

A Focus Stacking Setup for Shell Photography by Edward J. Johannes

During the 2014 September shell club meeting Linda Schroeder asked if I could photograph two very little shells using my focus stacking setup with my microscope. She was unable to get decent photos of them; they were pyramidellid snails collected during the shell club's June field trip to Little Beach, Ucluelet, Vancouver Island, BC. She sent the photos of these shells to Patrick LaFollette at the Natural History Museum of Los Angeles County, who tentatively identified them as *Boonea cf. cincta* (Carpenter, 1864) (see David McKay's article this issue for photographs and additional background). It occurred to me that the club members who photographed their specimens might be interested in the possibilities that focus stacking brings and in a focus stacking setup I built to allow me to use this technique.

One constant problem of close-up photography is the limitation of depth of field (DoF); a single plane where a photograph is sharply in focus, varying in degrees of thickness depending on several factors (f-stop, focal length [magnification], and objects distance to the lens). Obtaining a sufficient DoF to photograph an entire specimen in focus can sometimes be difficult in macrophotography but especially with higher magnification necessary in microphotography.

Before the digital revolution, one solution was stopping down the aperture in a camera (using a larger f-stop number) to increase the DoF, but beyond a certain point, this causes blurring due to diffraction, which counteracts the benefit of being in focus. In addition stopping down the aperture also decreases the light reaching the camera forcing an increase in exposure time. This could be especially problematic when photographing through a microscope.

Another solution in microphotography was the use of the scanning electron microscope (SEM) that mostly eliminated the DoF problem. However, it is unlikely, due to the expense, one would own one or even have access to a SEM. In addition, it is doubtful a shell collector would want their favorite micro shell to be permanently very thinly coated by a layer of precious metal, a necessary step to make it possible the specimen can be properly scanned. In addition color photography is not possible with SEMs.

Focus stacking (also known as focus blending, focal plane merging or z-stacking) is a digital image processing technique, which combines multiple images taken at sequentially different focus distances by eliminating the out of focus

areas to give a resulting image with a greater DoF than any of the individual source images. There are two possible ways images for photo stacking can be obtained, either by changing the focus of a camera or microscope or by moving by some mechanical means the object to be photographed (not changing the focus of a camera or microscope) at precise increments between each photographic shot. Several software options are available for processing focus-stacked images like Combine ZP (free), Photoshop, Helicon Focus and Zerene Stacker (only the last three have versions for Macintosh OS X). I use Helicon Focus because it is the only one with the ability to eliminate resulting dust streaks using a dust map program.

This is especially useful when photographing through a microscope where dust on lenses is a constant problem. It also can be used to stitch together panoramic shots.



To photograph micro shells I attached a Canon EOS 20D (with no lens) to a phototube mounted on a Leica Wild M3Z microscope with a X1.0 objective lens. With macro shells I use the camera alone attached to a special mount that allows its lens to be pointed downward. Initially when photographing shells I tried manually changing the focus of the camera or microscope to focus stack finding it produced an unsatisfactory result when I processed the photos. Realizing I needed to move specimens in small accurate increments to obtain better results I searched for a solution but found what was available commercially was expensive and did not fit what I needed. Searching the web I found that the Proxxon Micro Compound Table KT 70 (a micro milling table) was being used for photo stacking involving both macro and microphotography. This table has two adjustment dials graduated in 0.05 mm increments, with 1 revolution of the dial equal to 1.0 mm of movement. I mounted the Proxxon Micro Compound Table on a wood block 90° from the orientation that it's typically used. The wood block is clamped to a table. I built a focus stacking stage that is attached to the Micro Compound Table (see photograph). The specimen sits on this stage and can be moved in an X, Y, and Z-axis. The Z-axis is used for changing the focus (allowing for focus stacking) and the X and Y-axis for properly framing the shell inside the area of the photo. Accurate incremental movement in the Y and Z-axis as small as 0.025 mm is possible with the Proxxon Micro Compound Table KT 70 and coarser movements in the X-axis are obtained using the focus stacking stage by turning a gear meshed with a threaded rod. The stage slides on two smooth aluminum tubes.

Though halogen illuminators are shown in the photograph of the focus stacking stage, I no longer use them for this purpose due to vibration from the fans. I now use LED lamps with clamps. I have found that the color of the shells photographed under the LEDs is more true to life than I could obtain with the halogen bulbs.

The two *Boonea cf. cincta* snails figured in McKay's article were photographed through a microscope at 0.05 mm increments resulting in about 30 photographs for each snail that were separately processed in less than a minute using Helicon Focus software.