

Comparison of z-stacked light micrograph (about 25 frames) and single capture scanning electron microscopy (SEM); image of a marine snail. Notice the greater detail of the SEM, while color can only be recorded by images recorded with light. Light micrograph on Zeiss SteREO Discovery.V12 with 0.63x plan apochromatic lens, Zeiss AxioCam HRc, processed with Helicon Focus.

Considerations and Limits of Z-Stacking In Macrophotography

Daniel L. Geiger

In a recent article in photo technique Magazine (Nov/Dec 2012), Dan Burkholder provided a helpful overview of focus or z-stacking. The technique is most frequently applied in macrophotography. In this article I share some special tips and hints that may not be apparent and may even appear counterintuitive to anyone interested in trying this technique.

Lighting should be consistent both in directionality as well as intensity in all frames of the stack. Accordingly, light sources should not be mounted on the camera or lens, which is moved in the process of z-stacking. The changed position of the lights can alter the shadows and highlights on the object.

I mount the flash heads of my Canon MT-24EX on Wimberley Plamps. I modified one of the links in the arms by attaching flash mount shoes with a screw and setting it in epoxy resin (superglue does not work).

To ensure consistent exposure of all frames, manual exposure is advised, both with ambient light as well as with flash. Highlights have a tendency to blow out in the z-stacking process, so the familiar “expose to the right” maxim of digital imaging should not be pushed.

Rather, expose so that the histogram ends about one f-stop to the left of the white point. When capturing RAW files, overexposed stacks can be batch-processed in Photoshop with an exposure adjustment action.

F-stop

The intention of z-stacking is to achieve extended focus. It may sound reasonable to use the greatest



The Cognysis StackShot set-up in the author's “studio,” aka the kitchen counter. The image capture is controlled from the laptop computer through Zerene Stacker with StackShot window.



Comparison of z-stacked light micrograph (19 frames) and single scanning electron microscopy image of an orchid flower. Notice the greater detail of the SEM, while color can only be recorded by images recorded with light. Light micrograph on Zeiss SteREO Discovery.V12 with 0.63x plan apochromatic lens, Zeiss Axiocam HRc, processed with Helicon Focus.

f-stop to generate the greatest depth of field for each of the frames. However, it is a better approach to use the lens at its sharpest and let the z-stacking program do the work of generating the depth. Most lenses are sharpest around 1 to 2 f-stops down from fully open, which is the ideal f-stop for z-stacking. Also remember, that diffraction introduces image blur, so maximum advantageous f-stop (f_{max}) is $32/M+1$. At 1:1 = $f/16$, at 5:1 = $f/5.3$. For work on stereomicroscopes it is best to leave the f-stop (if available) all open.

Focusing Rails

There are a number of rail systems on the market. For my work, I found the Velbon version lacking stability. Adorama's reasonably priced rail is by far superior. Really Right Stuff also makes exquisite dual axis platforms, but all of them rely upon manual advance of the stage. Optimizing the optics for sharpness with rather open f-stops causes the depth of field of the each frame to be rather shallow. At 5:1, which is easily achieved with the Canon MP-E 65mm lens, depth of field with $f/5.6$ is around $0.08 \text{ mm} = 80 \mu\text{m}$! Steps between frames should be approximately 70% of depth of field; ideal steps are $\sim 0.05 \text{ mm} = 50 \mu\text{m}$.

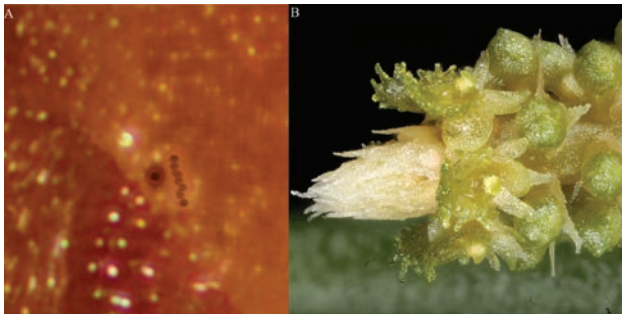
Cognysis' StackShot to the rescue! It is a motorized and computer controlled single axis focusing rail, which can either be run with its own controller box, or through the computer interface of Helicon Focus or Zerene Stacker. After setting start point, end point, and step size, a single push of a button takes the entire stack. Besides affording extremely precise stepping for z-stacking, it can also be used for time-lapse photography. To facilitate composition, I use a Really Right Stuff LMT Lens Mount on the Arca quick release. It permits side-by-side sliding of the camera, though not with a rack and pinion system. A little known fact is, that the MP-E 65mm can be used with tele extenders to increase magnifications to 7:1 with 1.4x ($f_{max} = f/4$) and 10:1 ($f_{max} = f/2.9$) with a 2x converter. The usual caveats regarding tele-extendors still apply: loss of 1–2 f-stops, some loss in resolution, additional play and vibration in set-up.

Stereomicroscopes

Z-stacking can be applied to microscopy for images at magnifications not easily obtained with photographic equipment. One problem on stereomicroscopes is the 7.5° stereoangle of the optics. To maintain the optical



Stereomicroscope Zeiss Discovery.V20 with blue Carl Zeiss AxioCam HRc microscope camera (peltier cooled CCD). Notice that the lens is off-center to ensure that the optical axis is parallel with the z-axis.



Dirt on sensor or optics leaves traces on the final z-stacked image. Linear tracks are produced by rack-and-pinion focusing systems, as seen in partial blow-up of a 15 image stack of a flower. Spiral structures result with the StackShot motorized focusing rail using a helical drive. Canon EOS 5D MKII with MP-E 65mm at 4:1; 74 frames at 0.75mm = 75µm step size, processed in ereneStacker using Pmax algorithm.

axis parallel to the z-axis, special attachments such as the objective slider for the Zeiss Discovery series microscopes can be obtained. Those options are only available for high-end instruments.

Dirt on the optics or the sensor poses problems, because it is always in focus. Due to the alignment procedure, dirt particles will change position on the image. In the final image, they typically appear as a linear series of dots on rack and pinion focusing rails, or as spirals on the Cognysis StackShot with its helical drive. The dust-delete function available with some cameras can help to reduce the necessary post-processing either in the stacking program or in Photoshop.

Z-stacking can extend focus and generate images of unparalleled appeal. However, z-stacking cannot in-

crease resolution. On the stereomicroscope the low numerical aperture (NA), typically <0.1 , means that the resolution is limited to around $6\ \mu\text{m}$ ($1.22\lambda/\text{NA}$). For a field of view of 1 mm (36x in photographic terms), only 167 line pairs can be resolved! Large sensors will not be able to capture more details, they only image detailed blur circles. For more highly resolved images other imaging techniques such as deconvolution or scanning electron microscopy have to be added.



Dr. Daniel L. Geiger is Curator of Malacology at the Santa Barbara Museum of Natural History, where he also oversees the electron microscopy facility. He uses a range of imaging techniques from 4x5" to photomicrography professionally as well as for his personal work. He has published z-stacked images in his monographs on abalone and the microscopic scissurellids, both published in 2012. See more of his work at vetigastropoda.com

Resources

Canon EOS 5D Mark II with battery grip, Canon M-PE 65mm, Canon extension rings, Canon EF 1.4x II tele extender, Canon MT 24-EX, Canon Speedlite 580EX II-usa.canon.com; Cognysis Stackshot with Arca QR and RRS LMT-cognisys-inc.com; Cressington 108auto with rota-cota stage sputter coater-cressington.com; FlashZebra TTL flash cables-flashzebra.com; Gitzo CF tripod-gitzo.us; Linhof Profi II ball head-linhof.com; Haoda matte focusing screen-ebay.com; Helicon Focus-heliconsoft.com; Really Right Stuff-reallyrightstuff.com; Tousimis Autosamdri®-815 critical point dryer-tousimis.com; Wimberley Plamps-tripodhead.com; Zeiss 100mm f/2.0 Makro-Planar T ZE lens, Zeiss SteREO Discovery.V12 stereomicroscope, trinocular ergohead with SLR tube or 0.63x TV coupler for Zeiss AxioCam HRc, objective slider, 0.63x and 1.5x plan apochromatic lenses; Zeiss EVO® 40 XVP scanning electron microscope with SE, VPSE and QBSD detectors-zeiss.com