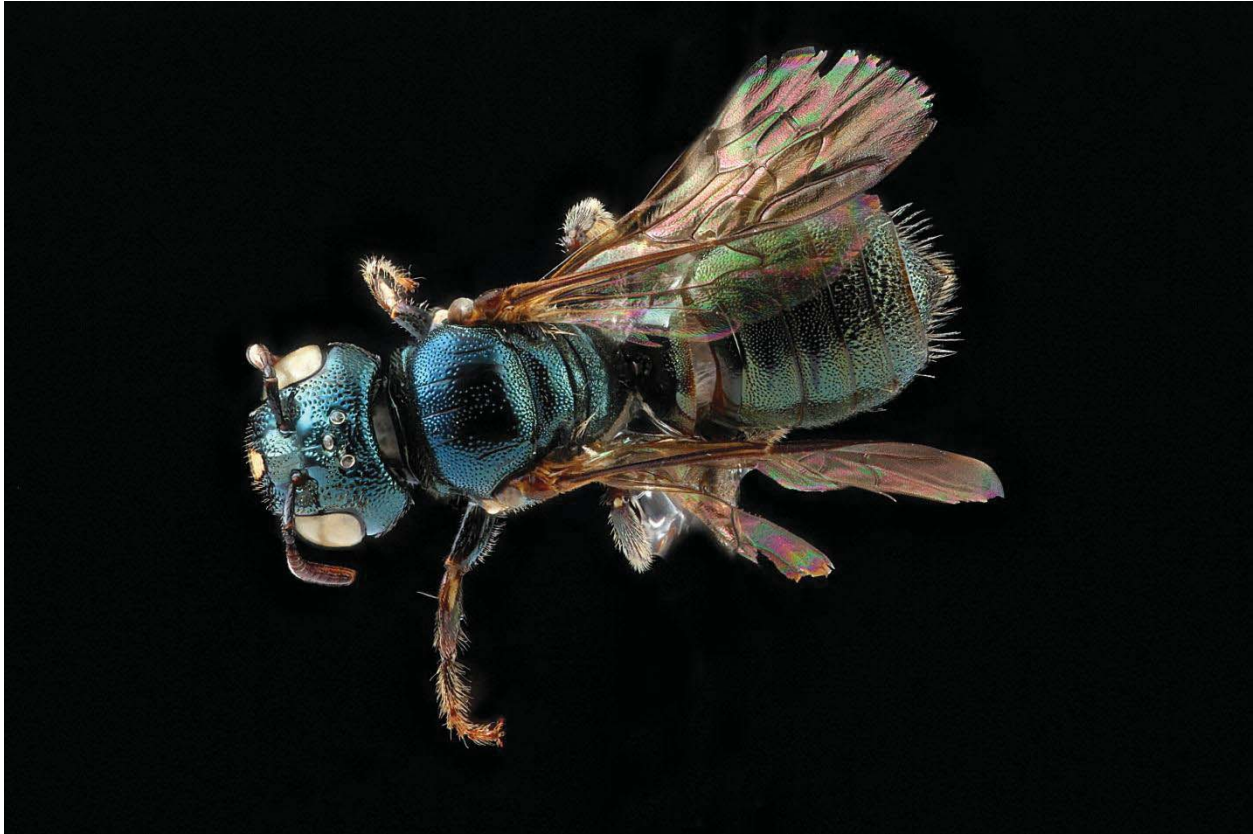


## Detailed Macrophotography of Insect Specimens: A Laboratory Setup



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This is a short introduction to how the USGS Bee Inventory and Monitoring Laboratory (BIML) takes photographs of insect specimens in the lab. These techniques are based on those first developed by Dr. Anthony G. Gutierrez ([Tony.Gutierrez@us.army.mil](mailto:Tony.Gutierrez@us.army.mil)) and Graham Snodgrass with the U S Army Institute of Public Health.

The working idea behind this systems is that specimens are photographed using a camera with a very large sensor area and a high quality macro lens that magnifies the image of the specimens so that it fills the sensor area of the camera. This combination permits the creation of images with extremely high levels of detail. Due to the narrow depth of field created by the camera lens and high degree of magnification, multiple photographs need to be taken and then combined to create a picture of a specimen completely in focus. The Cognisys StackShot Rail is used to move the camera (or, alternatively, the specimen) in a user chosen set of increments (from 1um to 999mm). The controller that comes with the StackShot Rail also fires the camera/flash unit. Specimens are taken against a black background and a flash is used for illumination by bouncing the light off of white Styrofoam, in our case an old cooler.

### Laboratory Setup at BIML

Equipment

- Camera: Canon EOS 5D MarkII
- Macro Lens: Canon Manual 60 mm 1-5X (MP-E65/2.8)
- Macro Flash: Canon Twin Lite MT24EX
- StackShot Rail: Revision 1.4 by Cognisys, Inc ([www.cognisys-inc.com](http://www.cognisys-inc.com))
- Zerene Stacker Version 1.04 Build T201110101145 ([www.zereneystems.com](http://www.zereneystems.com))
- Canon Battery Charger LC-E6, and Battery Pack LP-E6
- Canon ACK-E6 AC Adapter Kit
- Styrofoam Cooler (ours is 15"W x 16"D x 13"H, but other sizes will also likely work)
- Black Felt
- Wood base to hold the StackShot Rail

### Camera and Flash Settings

Note that the settings that follow will likely need to be altered to fit your particular set-up and needs.

#### Camera:

- Aperture: Primarily f/5.6 but at times f/6.3 and f/7.1
- ISO: Values commonly used are 125, 160, 200, 250
- Exposure time: Values commonly used are 1/80, 1/100, 1/125
- Flash is set to Manual
- Flash Time is either 1/8 or 1/16 sec
- For 5X magnification, we occasionally set the time to 1/4sec

### StackShot Rail Settings

The depth of field for any particular picture will vary with the magnification of the lens, the f-stop of the lens, and the type of lens used in taking the shot. To create a usable picture you will want to make sure that you have a sequence of pictures whose depths of fields overlap one another so that the final picture is totally in focus once the individual pictures are processed.

Below is a little table that Alexander developed that will be a good starting place for your efforts. It is based on the depth of field for the lens mentioned above at f/5.6 given in the Canon Instruction Manual. Of the 3 F-stops that we use, f/5.6 has the shallowest depth of field; so the slice sizes for f/5.6 will work for f/6.3 and f/7.1 as well. Higher F-stops are possible, but lead to deterioration of the quality of the images and we suggest you not use them.

<b>Magnification</b>	<b>Distance Camera or Specimen is Moved (microns)</b>
1X	650
1.5X	350
2X	250
2.5X	180
3X	150
3.5X	125
4X	100
4.5X	85
5X	75

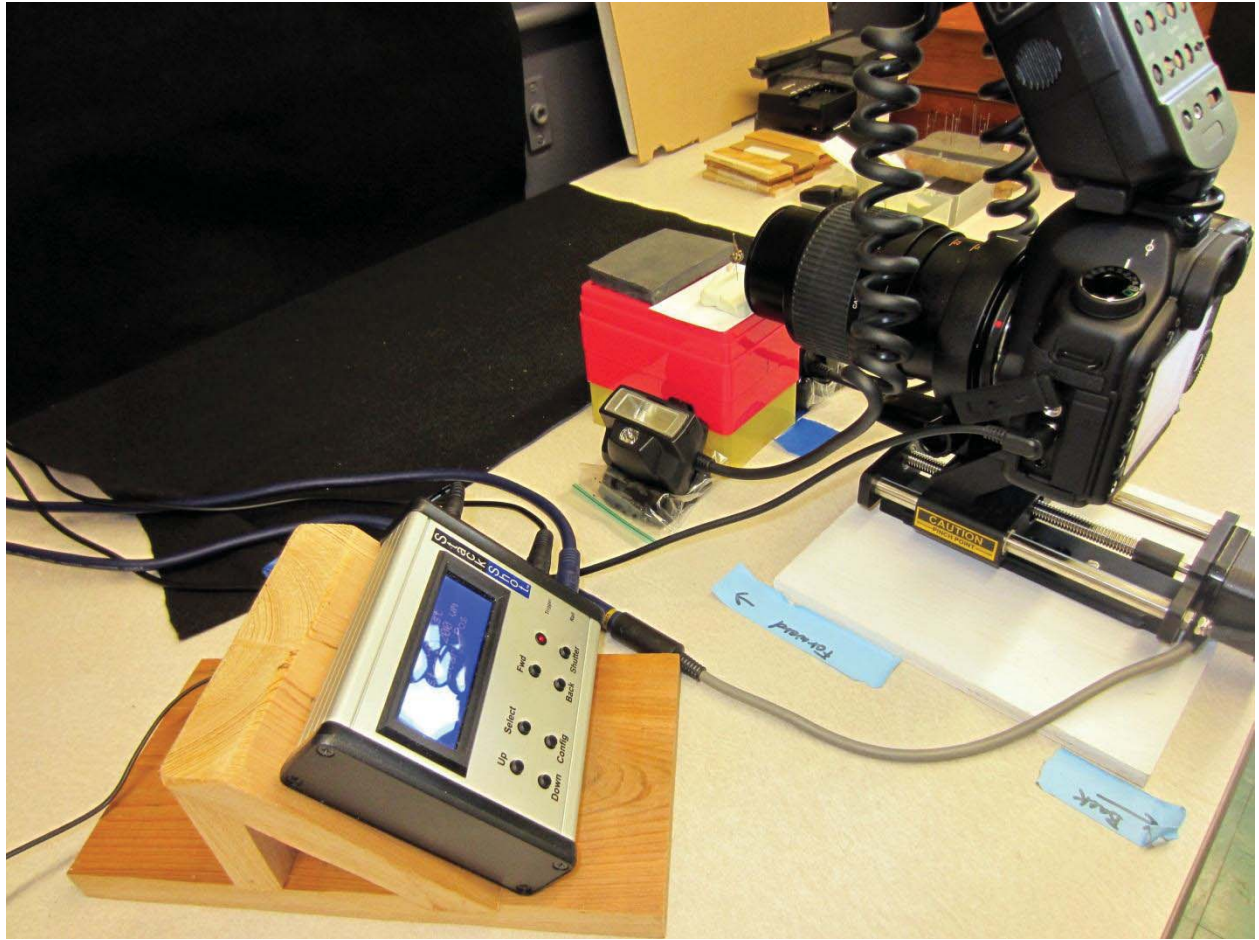
### Physical Set up

The table or desk that is used to take pictures needs to be heavy and stable so that vibrations you make touching the table or the movements of the StackShot Rail do not shift the specimen. The StackShot Rail does not come with a platform but we suggest you attach one to stabilize it, in particular if the camera is riding on the StackShot Rail rather than the specimen, to prevent it from accidentally tipping over.

The controller for the StackShot Rail was placed on a small wooden stand to keep it from sliding around and for better visibility by the operator. The heads of the macroflash are NOT kept on the camera lens, but placed to either side of the specimen platform resting on lumps of Plasticene modeling clay. The specimen platform can be made of anything, but needs to elevate the specimen to the level of the camera lens. Modeling clay (on top of the specimen platform) is used to create a base for holding the pin of the specimen. Clay is preferred as the pin and specimen are easily shifted to new positions when setting up the shot. A small square of absolutely black cardboard is used to form a background for the specimen, if the clay will possibly be in the shot. This cardboard forms a black background better than the clay...which tends to look gray and detracts from the final picture.

The Styrofoam box is inverted over the camera lens and specimen. The back of the box is cut out and a piece of black felt is placed a short distance away on a nearby wall. If the felt is too close, then its surface appears in the picture rather than creating a uniform backdrop of deep black. The front of the box is partially cut away to allow the lens to be INSIDE the box, with the camera body remaining OUTSIDE the box. Pieces of cut white paper are used to patch up any hole where the lens enters the box. It is important to have as much white present on the floor of the setup as well as the front of the box so that sufficient light is reflected to the bottom and the front of the specimen. White paper is also used underneath the specimen to reflect light to the specimen bottom. Experiment.

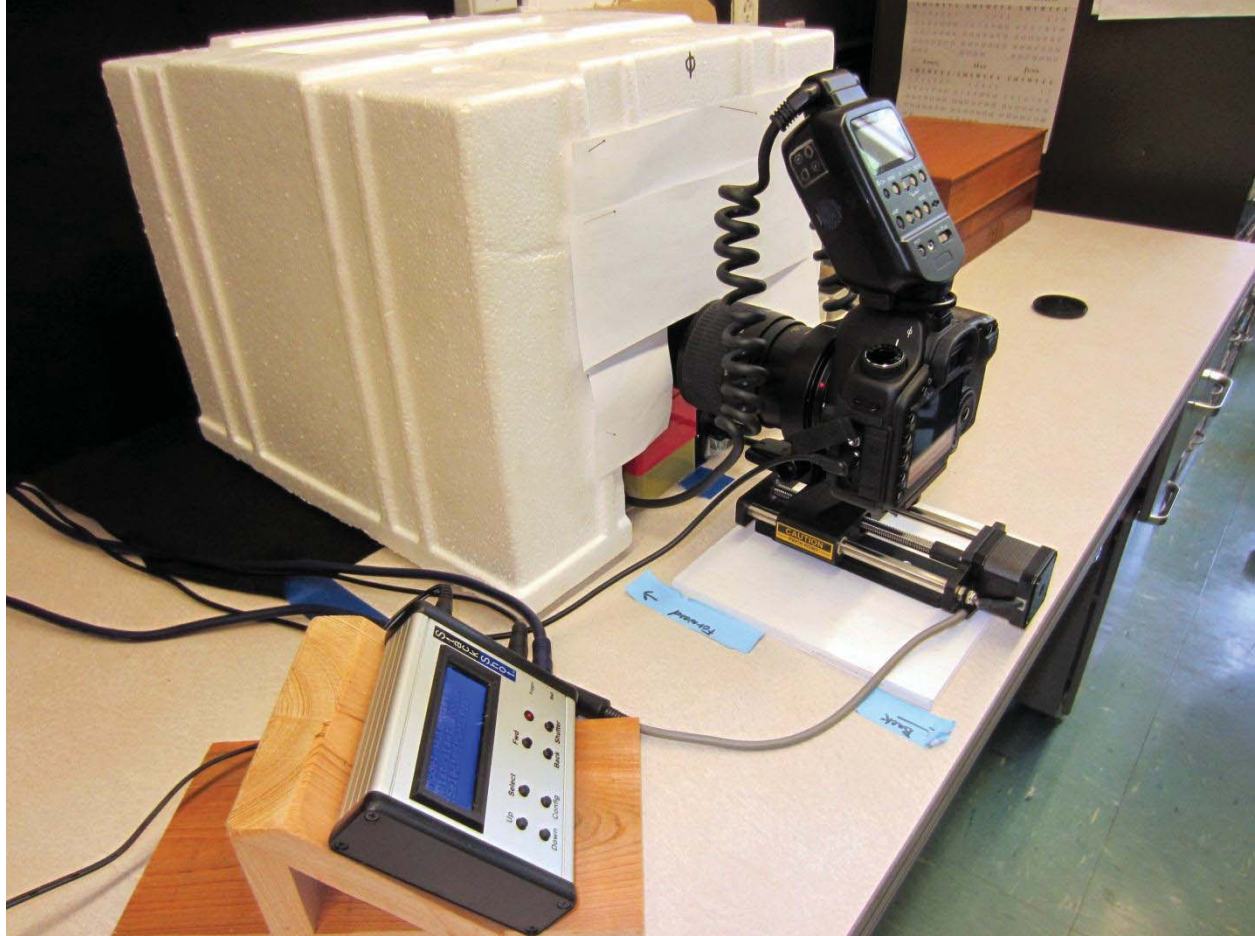
Below is a set of shots of our set up with the box off and a specimen in place and the box ready for photographing.



Control unit with a screen and buttons. The screen displays a blue image. The buttons are labeled: Up, Select, Down, Control, Pref, Black, Shutter.

← Forward

← Back



## Specimen Preparation

Because the pictures are very detailed any form of fiber, dust or scales will be obvious and distracting. Thus you will want as clean a specimen as possible. If the specimen is dirty, then a good way to remove these fibers is to take a very small piece of tape and attach it to a pin or probe. The tape should be stuck repeatedly to your clothes to decrease its stickiness but still allow dust to be removed.

Specimens can be positioned however you like. With practice you will find the best angles and shots for your particular taxonomic group. It is helpful if the pinned specimens have black pins as that makes removing them in PhotoShop easier. If the specimen is unattached to a pin then the specimen can be attached with Superglue to pins.

## Picture Taking Steps

We start with the aperture at  $f/5.6$ . We zoom in and fill the frame with the center of the bee and take a test picture. By looking at the test picture and the resulting histogram we adjust the aperture, ISO, and flash setting until we like the results. We then choose a magnification that almost fills the field of view of the camera. Anything on the bee or insect you want ultimately in focus in the final picture needs to be in this first photo now and in each subsequent photo. Another test shot is then taken to see if everything looks good prior to taking the full run of pictures. Over time you can pretty much predict what settings were going to work best.

Using the controller for the StackShot Rail set the end points for the start and the finish of the picture. It is best if you move the start and end points slightly BEYOND where the most projecting part of the specimen appears to be in focus so that you are guaranteed a final picture completely in focus. Set the increment distance for each individual shot using the table above.

If you have questions about whether the increments you have chosen for the StackShot Rail is appropriate then you can use the live view screen of the camera with the Depth of Field Preview set to on.

### Stacking the Pictures into A Final Shot

While the instructions below are for the Zerene Software we use, there are a number of software packages that will do the same thing. The US Army Institute of Public Health has found that the PMAX setting gives the best photographic rendering of specimens in Zerene and we also agree. Note that in some areas of a photograph the stacking process can be fooled where legs or antennae cross, creating a blurred area. This can be fixed in Zerene by setting areas of your choice of photo slices as the Master for the final stacked picture. Photos should be saved in .TIFF format for the best results.

### Retouching in PhotoShop

The final .TIFF picture produced in Zerene can be transferred to Photoshop or any similar software package to tweak the color, sharpness, and clean up small issues of dirt and remove the presence of any pin, if so desired. We have found the following processes in Photoshop to be useful:

- In the Curves of Levels setting use the Auto function to create an initial color adjustment and then bend the curve to more finely adjust the color or move the sliders in the histogram.
- We usually deepen the Vibrance and Saturation settings a little bit.
- We use the Unsharp filter to sharpen the photograph by playing with the first 2 levels but not the Threshold setting.
- We use the Rubberstamp tool to eliminate the pin from the picture as well as move some of the hairs around to cover up where the pin was inserted (if necessary).
- We spend a lot of time at a very fine level to rubberstamp or healing brush out the dust and fibers that magically have appeared on the specimen, concentrating, in particular, on the eyes.
- We save the final picture as a .JPEG at the 50-100% setting. Note that there appears to be relatively little difference between the final JPEG and the TIFF formats.

### **The U S Army Institute of Public Health's Field Set up**

This set up is designed for rugged, in the field use for relatively untrained US Army personnel in remote bases, as such, it represents a well-tested field rig that you may want to emulate. Further details about where to get the modified tripod are available from Dr. Anthony G. Gutierrez, Laboratory Sciences, U S Army Institute of Public health, Aberdeen Proving Ground South Maryland 21010 (tonygt19@gmail.com).

