

## PROVIDING SOLUTIONS...

# SEM Specimen Mounting

It is easy to overlook the importance of mounting samples for SEM observation, but this step is just as critical as all the ones you took in order to ensure accurate morphology in the first place. So, taking a little extra time to mount the specimen with purpose will pay off when imaging in the SEM.

### Proper mounting ensures:

1. **Desired orientation**, which reduces confusing, time-wasting stage manipulations
2. **Good mechanical stability**, thus reducing potential vibrations and drift
3. **Proper electrical grounding**, which reduces potential charging

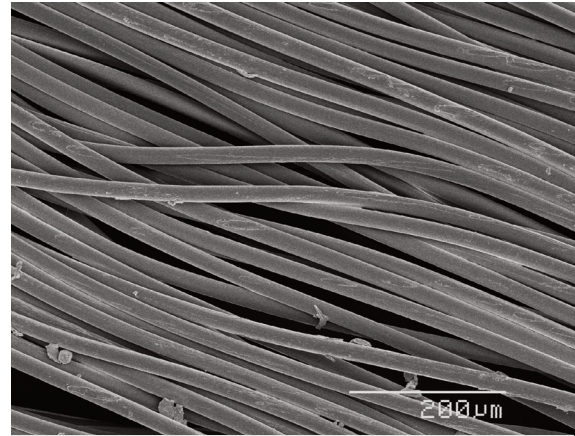
## Achieving the Perfect Mount

### Orientation of the ROI to the beam/detector system

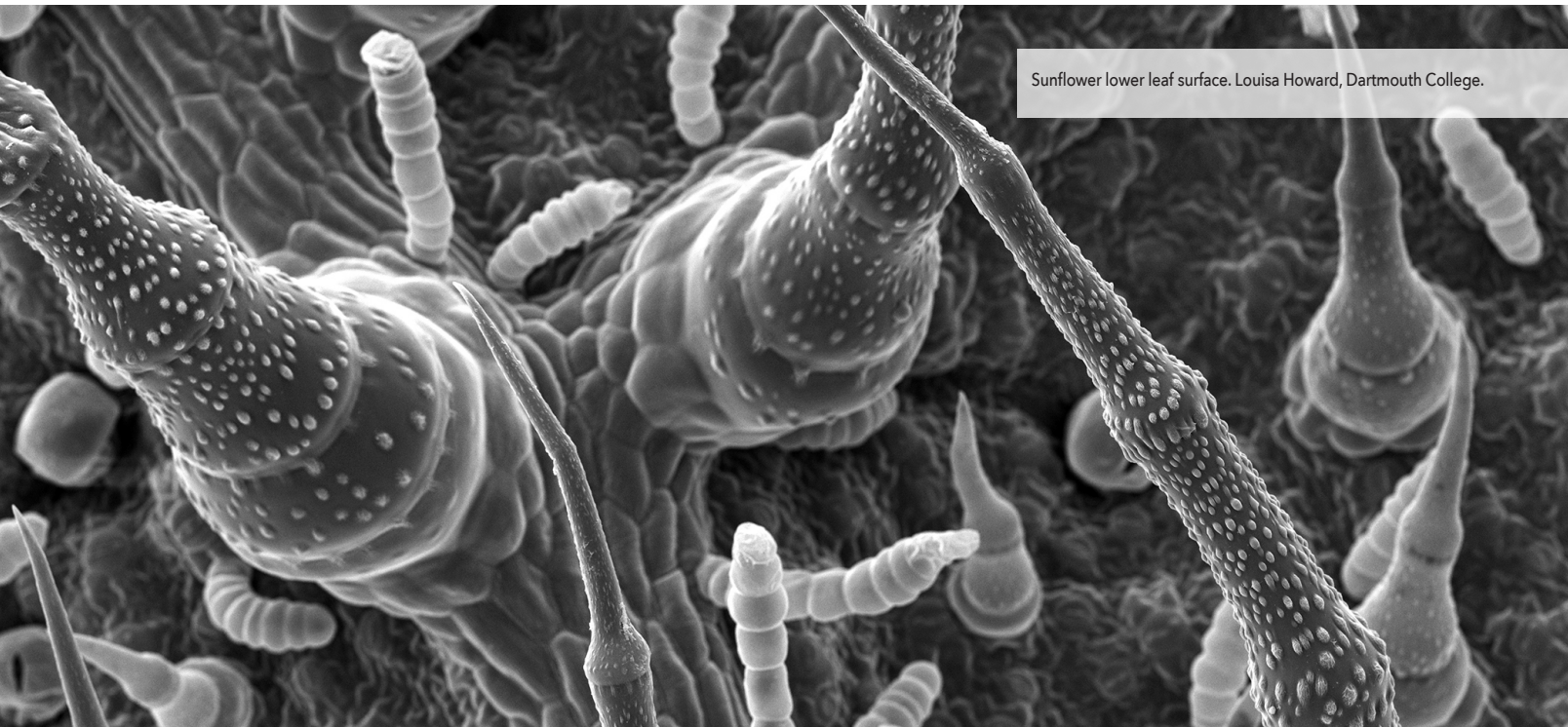
The first parameter to take into consideration is the orientation of the ROI to the beam/detector system. Visualize the beam as your eyes' line-of-sight and the detector as the sun, which determines apparent illumination direction and shadows. If the sample has a specific polarity, like a leaf or insect, the top of the sample should be oriented towards the detector, giving it the appearance that the sun is shining down on its upper surface just as you would see it in nature.

This beam/detector system is also important to keep in mind when mounting multiple samples on the same stub or samples with considerable height variations, with the taller samples mounted to avoid casting a shadow on other areas of interest. When mounting multiple samples on the same stub, it is also a good idea to cut different samples into different shapes and draw a map of them so they can be identified in the SEM.

continued >>>



Dental floss.



Sunflower lower leaf surface. Louisa Howard, Dartmouth College.

Achieving the Perfect Mount (continued)

**Secure Adhesion of Sample to Stub**

The second parameter is secure adhesion of the sample to the stub. There are several materials available: double-sticky carbon tape or tabs, copper or aluminum tape, and colloidal carbon or silver fluids. These work well for most applications and are ready for use in a few minutes. There are also a variety of pastes, glues, and more permanent epoxy conductive materials, which take anywhere from one to many hours before they are ready to use.

**NOTE:** Care must be taken when using colloidal silver with biological samples, since it has a tendency to wick or migrate from the point of application.

**Providing Good Electrical Grounding**

The third parameter is providing a good electrical grounding pathway, which is essential to avoid sample-charging artifacts.

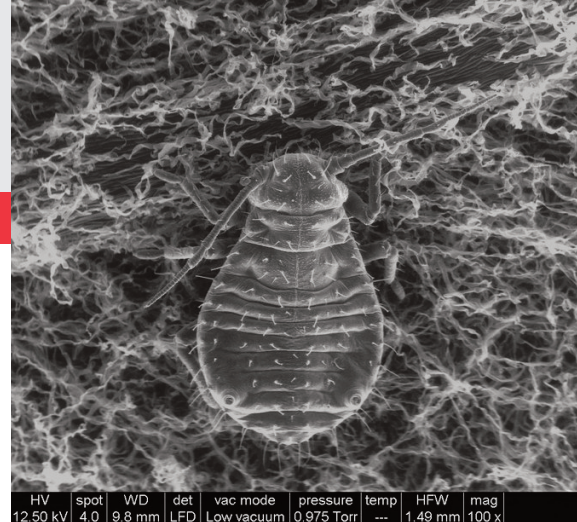
**For specimens that are flat or lying down**, it is convenient to use the double-sided sticky carbon tabs or one of the other types of tapes for initial adhesion, followed by several spot welds of colloidal carbon to ensure stability and electrical contact to the surface to the stub. *Simply sticking the specimen down on the tape does not provide sufficient electrical grounding to the stub, despite several heavy metal coatings, and is one of the main causes of charging.*

**When mounting a sample with a high aspect ratio**, like a stem on end, the double-sided sticky materials don't work well. Instead, deposit a small drop of colloidal carbon on the stub, and, holding the sample away from the ROI, insert it into the drop.

Then, let go of the sample but keep the tweezers on each side and support the sample until the colloid dries. At this point, a slight tilt can be introduced to facilitate imaging. After it dries, take a fine wire or tooth-pick and paint a conductive path, with the carbon paint, to an area which will provide a path for electrical conductance. Also make the sample, twig, as short as possible to minimize sample drift when imaging.

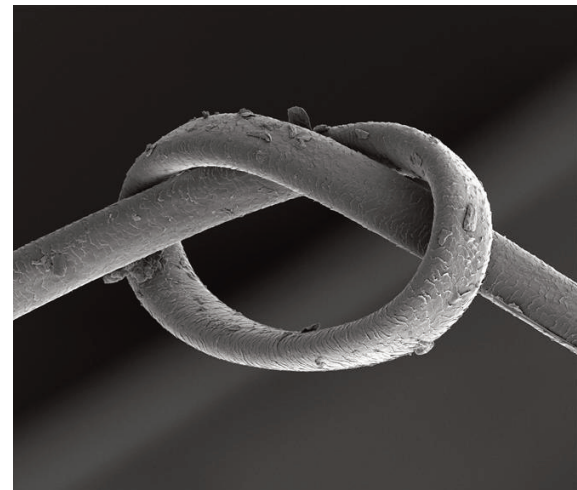
**NOTE:** Use a pair of self-closing tweezers to hold the sample in its desired orientation to avoid rushed mistakes during mounting.

When inserting the samples in the SEM, be sure to orient the stub so that your carefully processed and mounted samples correspond to the position of the SE detector so rotation of the stage is not required.



HV	spot	WD	det	vac mode	pressure	temp	HFW	mag
12.50 kV	4.0	9.8 mm	LFD	Low vacuum	0.975 Torr	---	1.49 mm	100 x

Aphid on white poplar. Courtesy of Riccardo Antonelli.



mag	500 μm
400 x	

Knot of human hair. Courtesy of Frans Holthuysen.

EMS Catalog supplies mentioned **Cat. No.**

**Adhesives**

Conductive Lift-N-Press Carbon Tabs, 12mm diameter, roll/250	<b>76762-01</b>
Double Stick Carbon Tape, 12mm wide x 20m	<b>77817-12</b>
Carbon Conductive Adhesive, 30ml w/brush	<b>12691-30</b>