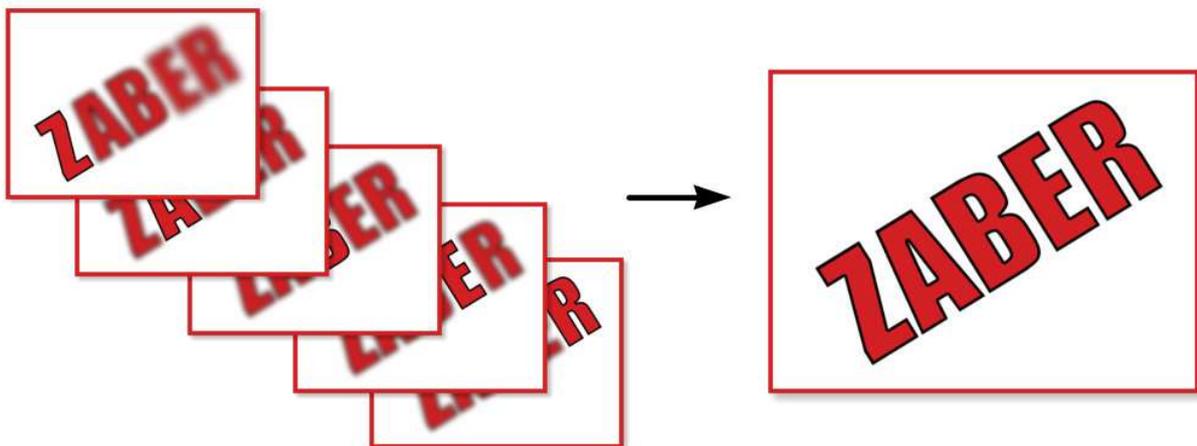


Z-Stacking With the MVR Microscope and μ Manager

By Mike Fussell

Z-stacking or focus-stacking is a useful multi-dimensional imaging technique for capturing sharp and detailed pictures of large samples which exceed the narrow depth of field of high magnification objectives.



This tutorial will cover how to quickly automate the capture of Z-stacked images with a Zaber MVR inverted microscope, μ Manager and ImageJ.

Using μ Manager and the MVR Microscope for Z-Stacking Z-stacking or focus-stacking is a useful multi-dimensional imaging technique for capturing sharp and detailed pictures of large samples which exceed the narrow depth of field of high magnification objectives.

Equipment

In this example, we will use the hardware and software listed below. Please contact Zaber if you are interested in configuring a system for your application.

Hardware

- [MVR100E025AC](#) - Automated inverted microscope.
- [MLR3B-T13A](#) - Microscope reflected illuminator (385, white, 625 nm).
- [X-LCA4](#) - 4-Channel LED Controller.
- [Teledyne FLIR - BFLY-U3-23S6M-C](#) USB3 Monochrome camera.

Software

Set-up

A detailed guide for how to set up MVR with μ Manager is available in the Micro-Manager Software Setup section of the MVR manual. Download and extract Fiji, then download the Extended Depth of Field plugin and copy Extended_Depth_Field.jar to your Fiji Plugins folder.

Use the live view function to position your sample (Fig. 1). This example uses a pre-prepared slide from AmScope of the tarsal claws of a honeybee foot.

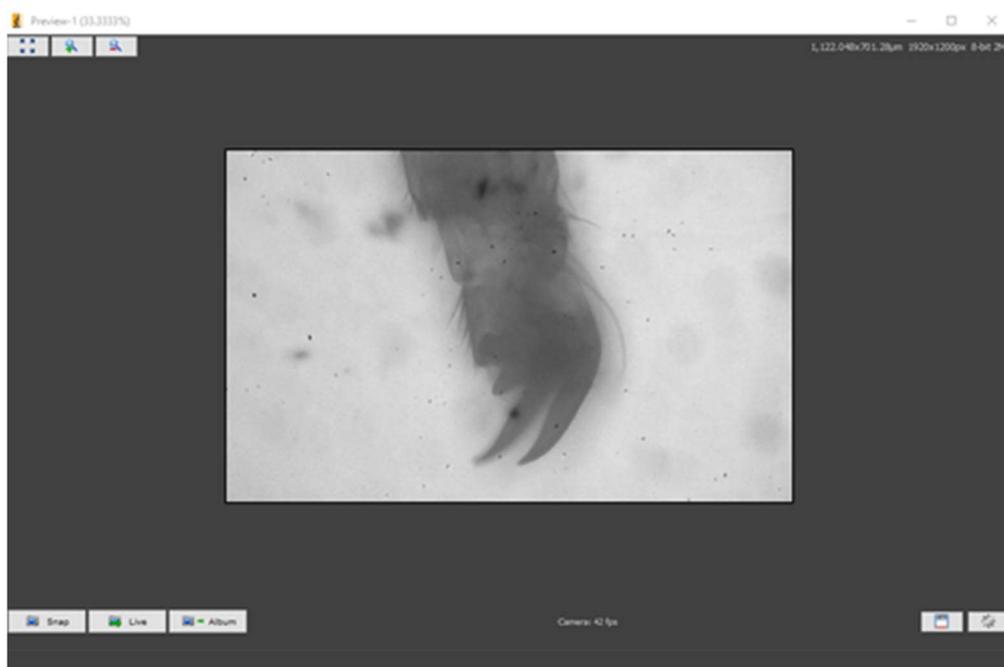


Figure 1. Honeybee foot at 10x magnification. One claw is in focus, while the other claw is not.

Capture an Image Stack

Use the multi-dimensional image acquisition tool to set the parameters of your Z-Stack (Fig. 2). Set the Start Z depth by using the focus control to focus accurately on the furthest point that should be in focus. Then, focus on the nearest point that should be in focus and set the End Z point. The optimum Step size will depend on your objective. A smaller step size will yield better results, but will also require more system resources and take longer to process. Select the directory you wish to save your images to and set Image Stack File as the output format. Click **Acquire!**.

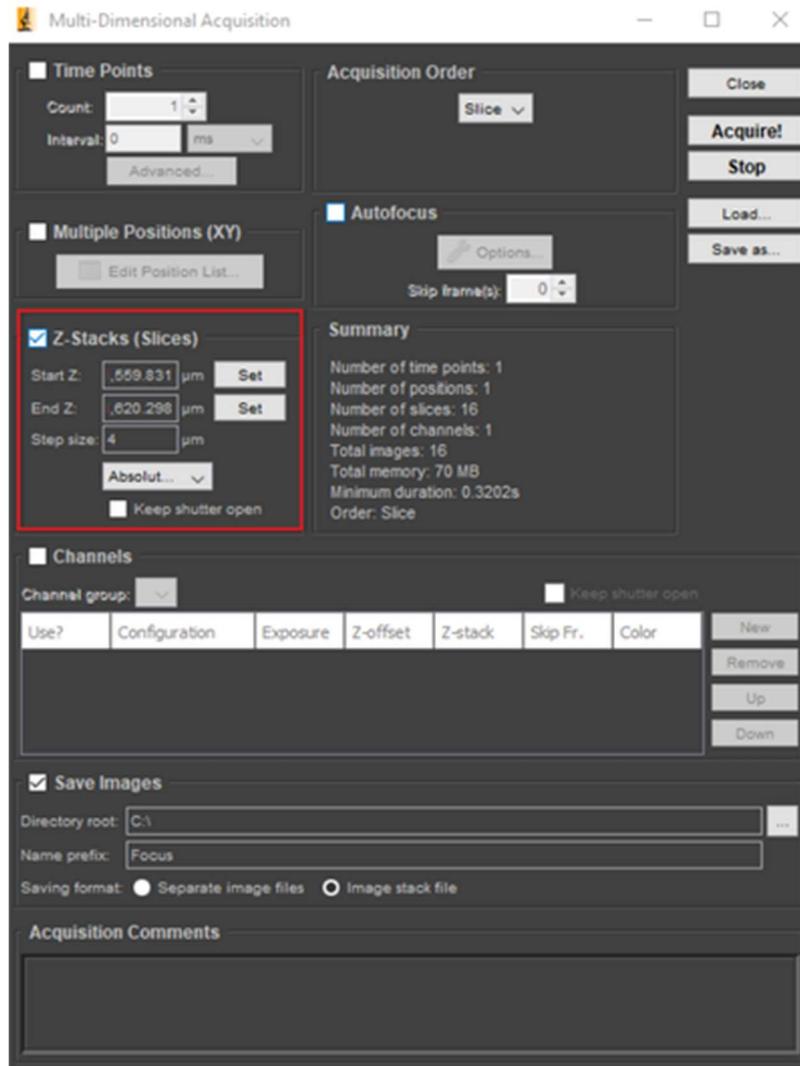


Figure 2. Defining endpoints and step size for the Z stack.

μ Manager will automatically capture the sequence of images defined by your Z-Stack settings and display the resulting image stack when it is complete (Fig. 3).



Figure. 3. Output Z-stack file from μ Manager.

Open the Fiji application. Select File, then Open... and select the image stack you created in the previous step. Once the stack is open, select **Plugins** then **Extended Depth of Field**, then **EDF Easy Mode**.

The Speed/Quality setting (Fig. 4) changes the balance between fast speed but lower quality, and higher quality at the expense of longer processing time. Click **Run**.

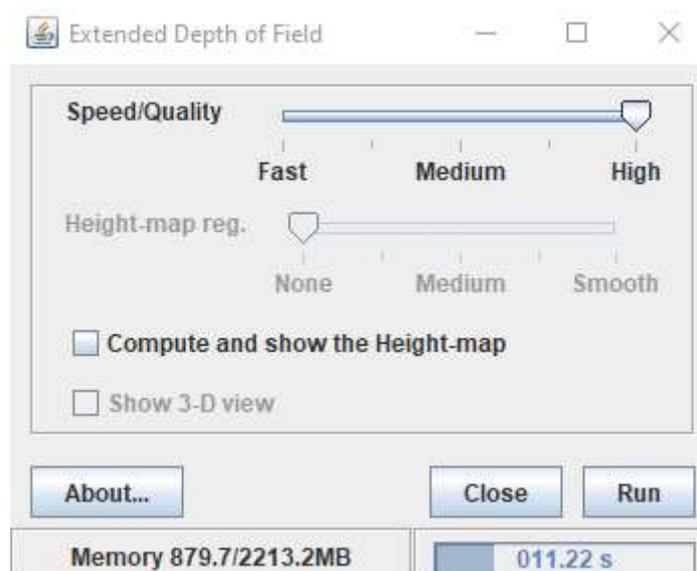


Figure 4. Settings for Extended Depth of Field processing

The Extended Depth of Field tool will take several seconds to several minutes depending on the resolution of your images, the number of images in the stack, the quality settings and how powerful your computer is. When processing is complete, the resulting image will be displayed (Fig. 5). Save the image using the File menu in Fiji.

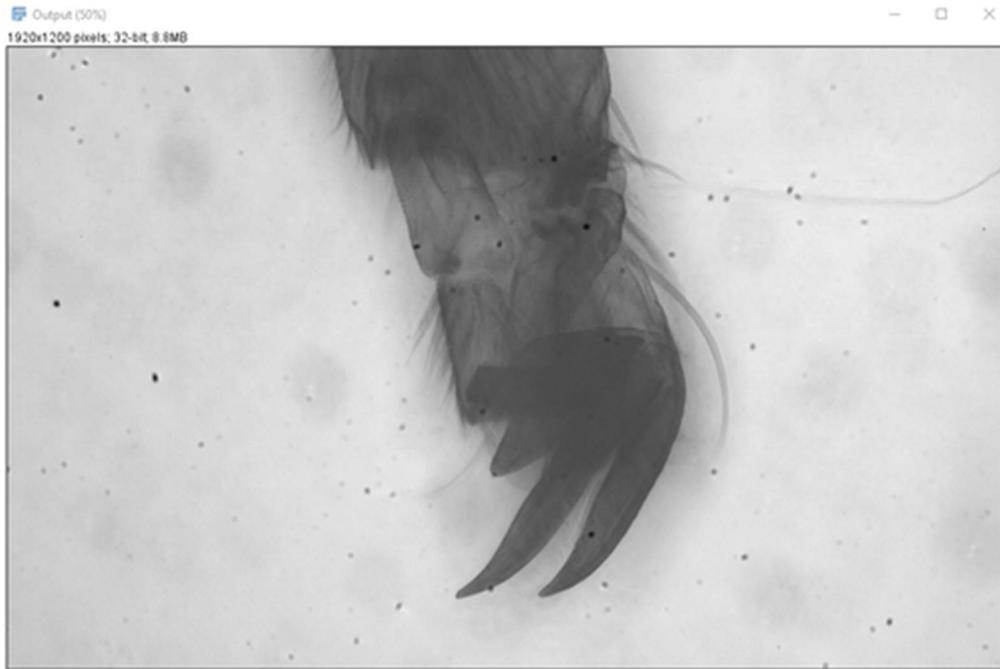


Figure 5. Honeybee foot with both claws in sharp focus.