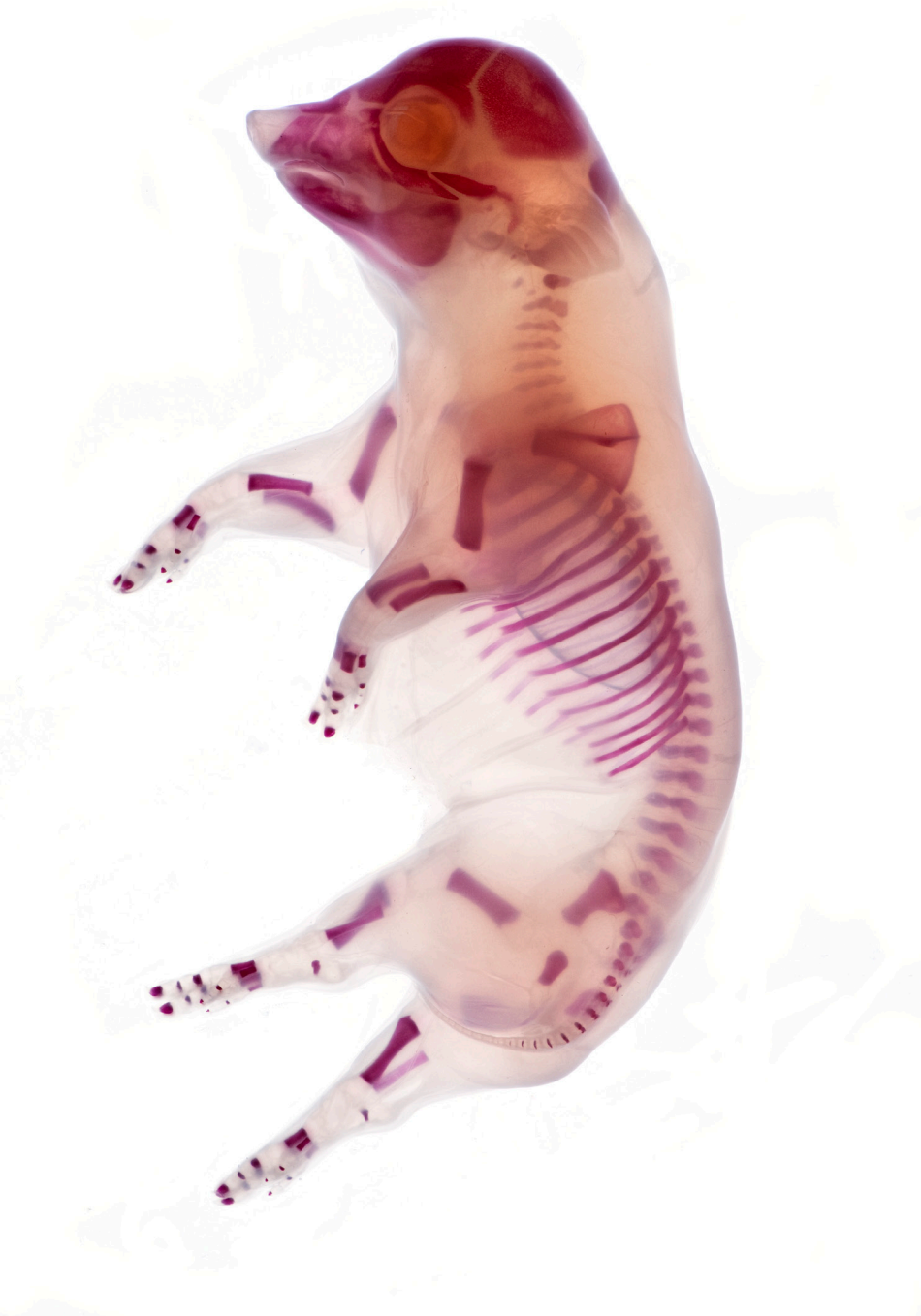


# Fetal Pig Skeleton

cleared and stained

shelby leeman



# About Specimen

The skeleton of a fetal pig is not mature, comprising itself of mainly cartilage, which later will develop into bone. Cartilage is a flexible connective tissue assisting in mechanical support, although it is not as elastic as muscle. It is a storage site for calcium and other minerals, covering the ends of long bones providing cushion for the joints. Unlike other connective tissues, Cartilage does not contain blood vessels resulting in its slow growth and healing process.

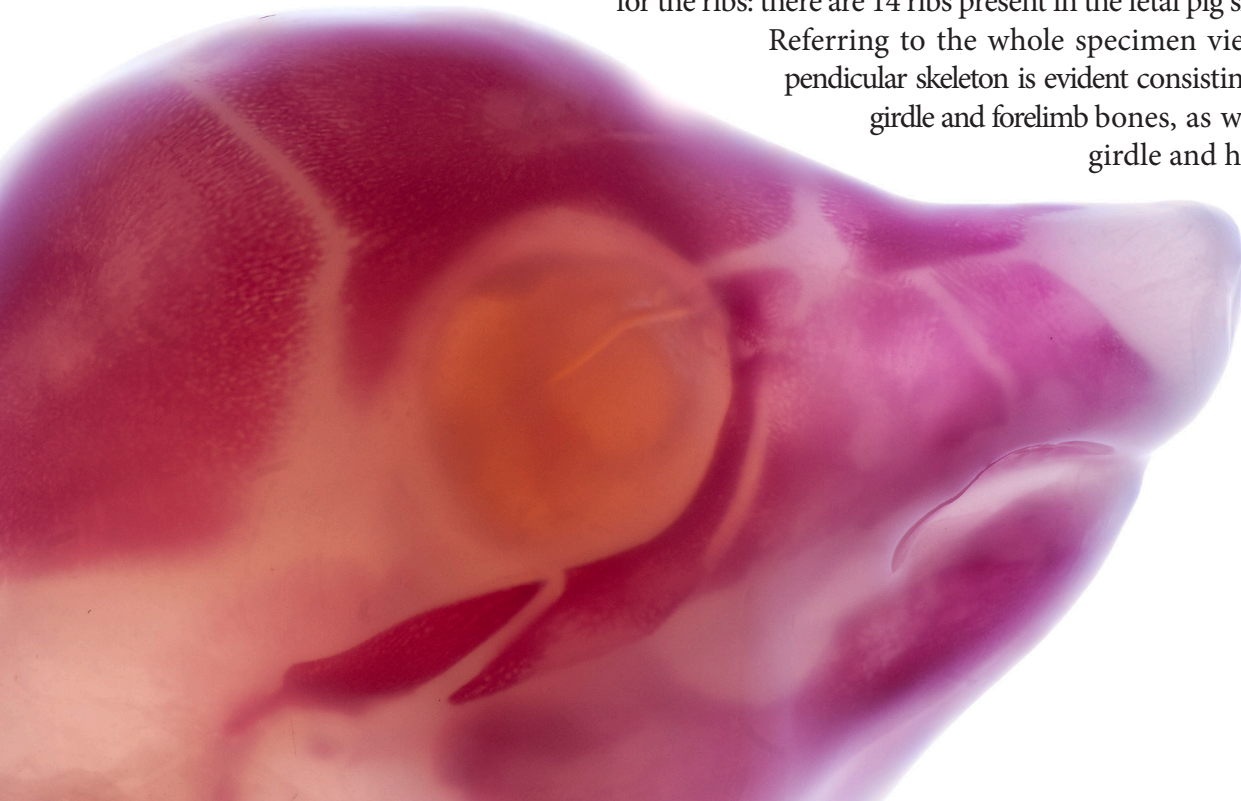
This pig has been stained using an Alizarin Red, anthraquinone derivative technique. The technique is used to identify calcium in tissue sections. Although it is not strictly specific for calcium the other elements usually do not occur in sufficient concentrations to interfere with staining. In the pig specimen this staining is evident for it displays the axial and appendicular skeleton. If photographed correctly, this stained specimen can provide information about the developing skeletal structure of the fetal pig.

When observing the specimen you can easily identify the Axial skeleton, which consists of the vertebral column, ribs, sternum and skull. Referring to the full specimen image examine the axial skeleton. As you glance over the fetal pig the nostrils at the tip of the head, jawbone and skull are evident. Additionally, you can observe the growth of the spine occurring throughout the specimen.

It is important to note that the densest area of calcium occurs in the skull, which is made evident by the Alizarin Red staining. The skull is divided into the cranium (frontal bone, parietals, temporals, occipital, sphenoid, and ethmoid) and the facial bones (nasals, maxilla, lacrimals, zygomatic, vomer, palatines, mandible, and hyoid). The cranium serves as protection for the brain, once again displayed by its greater staining density. The facial bones surround the digestive and respiratory portion of the head.

Similarly the ribs and sternum have an effective density to them at this time in development. Since they protect the internal organs of the pig, including the heart, the ribs need to be stronger than other skeletal bones in the body. The sternum is composed of three portions: the cranial called the manubrium, the body of the sternum called the gladiolus and the most caudal portion, the xiphoid process. The sternum serves as the attachment site for the ribs: there are 14 ribs present in the fetal pig skeleton.

Referring to the whole specimen view again the appendicular skeleton is evident consisting of the pectoral girdle and forelimb bones, as well as the pelvic girdle and hind limbs. Take



the time to distinguish these skeletal structures against the last.

The pectoral girdle and pectoral appendage (forelimbs) are essentially the same in a pig as in a human. The humerus, is the only bone found in the upper arm. The distal end of the humerus articulates with the lower arm at the elbow. In the forearm there are two bones, the longer one being the ulna and the radius being the shorter bone. The wrist is comprised of eight bones, referred to as the carpals. Four bones at the forefoot of the pig are known as the metacarpals. Phalanges, are the four digits or toes in the pig. All structures discussed are displayed in the image to the right.

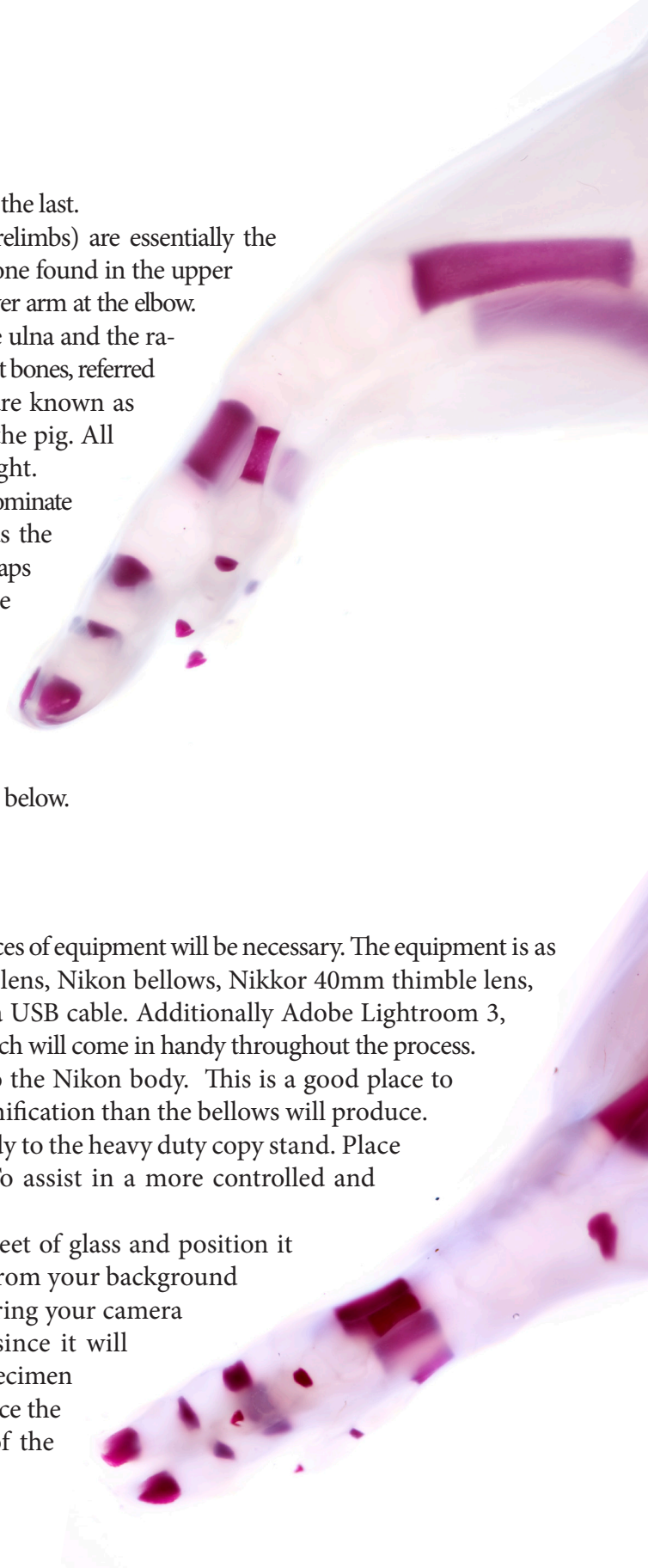
The pelvic girdle is divided in two halves, the innominate bones and the upper part of the hind limb is known as the thigh. The femur is the only bone in the thigh and is perhaps the strongest bone in the body. At the distal end of the thigh are the lateral and medial condyle, which articulate with the lower bones to form the knee. Seven bones make up the ankle and the fibula is the long slender bone on the lateral side of the tibia, or shinbone. Similar to the forearm there are five metatarsals in the foot of the pig and four toes. These structures are displayed in the image below.

## Photo Setup

To photograph this specimen effectively a few pieces of equipment will be necessary. The equipment is as follows: A Nikon D700 DSLR, Nikkor 105mm macro lens, Nikon bellows, Nikkor 40mm thimble lens, a light table, heavy duty copy stand, glass sheet, and a USB cable. Additionally Adobe Lightroom 3, Photoshop CS5, and Zerene stacker are all software's which will come in handy throughout the process.

First, attach the Nikkor 105mm macro lens to the Nikon body. This is a good place to begin as it will result in an image with a little less magnification than the bellows will produce. Once the lens is on the camera connect the camera body to the heavy duty copy stand. Place the lightbox directly bellow the camera lens. Tip: To assist in a more controlled and even lighting center the lightbox.

Once the lightbox is in place, clean a large sheet of glass and position it above your light source. This will create separation from your background and avoid scratches showing up in your image. Tethering your camera will make the imaging process significantly easier since it will immediately display the results. Remember: your specimen must be cleaned so once removed from the glycerin place the fetal pig in water. This will improve the final look of the specimen, removing any dirt or remaining glycerin.



# Photographing

Once you are all set up it is now time to shoot. When photographing it is important to reduce as much vibration as possible; tethering your camera will assist with this since you will not have to press the shutter release button. Begin by turning your camera on and making sure your lens is set to 1:1 imaging.

Once your camera is on set your camera exposure. Ideally, adjusting the shutter and aperture settings till they align in the middle of the metering scale would create the best image. With this specimen I would recommend overexposing by half a stop. When it comes to digital imaging it is always better to overexpose than underexpose because you can easily shift the histogram/exposure. Overexposing by half a stop I found that the background was a little brighter and more detail was captured throughout the fetal pig. Select your area of focus and change the your working distance to focus on your subject. You will now photograph your specimen with the intent of later stacking. To do this you must create multiple images with varying levels of focus. By changing your working distance throughout the specimen over a series of images, you will be able to achieve this. Note: For these images do not change your exposure, only adjust the working distance. I would recommend shooting in RAW mode, as it will provide the most information for the optimization process.

After you have captured the specimen with the macro lens attach the bellows and thimble lens. This portion of the photographing process will create high magnification images, such as the ones displayed throughout this paper. This process is a little harder and will require longer exposure times to gain enough light. To focus you will set the magnification you desire by extending the bellows. Then you will change the working distance until the ideal focus is achieved. Although all my images are focus stacked it is not required.

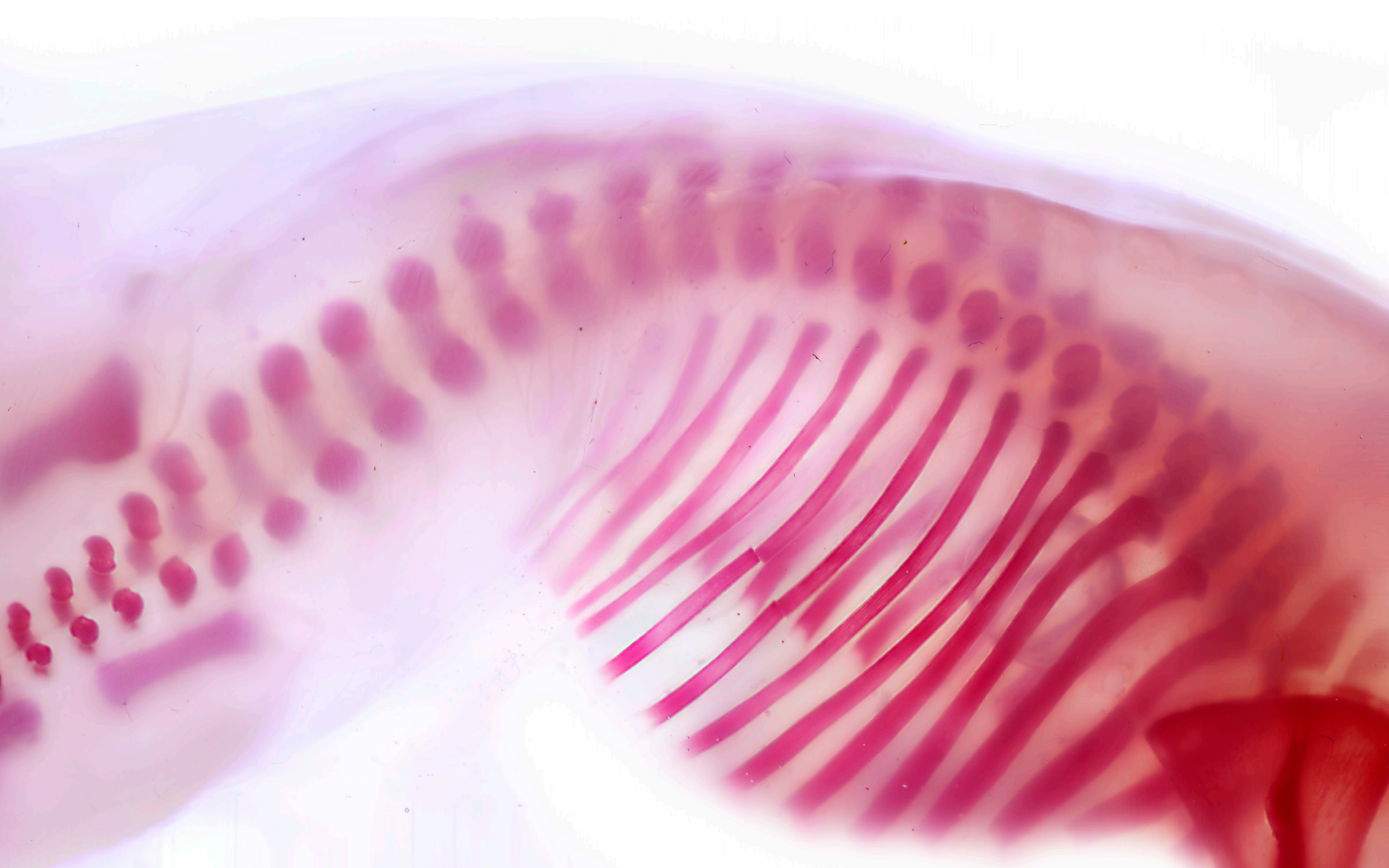
# Image Optimization

After you have completed photographing, use Adobe Lightroom to rank your images by favorites. Five stars should be the best, while one star represents the worst. I personally only rank to three which indicates the possibility of image use. Once you have selected your favorite images export your files as 16 bit Tiff files; this will provide more information during the optimization process.

When the images are exported, find the same images with varying focus and open them in Zerene Stacker. This program will automatically stack your images, creating an image with a full range of focus. Select Stack from the main tool bar, and then use the PMAX option to stack your images. I have found that commonly this option results in the best final image but feel free to try the others. After Zerene has finished stacking your images save the output image, save the file. Include PMAX in the name to identify the image as stacked.

Open your PMAX image in Adobe Photoshop CS5, here you will begin the optimization process. First, white balance your image since the light emitted from the lightbox has a bluish tonal value. White balancing will remove this hue and creates a white background. You can use the levels option to do this.

When your image has been white balance edit your image based on personal preference. Usually every image can benefit from a levels adjustment and a sharpening algorithm (unsharpen mask). Remember, the final image should not show the optimization process. Your images should appear to have been the result at capture. Lastly, use the color balance option to create a common tonal value across all your images. This is the final touch and will really make your body of work look good. At this point you have effectively photographed and edited a fetal pig skeleton. Congratulations!



# Contact

My name is Shelby Leeman and I'm a third year Biomedical Photographic Communications major at Rochester Institute of Technology, with a minor in Imaging Systems. Anticipated graduation day November 2012 with a Bachelor of Science degree. In summer 2011 I completed my co-op with Albert Einstein College of Medicine in their Analytical Imaging Facilities lab.

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