Everhart-Thornley (SE)	8 - 12 mm	Acceptable: 6 - 15 mm
In-lens / Through the lens (all detectors)	2 - 4 mm	Manufacturer dependent

CALIBRATION CAPSULE USE

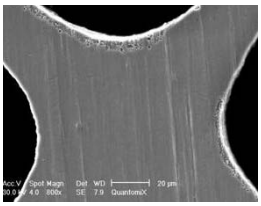

Figure 1

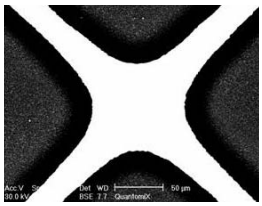

Figure 2

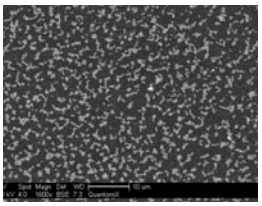

Figure 2

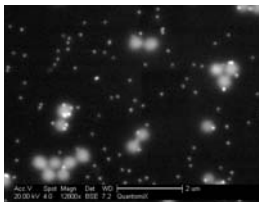

Figure 4

For first time imaging, always use the Calibration Capsule.

01 Reconstitute the Imaging Buffer with 1ml of double distilled water.
02 Place the Calibration Capsule in the Multi-well Plate, and remove the sealing stub.
03 Apply 15µl of Imaging Buffer into the Calibration Capsule, and seal with the stub.
04 Place the hydrated Calibration Capsule in the SEM.
05 Set the acceleration voltage to 30kV.
06 Set the spot size at 35% of the spot size range.
07 See Table 1 for a working distance suitable for your SEM configuration.
08 Initially, use the SE detector and focus on the metal support grid (see Figure 1).
09 Once focused, change to the BSE detector.
10 It is recommended to start sample imaging with a slow scan speed (a few seconds per frame).
11 Increase contrast drastically. The grid should give a strong, white signal (see Figure 2).
12 Move from the grid to one of the windows.
13 Increase magnification until the 500nm silica beads are visible (see Figure 3).
14 If no beads are visible, increase the contrast further.
15 If no beads are visible yet, gradually increase the spot size. (Note that spot size correlates to probe current. See Table 1 for the maximum probe current that should be used for WETSEM™ with your SEM configuration).
16 Once the silica beads are visible, improve the focus and zoom in.
17 Identify the 40nm gold beads, focus and correct astigmatism. The gold beads should appear perfectly round after the correction (see Figure 4).
18 Obtain a slow-scan image and save.

SAMPLE IMAGING

01 Apply steps 1-12 in the above procedure to your sample.
02 Adjust contrast and brightness until features of interest are visible.
03 If no features are visible, gradually increase spot size.
04 Once features are identified, focus, adjust contrast and brightness, and obtain the required images.
05 Zoom in as required, adjusting focus and correcting astigmatism.