Designing a Macro Imaging Solution

Photographing Butterflies

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1. Introduction

Scientific photographs can be visually stunning, intriguing, and informative. To produce these photographs consistently it is important that the photographer forms a reproducible lighting setup and imaging workflow.

Three butterfly species were photographed on their dorsal and ventral sides to compare their morphological differences. The structure of this article will follow the imaging process from start to finish. Section 2 covers the species and preparation, Section 3 details the lighting setup, Section 4 discusses imaging procedures, Section 5 reveals the processing methods, and finally the image gallery is in Section 6.

2. About the Specimens

It is important to acknowledge that access to these specimens were generously provided by the entomology department of the Rochester Museum & Science Center.

Kingdom: Animalia
Phylum: Athropoda
Class: Insecta
Order: Lepidoptera

Anteos *clorinde* - White Angled sulphur

A pale green colored butterfly of the Pieridae family and Colladinae sub family. A yellow bar on each of its forewings aid in proper identification as well as the apex of their forewing being hooked. As the subject ages the green wings begin to loose color. Their distribution ranges from Argentina to Mexico commonly, though in southern areas of North America, such as Texas, they have been widely reported. They thrive year round in tropical areas though in cooler climates are found generally between August to December. Wings are between 70 to 90mm in width.

Phoebis *philea* - Orange-barred Sulphur

Phoebis philae's habitat ranges from Brazil and north to the southern ranges of the state of Florida in the United States. Rare sightings of the butterfly have placed the outer reaches of the habitat as far as Colorado and Texas. In Florida generally two to three flights are seen per season, though in the more northerly extent of their range only one flight is observed. Wingspans are generally between 68 to 80 mm.

Graphium *antheus* - Larger Striped Swordtail

A native to tropical Africa, the Striped Swordtail displays brilliant blues contrasting with blacks on its dorsal side. This specimen differs from the other two as it is a member of the Papiliondiae family as opposed to the Pieridae family. Populations peak from November to December. Wingspans range from 65 to 75 mm

Preparation of Specimens

After an insect dries it can often be difficult to form the insect back into its natural opened state. Through a technique referred to as relaxing and some careful handling, all three butterflies were opened from their closed state. It is important to note that the following method was performed due to the lack of proper relaxing supplies, for important samples it is recommended to seek professional entomology advice.

Gather a small hot plate, ~200 ml glass beaker, some gauze as would be found in a medical kit, toothpicks, and a plastic set of tweezers. Fill the glass beaker about halfway with water. Tape a doubled up strip of gauze over the beaker so as to make a stage for the specimen to lay on. Place the beaker on the hot plate after pre-warming. Now gather the specimen and carefully place it on the gauze stage. The steam coming off of the water will allow the subject to be opened incrementally. Patience and a steady hand cannot be stressed enough during this process. By taking the plastic tweezers and gently opening them between the wings of the specimen over the steam the subject will eventually open. Nonetheless, without the pressure of the tweezers the wings will quickly close again.

An extra set of hands is useful in this next step though not required. Take another strip of the gauze and lay it over the opened subject while simultaneously holding it open. Figure 2.1 displays the hot plate setup before gauze has been placed over the specimen. Tack the base and top layers of the gauze together by sticking toothpicks on four edges around the beaker. This will apply even pressure as well as create a steam chamber of sorts to further relax the specimen. Once the subject is safely tacked down turn the hot plate completely off unless another specimen needs to be prepared. Figure 2.2 displays the hot plate setup after the subject has been tacked.

Fig 2.1: A White Angled Sulphur during relaxation through the use of steam, a hot plate and gauze. Do not leave the subject above the steam for too long as this will cause damage to the specimen.

Fig 2.2: Toothpicks have been tacked between the upper gauze and the base gauze in this image to apply even and gently pressure to the subject. Once the subject cools it will remain in this position.

3. Lighting Setup

The first step in creating a scientific photograph in a laboratory or studio environment is the creation of a lighting plan. A good design will allow flexibility while also offering a repeatable setup. Recording these steps will also help the photographer learn what worked, didn't work, and most importantly *why.* For the three butterflies that were photographed for this paper the lighting setup remained stationary with the exception of a white or black background and the subjects.

While there are many ways to light an object it is also important to keep in sight the fact that for scientific imagery the lighting method aims to reveal and portray the subject as accurately as possible. The lighting modality therefore must be catered to the subject with this goal in mind. Butterflies are highly textured subjects with bristles, tarsals, and hairs covering the majority of the subject. Therefore highly diffuse light is a must to completely illuminate the subject. Diffused light has the dual benefit of being one of the aspects of what focus stacking software needs to perform an accurate stack. For those unfamiliar with the process, which may be referred to as 'z-stacking' or 'extended focus', images are captured at varying focal points along the subject. If the process is controlled well with out camera shake, proper slice overlapping, and consistent lighting, specialized computer software is able to combine only the areas in focus. Algorithms within the program look for areas of high frequency and by comparing contrast between adjacent areas. The majority of images in this publication are focus stacked.

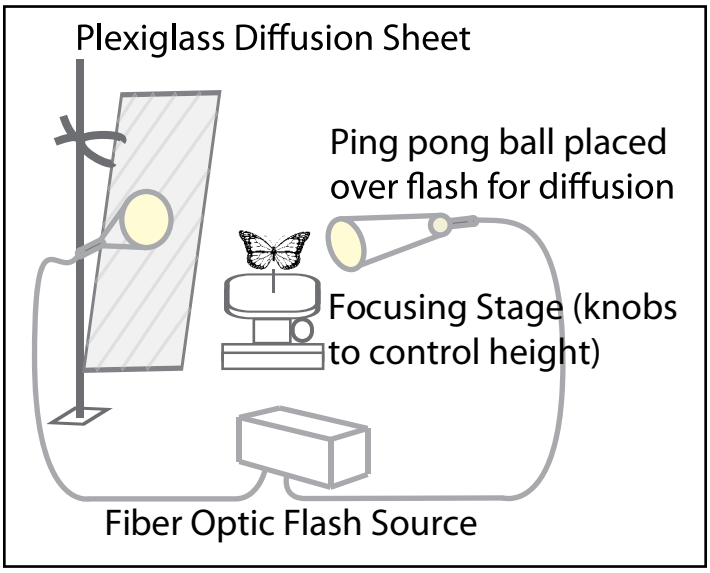

Fig 3.1: A lighting diagram of the setup used to photograph the butterflies. Producing images that can be processed easily by focus stacking programs requires highly diffused light.

As displayed in Fig 3.1 the use of a flash was necessary to produce adequate light output and to eliminate camera shake during exposures. One flash is firing through a sheet of frosted Plexiglas while the other has a ping pong ball placed over the flash. The diagram does not display that whereever possible white sheets of mat board were positioned towards the subject so as to form a white room of sorts surrounding the whole setup. Any stray light is subsequently diffused back towards the subject which creates a pleasing, well diffused, exposure.

4. Imaging Procedures

Now that the subjects have been relaxed and positioned to their open state and a controlled lighting setup has been created, imaging of the subject can begin. Choosing the 'correct' imaging setup can be a subjective topic. The images created for this paper are likely going to be printed larger and therefore need adequate resolution. A Canon 5d Mark II was attached to a copy stand above the subject and the lights. A sync cord between the camera and the flash pack triggered the powerful flashes during each exposure. The camera was tethered to a laptop running Adobe Lightroom 3.2 to allow immediate viewing of the files after each exposure.

For the whole body images of the subjects a 50mm macro lens was attached to the camera. Leaving the lens set at a 1:2 life size ratio and then focusing through the conjunction of the copy stand moving up and down as well as the focusing stage ensured that touching the camera directly wasn't a necessity. For the higher magnification images which were shot at 3:1 life size and 5:1, a 65mm 1 to 5x macro lens was used.

The most useful element of the imaging system used for these images has to be the ability

to see a live view of what the sensor is seeing before committing to taking an image. By using live view to see how changing the focusing stage in increments affects the focal point, one can begin to plan out how many images will be needed to create a fully stacked image of a subject. In essence we are planning how many very thin slices of focus will amount to the whole. For subjects of these size and magnification ratio between 20 to 100 slices were sufficient. It is important to remember that as magnification of the image increases, the depth of field decreases. This explains why focus stacking is mainly a method used in scientific photography as opposed to normal non-magnified imaging.

5. Processing

After capturing the images and loading them directly to Lightroom 3.2 on an adjacent laptop the images were corrected for minor exposures issues, gently sharpened, and run through a noise reduction filter that focused on luminance noise. Groups of images were exported as 8 bit uncompressed TIFFs originally. As the capture process went on, LZW compression proved to be just as effective while producing smaller file sizes. This subsequently sped up the whole processing portion due to dealing with approximately 20 mb files as opposed to 60 mb files.

Zerene Stacker was the program of choice for the butterflies. Based on personal experience, this program's ability to handle hairy and textured structures surpasses Helicon Focus, another popular focus stacking program. Either program has its advantages and disadvantages which are drastically affected by the subject characteristics. Images were aligned against each other prior to stacking both pyramid type stacking and depth type stacking. After about half an hour the average stack would be completed. Retouching inside the program allows the user to correct for program side stacking errors by manually painting in selected photos that are at the desired focus level

The program based retouching is the most time consuming part of the whole project. Once this is completed the final image was exported as an 8 bit TIFF without compression. The resulting image was the equivalent of one TIFF, 60mb in size, but had the focus level of one hundred photos. While the stacking is complete, processing is not complete. Images were brought into Photoshop CS5 to perform final tuning to the file. High pass type sharpening, a common choice of scientific photographers, was applied to sharpen textured areas. Fortunately, as the image was captured at ISO 100 and processed for noise in Lightroom, the image had little to no noise even after sharpening. As a final step in the process the images had Curves adjustment layers applied to set a white and black point if needed.

Fig 5.1: Three image slices representing the various levels of focus required to make the whole subject in focus. Eighty two images were required for this stack. Reference Fig. 6.4 to see the final image.

Fig 5.2: A zoomed in section from one of the focus stacks. Note the level of detail and artificial levels of sharpness caused by stacking artifacts. The full image is seen in Fig. 6.2.

6. Image Gallery

Fig 6.1: Dorsal (top) and Ventral (bottom) images of Phoebis *philae.* The subject was missing an antennae before being photographed.

Fig 6.2: Dorsal (top) and Ventral (bottom) images of Anteos *clorinde.*

Fig 6.3: Dorsal (top) and Ventral (bottom) images of Graphium *antheus.* The subject was missing an antennae before being photographed.

Fig 6.4: A stacked image of Graphium *antheus* at 3x magnification at capture. Most of the tarsal hooks are missing in the image with the exception of the hook in the lower third of the image.

Fig 6.5: A stacked image of Graphium *antheus* at 5x magnification at capture. Stacking software has a great deal of difficulty with stacking areas of hair as you may recognize in this image. The compound eye of G. *antheus* is of particular interest here.

Sources

Montana State University. “Butterflies and Moths of North America.” Butterflies and Moths. Montana State University, 2011. Web. 12 Nov. 2011. <http://www.butterfliesandmoths.org/>.

Acknowledgments

Liz Marchiando
Ted Kinsman
Gillian Gail
Teresa Deforge
Taylor Pannell
Daniel Jones
Rochester Museum and Science Center

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Tim Tiebout is a 3rd year Biomedical Photographic Communications Major with a concentration in High Magnification Photography. He is minoring in Applied Imaging Systems, History, and Philosophy. Tim is a PADI Open Water certified underwater photographer. This past summer Tim interned with the Research and Development Department at Canfield Scientific Inc. Besides enjoying late nights photographing specimens in the Biomedical Photography lab, Tim enjoys hiking, soccer, and biking.