Alcian Blue, Alizarin Red Chameleon Embryo

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Why Stain?

In the field of embryonic research, scientists need to visualize the development of skeletal structures. How is it possible to see the development of bones or the formation of cartilage without the use of X-Rays? How are scientists able to track the recession or growth of calcium and cartilage deposits? Is it possible to see into an organism without taking a blade to it? The answer lies in a chemical process that allows researchers to look at the organism in a new light.

Utilizing the Alcian blue and the Alizarin red chemicals, the hard tissues of mammals, reptiles, and even cephalopods can be pigmented. After the stains have adhered to markers in the solids, calcium and cartilage, trypsin is used to clear the soft tissue. Over a period of time, depending on the density of the specimen stained, the stains and natural pigmentation are slowly leeched from the specimen. What is left is a sample that has a translucent, fleshy envelope. Encased within this clear containment is the specimen's pigmented internal structure.

Colors range from deep blue-violet to bright reds. These pigments depend on what has been stained and its density. A thin piece of cartilage will be a vivid blue, while thicker pieces will appear as a darker blue. External characteristics can also be highlighted with this technique. Scales and barbs on aquatic organisms are distinguished easier when sent through the staining process.

Creating the stained samples is one thing but how is a researcher supposed to share their findings? Words can only go so far. A researcher can publish all the words they please but substance, impact, and that "wow" factor are lacking without the proper use of images. Since the specimens were originally embryos, a macro or "close-up" photographic configuration is beneficial to capture small details. The inherent characteristics of these specimens also make them an interesting practice in lighting. With a translucent exterior and, sometimes, extremely dense structures a photographer may have to make a decision to either expose for the soft tissue or for the stains.

Because the specimens are, by design, moist they need to be stored in glycerol to prevent drying out. This makes photographing the chameleon a challenge – with its contours and moisture, highlights and specular highlights are difficult to avoid.

Photo Setup

Equipment: Nikon D300, Nikkor 60mm macro lens, Nikon Bellows, Zeiss 16mm macro lens, USB cable, light table, heavy duty copy stand, Mac computer, Nikon Camera Control Pro 2, Adobe Bridge CS5.1, Adobe Photoshop CS5.1, Zerene Stacker.

A Nikon D300 was attached to a copy stand and tethered to a Mac computer via a USB cord. Nikon Camera Control Pro 2 and Adobe Bridge CS5.1 were used to capture and view the images in real time. A light table was used as the main light to backlight the chameleon. Adhering to fundamental photographic techniques will prevent long hours of editing post processing. Before photographing, take these simple steps that will take only a few moments and will save you hours of screen-staring later.

1.) Keep you work area clean and free of dust and particulate in the air.

a. That may mean either replacing the glycerol the specimen is in, building a dust tent over the photographic setup or doing a combination of the two.

2.) Make sure your camera sensor is free of dust. If need be get it cleaned by professionals before you shoot.

a. Be sure to keep the camera facing down when switching out lenses and bellows.

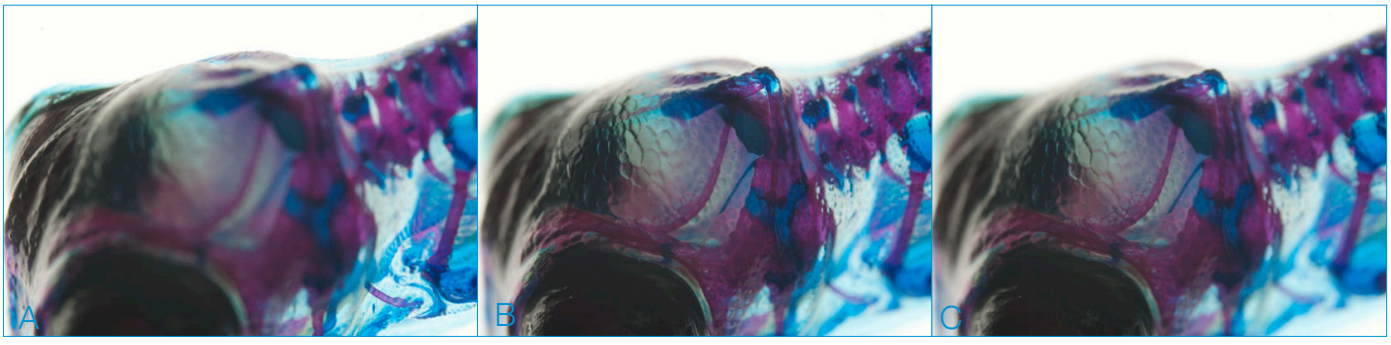
3.) Keep vigilant of any sort of debris that may fall into the setup while you're shooting.

How to Capture an Image with Extended Focus (Z-Stacking)

With a thick specimen at high magnifications, there is only going to be a think slice of the specimen in focus. How is it possible to get a specimen entirely in focus? Z-Stacking involves photographing multiple frames of a single composition and loading those images into dedicated stacking software. The images each have a particular plane in focus, with the preceding and following images in the sequence having incremental differences in the location of focus. The software essentially finds the greatest contrast between pixels and sees that as "sharpness." These slices of sharpness are composited together to create a focused image.

A Single capture, point of focus is just below the tip of the tail.
B Single capture, point of focus is midway through width of tail.
C Single capture, point of focus is midway through the forehead.

The chameleon image on the front page is a stack of 32 images.



A Single capture, point of focus is just at the base of the image.
B Single capture, point of focus is midway through head crest.
C Single capture, point of focus is at the tip of the head.

This final image was composited from 13 individual images.

Photographing

The light table could not be easily maneuvered in small increments so the only alternative I could think of was to move the camera via the copy stand. As I shot, I had the files go to the computer's "picture" folder. Each composition was labeled, (ex: for the full body stack, "Chameleon_stack_01_001, …_002, 003, etc.). That way I did not have to distinguish one "IMG_####" from another "IMG_####" when loading the files into the stacking program. For each compisition, I photographed the series and went back to check that I had the necessary amount of images and overlapping planes of focus. It is annoying and time consuming to photograph for hours only to find that in the stacking software, there is a gap in focus. This gap usually happens when there were frames that should have been photographed but the camera was moved too much without photographing in between. I kept the camera settings at ISO 200 and had shutter speed anywhere from 1/2 second to 1/160th second depending on the magnification and what particular feature of the chameleon I was focusing on. The great thing about the Nikon D300, and now many cameras that are available, is its Live View mode. I can simply switch the camera to that mode, click the "LV" button in the Nikon Capture software and I have an image that went from being two inches on the LCD to over four inches on the computer screen. The best thing I can suggest to work slowly and thoroughly with your imaging. Keep an eyedropper of glycerol on hand to rehydrate your specimen ever so often. This may be scientific imaging but don't forget to have fun with the specimen. Don't just think and wonder what the specimen may look like at "this" or "that" angle, photograph it!

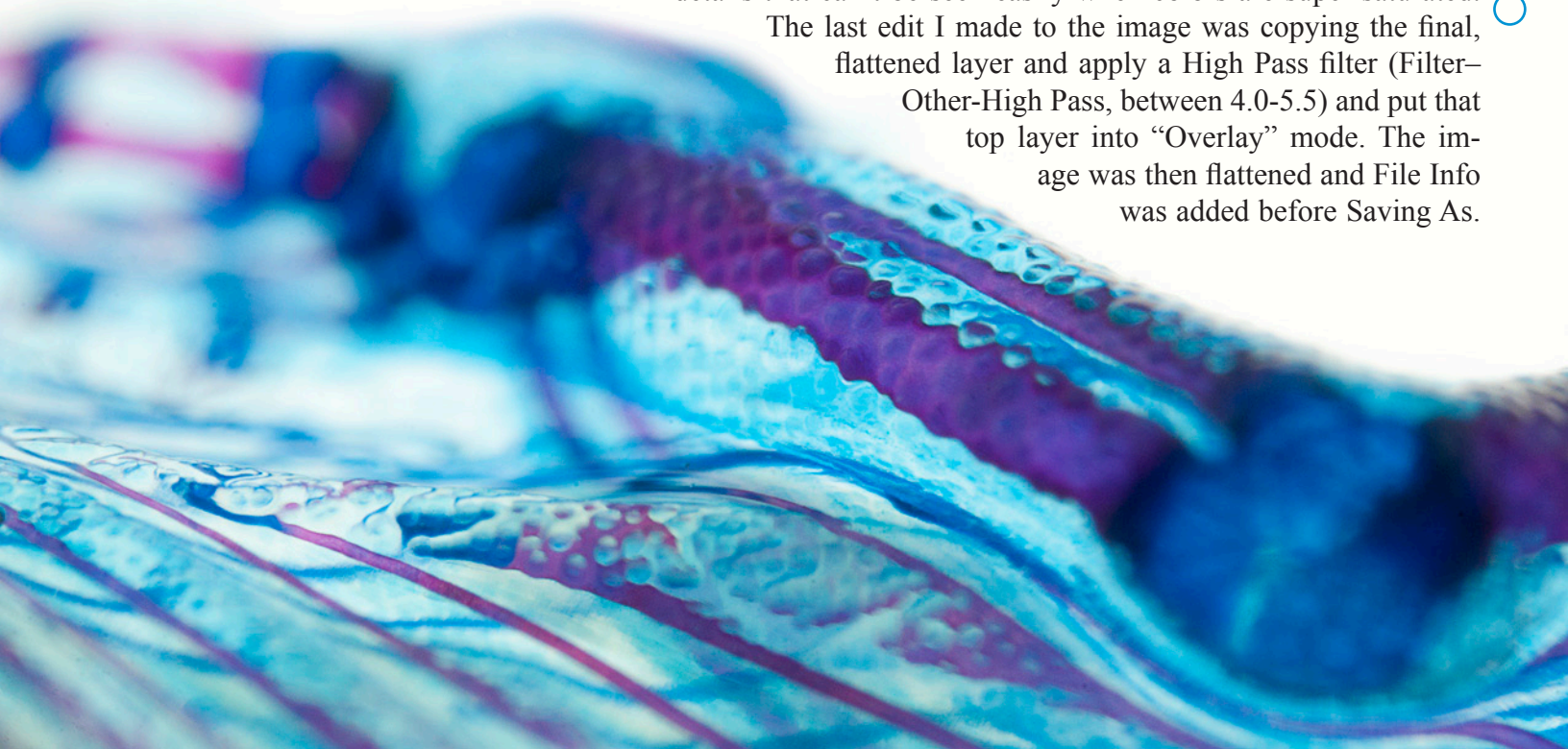
File Format

It is part of my workflow to photograph in the camera's RAW file. If your camera is capable, I highly suggest converting it to work with RAW files for optimization in post processing. The RAW files were loaded into Adobe Photoshop CS5.1 where global batch edits were made. I saved those images in a new folder as .tiff files. Please, if you have ever never used a stacking program before, do not use RAW files, even if the program accepts them! It will slow down and bog down the software and your computer. After using multiple stacking programs in the past for various projects, I chose Zerene Stacker for the chameleon. In Zerene, I utilized the DMap stacking option; it has a threshold that allows the user to choose what areas of the image to "ignore" when stacking.

When making edits or changes to my images, flattening layers, making major changes to white balance, etc. I always "Save As." Keep the master (RAW) files saved and work off of them but don't make any damaging changes to them. Work in layers and use non-destructive modes of image editing. Insert metadata to the images so that others can find you through your work (File - File Info in Photoshop). Back up your images in different and multiple locations; invest in a hard drive, a portable hard drive, an online "safe" or some combination of these methods.

Image Optimization

Before I stack the images, I use Photoshop to fine-tune the white balance. If the images need to be stacked, I go through the steps in Zerene Stacker and export the final image as a .tiff file. From there, I work more in Photoshop. I crop the image, sometimes Zerene creates image artifacts including elongating portions of the image to fit the frame. No matter how vigilant I am when I photograph, there is always dust or tiny hairs/fibers on the specimen. I just can't avoid it! This is where the Spot Healing tool becomes an asset. Small particles that have fallen on or around the specimen can be removed without destroying the integrity of the image. Final steps for the chameleon include desaturating the overall image just a bit. This allows for better prints and, sometimes, there are details that can't be seen easily when colors are super saturated. The last edit I made to the image was copying the final, flattened layer and apply a High Pass filter (Filter–Other-High Pass, between 4.0-5.5) and put that top layer into "Overlay" mode. The image was then flattened and File Info was added before Saving As.



I'm Liz Marchiondo, fourth year for Biomedical Photographic Communications at the Rochester Institute of Technology. Anticipated graduation date is May 2012 with a Bachelor of Science Degree and a Minor in Archaeology. In the summer of 2010, I completed a co-op at the Harbor Branch Oceanographic Institute and in 2011 I completed a co-op with Carl Zeiss MicroImaging, Inc.

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