

## Tip: Microscopy with Extended Depth of Field and High Dynamic Range Imaging

### INTRODUCTION

The Menil Drawing Institute paper lab recently purchased a Meiji stereomicroscope (fig. 1) with an attachable Promicra camera, tethered to a computer with QuickPhoto Micro 3.2 software.



*Fig. 1. Meiji stereomicroscope in the Menil Drawing Institute lab.*

The custom stand of the microscope is capable of 360 degrees of rotation, has an arm with multiple points of articulation that extends out to two feet from the base. A 42 inch 4k monitor display is mounted on a medical grade articulating arm, freeing the desktop and allowing the screen to be raised for large group viewings. A wireless mouse and keyboard allow for increased workspace flexibility and collaboration. This set-up has three main functions – it can be used to view a sample in the same manner as standard stereomicroscopes,

to display a live-view of the sample for a group to see (fig. 2,) or to image capture that live-view for later reference. The QuickPhoto Micro 3.2 software allows for still images of the live-view to be captured, or for the images to be modified with extended depth of field and/or high dynamic range imaging.



*Fig. 2. Brianna Warren and Ashley Stanford discussing an imaged sample on the monitor display.*

The Extended Depth of Field imaging technique – EDF – allows the user to capture a sample that would otherwise have too much depth to be entirely in focus at a single magnification and create an image that is in focus from edge-to-edge. This can be done with the EDF module of the QuickPhoto software. The user simply needs to set the highest and lowest points of focus (fig. 3.) There is a motorized focus drive dial attached to the microscope's coarse and fine focus that can be controlled through the software (fig. 4.) The distance between the two set points is then computed by the software. This distance is used

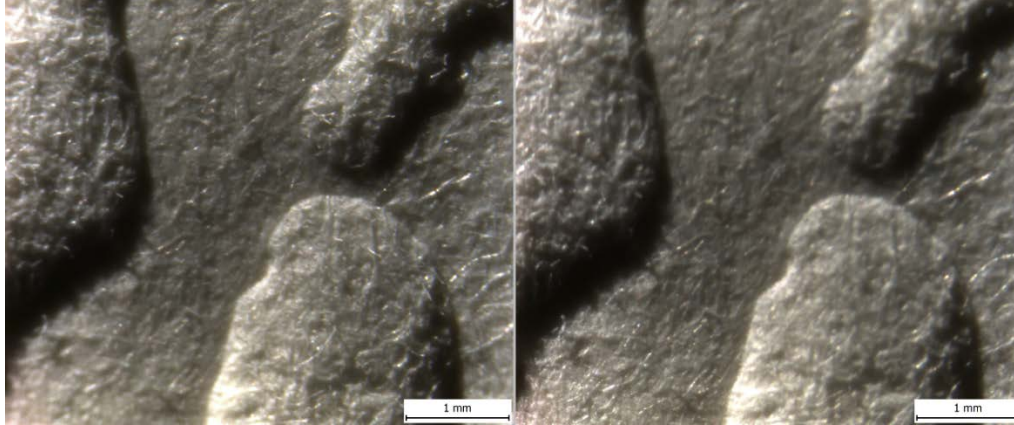


Fig. 3. Lowest set point of focus (left) and highest set point of focus (right) as seen prior to EDF imaging.

to calculate the number of images needed to capture focused image data across the entire depth of the sample. With a click of a button the motorized focus drive automatically collects stepped images at and between these points. The software stitches these shots together to create one image that is sharply in-focus from edge-to-edge (fig. 5.) The user can save the single composite image, and any or all of the steps used to create it.

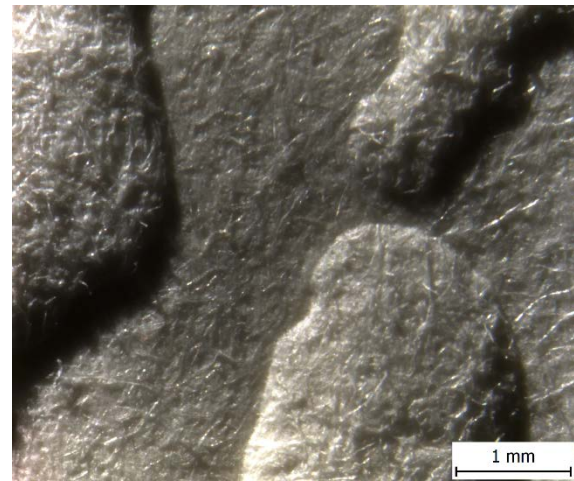


Fig. 5. Composite image of sample after EDF imaging.

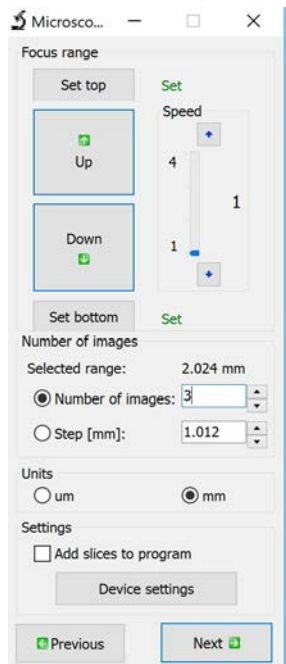


Fig. 4. Software interface menu for the motor focus drive function.

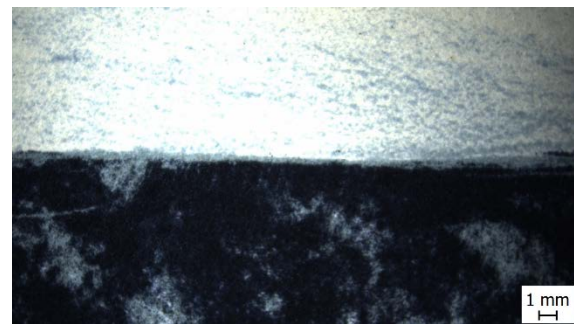


Fig. 6. Sample at 41.667 ms exposure time before HDR imaging.

The high dynamic range module – HDR - also uses software for image adjustment, but with a different purpose. The HDR imaging technique allows the user to image a sample that would

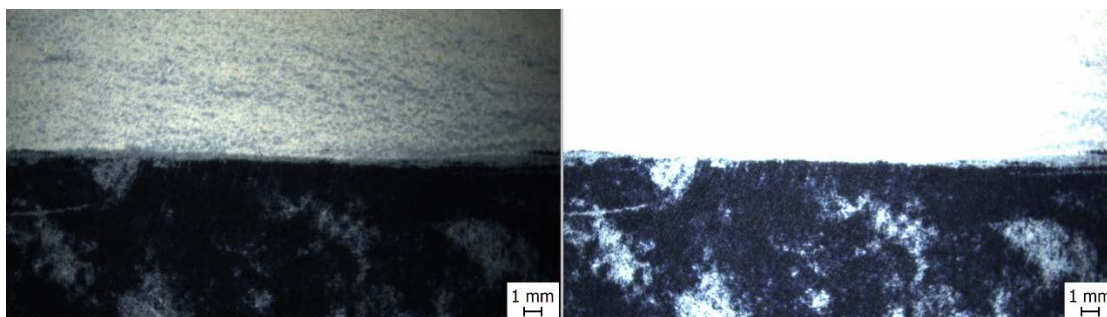


Fig. 7. Sample with set exposure time parameters of 27.776 ms (left) and 100 ms (right).

otherwise have areas too dark and too light to be captured with the same exposure (fig. 6.) To do this, the HDR software module is used to set an exposure short enough to properly image the lightest areas, and long enough exposure time to distinguish detail in the darkest areas (fig. 7.) The software then captures a series of images at and between these parameters. Those images are then stitched together by the software into one image with information in areas that were previously not detailed enough to be useful in documentation (fig. 8.)

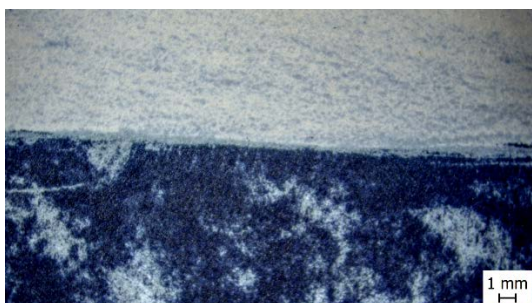


Fig. 8. Composite image of sample after HDR imaging.

For more challenging samples, the EDF and HDR modules can be used simultaneously to create images that are fully focused and filled with visual data (fig. 9.)

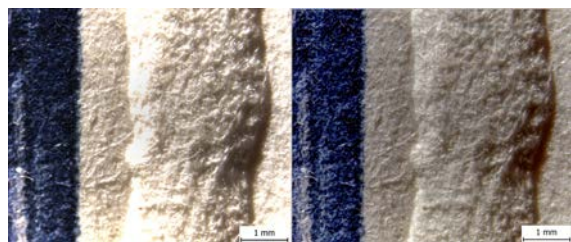


Fig. 9. Sample at 250 x magnification and 500 ms exposure before EDF+HDR (left) and composite image of sample after EDF+HDR (right.)

The software is very user friendly. The lab commenced imaging after only a brief demonstration, and has since established a formalized workflow to accompany use of the software. This imaging workflow accounts for the modified nature of these images by using particular naming and metadata entry protocols that specify the technique used and the set parameters of each captured image and image step.

#### ACKNOWLEDGMENTS

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