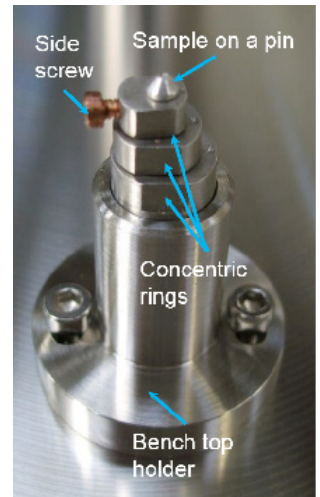


3View System Quick Start Guide

Step 1: Mount the sample in the specimen holder

- Place the sample (mounted on a pin) in the rivet specimen holder, then fasten the screw on the side to keep the specimen in place
 - Make sure the longer sides of the specimen block are parallel to the side screw
- Unscrew the clamp at the bottom of the rivet holder, then place the specimen holder in the bench top trinocular mount holder
- Center the region of interest under the trinocular by rotating the concentric rings
 - Make sure that the side screw stays on the left hand side and parallel to your shoulders at all times
- Fasten the clamp at the bottom of the rivet holder, then check to see whether the region of specimen is still centered under the trinocular



Step 2: Mount specimen to the microtome

- Vent the microscope chamber before you open the door
- Mount the trinocular on scanning electron microscope (SEM) door, then turn on the LED
- Using the 3View® system control in DigitalMicrograph® (DM) software, clear the knife
- Make sure the sample stage is lowered: Use the 3View system control in DM to lower the stage; next turn the coarse mechanical adjustment (the brass screw underneath the specimen) counter-clockwise; then bring the stage all the way down
- Place the sample in the microtome
 - Make sure the longer sides of the sample block are parallel to the diamond knife edge and the side screw on the sample holder is towards the SEM door
- Tighten the sample in place using a hex key and the screw beneath the sample

Step 3: Check the specimen height

- Place a hex key between the knife holder in clear position and the sample holder
- Unclear the knife in DM and remove the hex key while holding the knife holder by your other hand
- Bring the knife towards the sample very slowly to make sure the sample is not too high and does not crash into the sample

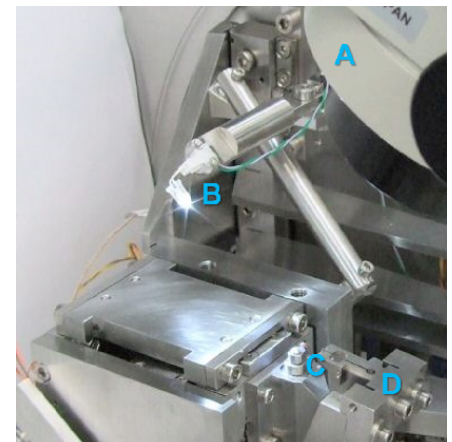


Figure 1. (A) Trinocular mounted on the SEM door. (B) Unfolded LED. (C) Sample on a pin. (D) Knife in clear position.

Step 4: Set the approach

- Look through the trinocular and focus on the edge of the knife
- Retract the knife in DM and select "Move to" in the 3View control to move the knife to the center of the block-face (usually around 400 – 600)
- Set the LED position so that you can see the knife shadow on the block
- Use the brass screw to bring up the sample as close as it is safe by turning it clockwise
 - Usually the knife shadow is the thinnest when the sample is very close to the knife edge
- Tighten two bolts on sides of sample holder stage to keep the sample from moving, then use the 3View control to designate an approach set of cuts (200 nm thick)
- Start the approach cuts and watch the block-face in the trinocular, continue until the knife starts cutting the region of interest

Step 5: Data acquisition

- Fold the LED, remove the trinocular from chamber door, pump up the chamber, then set up the SEM settings for back scattered imaging (focus, stigmation, brightness and contrast)
- Set the knife parameters so the oscillator is always on to get smoother cuts
- Set the cut thickness, dwell time, magnification, spot size, pressure and accelerating voltage, then acquire a test data set
- Check the test data set to make sure the images look good, there is no charging and the knife cuts the surface
- Optimize the parameters mentioned above before you start data acquisition