

## Capturing Stereo Images for MeX

- 1) Set the working distance to ~10mm so that you have room to tilt the stage (this length may differ for various samples).
- 2) Go through the microscope alignments and rotate the image 90° using the raster rotation option under the **Image** tab.
- 3) Calibrate the stage for tilting. Calibrating is important for eucentric tilting. When you tilt the stage, you want to be able to stay in the same general area on your feature of interest.
  - a. Find a small (<5µm) unique feature that you can find again, and get it in focus at relatively high magnification. It may be easiest to choose a feature right on the corner of the image.
  - b. Increase the magnification to at least 10,000X on the feature, and capture an image.
  - c. Under the **Stage** tab make sure that **Eucentric Tilt** is not checked, and select calibrate. Follow the onscreen instructions for calibrating, and when you are done, make sure you select **Apply** in the bottom left corner of the calibration window. It will be difficult to find your feature again once you start the calibration process, but when you do, make sure you reference the image you took in order to get the feature in the same orientation and at the same magnification as the first position.
  - d. Once you have calibrated, you can test how well you did. Turn on **Eucentric Tilt** under the stage tab. Get your feature centered, and tilt the stage 5°. Your feature should move back to the same place it was in before you tilted. The closer you are to the original position, the better you did calibrating. If your feature is still not returning close to where you began, you should calibrate again until you are able to keep it close, but it doesn't have to be perfect. You can always move the trackball to get the feature in its original position.
- 4) Take an image of the area of interest at 0° tilt. It is important that this is a good quality image (no astigmatism), and that the contrast is set so there are not extreme bright or dark areas. You also want to maintain similar contrast for each image.
- 5) Save the image as a .Tif file to the SemServer
- 6) Eucentrically tilt the stage at least 5° (tilt further if your sample doesn't have large topographical features), and make sure your feature returns to the same place it was in (you can use the track ball to perfect this).

Note: Tilting the stage further than 5° and increasing the magnification are ways to improve the disparity between images.
- 7) Make sure the image is in focus with the same magnification and similar contrast as the other image and save it.

- 8) Taking a third image at a greater tilt angle is optional, but recommended to achieve more accurate results.
- 9) Transfer your files from the SemServer to a flash drive to put into the MeX computer located to the right of the SemServer 1 computer. You can import images from the SemServer by FTP on the MeX computer.