Preparing Vertebrate Eyes for Shipment

A Few Remarks Before Getting Started

First, thank you for reaching out to send us samples! We are excited to begin new collaborations that can provide great information about the incredible visual world we live in, and how the great diversity of animals experience our visual world from their own physiological and ecological perspective. By studying the visual system, we can obtain knowledge that will help understand not only species-specific visual physiology, morphology, and behavior, but also learn about applications in wellness and conservation.

In order to harvest the vast amount of data the eye has to offer, one must consider the fragile nature of ophthalmic tissue to ensure minimal data is lost during tissue collection. However, one must not delay in sampling the tissue: fixation of the ocular tissue must occur quickly as the neural tissue within the eye (i.e. the retina) begins to degrade within minutes after death. In short, work delicately but quickly!

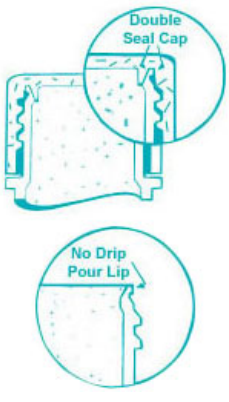
Considering the uncountable species differences in ocular anatomy & morphology that may require minor modifications to this protocol, there is no way for us to include explicit detail here to account for differences across all species. Please inform us of the species you are planning to send or have the potential of obtaining prior to tissue collection and we will gladly provide specific details for that particular species that vary from what is stated in this protocol.

One such source of variation is in how we orient the eye to its natural position. Before removing an eye, it is important to be able to distinguish the natural orientation of the eye as it would be positioned within the orbit of a living animal. Avian species, for example, have internal structures (e.g. pecten) that enable orientation once the eye is opened. Other classes of vertebrates are more difficult to orient and often require an artificial external marking (e.g. a cut/puncture). As stated before, please inform us of the species you intend to send and we will gladly provide the necessary details if artificial markings are necessary for orientation. Remember, this will have to be done *prior* to removal of the eye!

Not using anything above the neck for your own research? Great, you can just send the entire head! We actually prefer this as we often use the skull and brains in visual studies. The more we can contribute to the knowledge of your study species, the better!

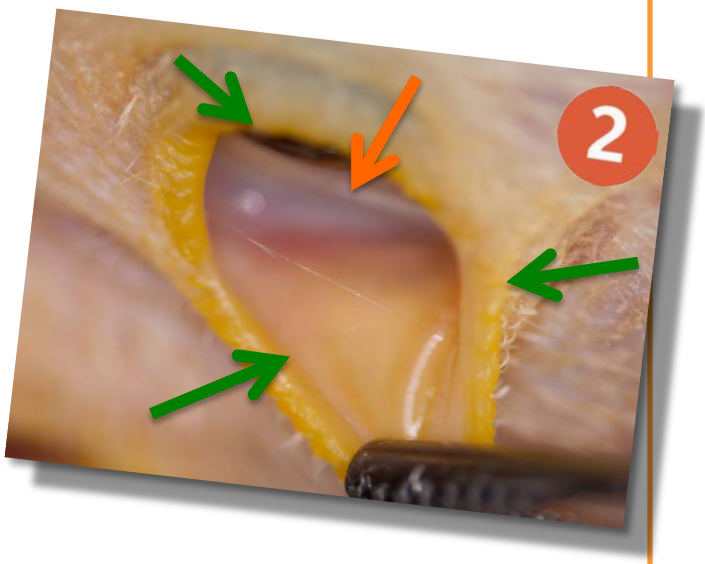
What You'll Need

- Tissue Collections
  - Forceps (curved edge, small point but not sharp → may puncture the eye)
  - Spring Scissors work best for cutting, but depending on the size of the animal you may be able to use something else. Iris scissors with also work.
- Tissue Fixation
  - Recipe for Proper Fixative:
    - 95% Ethanol 30mL
    - Formaldehyde 20mL
    - Glacial Acetic Acid 10mL
    - Distilled Water 30mL
  - To get proper penetration and fixation of tissue, you must place the tissue in 10x the amount of fixative per volume. Make sure you prepare enough fixative to accommodate the samples you intend to gather; making extra never hurts!
  - Container for storage and shipment:
    - Screw-top glass or Nalgene jars work great. You want a container big enough to hold the proper amount of fixative per tissue volume, and one that seals tightly to prevent leakage during shipment.
- Shipment
  - If proper jars were used for fixation, they can serve double as shipment containers.
  - 70% ethanol: this is for shipment. Chemical fixatives may not be shipped in the mail, so 70% ethanol is the medium of choice during shipment.



Removing the Head and Integument

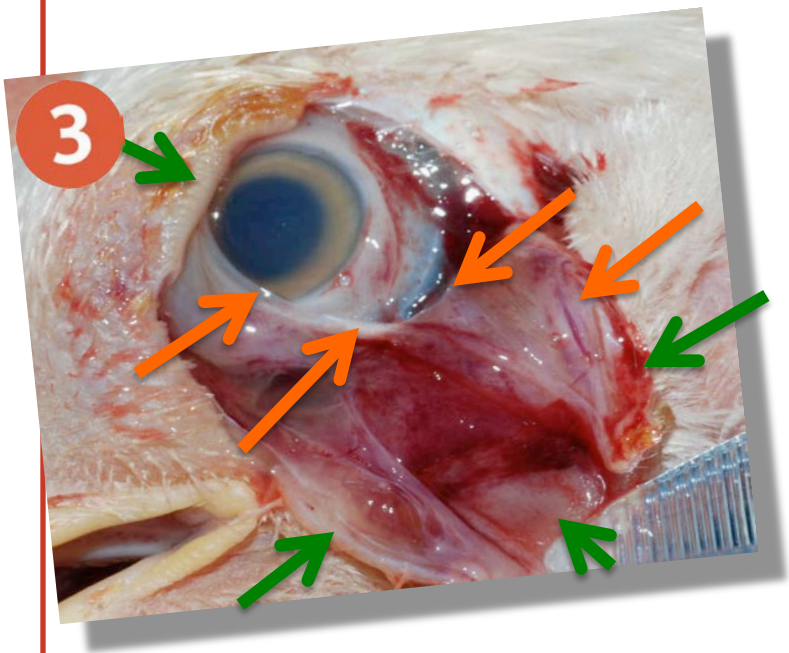
Immediately after euthanasia, begin by removing the head (cut the vertebral column close the skull). Pull the skin on the head rostrally (see Figure 1). You may not want to remove the skin completely, leaving it intact with the conjunctiva, which will provide a point of leverage from which to pull and cut. NOTE: You may skip head removal and procede to the following steps if you require that the head and skin remains intact.



Enucleation #1: Palpebral Conjunctiva

Figure 2 and 3 are of heads with the skin intact so you can more clearly see the conjunctiva in place. Eye removal may be performed using the same process whether or not the skin has been removed from the skull. To begin, start cutting the palpebral (outer) conjunctiva around the eye (green arrows). This holds true whether working behind the skin (as would be the case if you pulled the skin forward as in figure 1), or from the front (as in Figures 2 and 3). Removing the palpebral conjunctiva will allow access to the bulbar (inner) conjuctival margins (orange arrow).

CAUTION: The sclera (outside layer of the eye) can be quite fragile, especially in smaller animals, and can be cut/punctured if care is not taken. Take care not to push to hard on the eye causing a dent that may disfigure the retina and other internal structures.

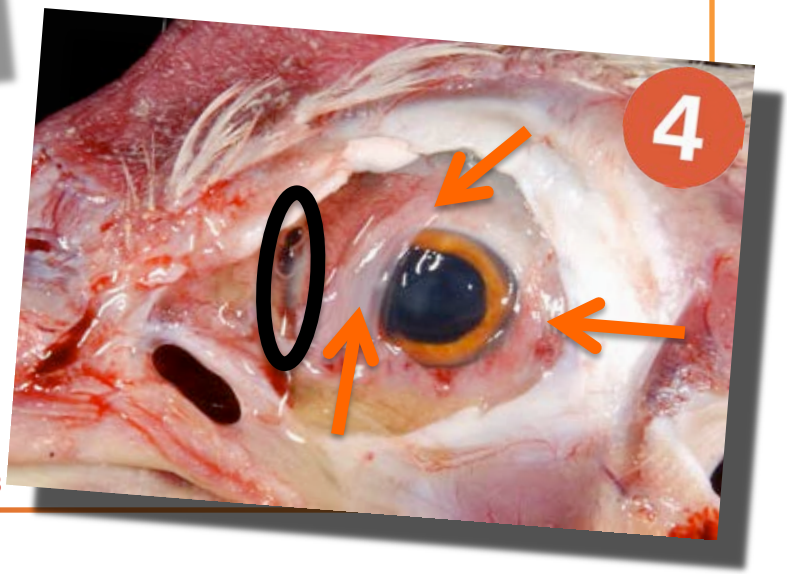


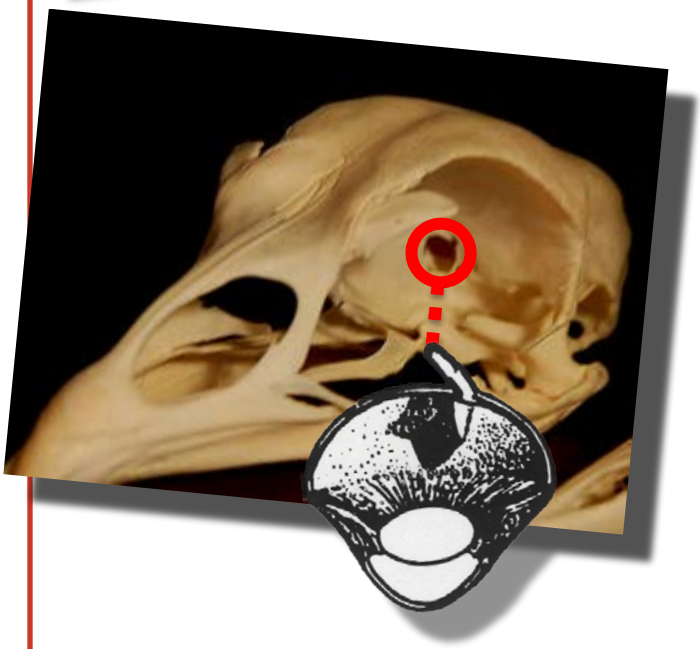
Enucleation #2: Bulbar Conjunctiva

Remove bulbar conjunctival tissue until you can see into the orbital cavity around all sides of the eye. Pulling outward on the conjunctiva will help you visualize the tissue better and will make it easier to cut. Again, take care not to damage the eye.

Enucleation #3: Finishing Up

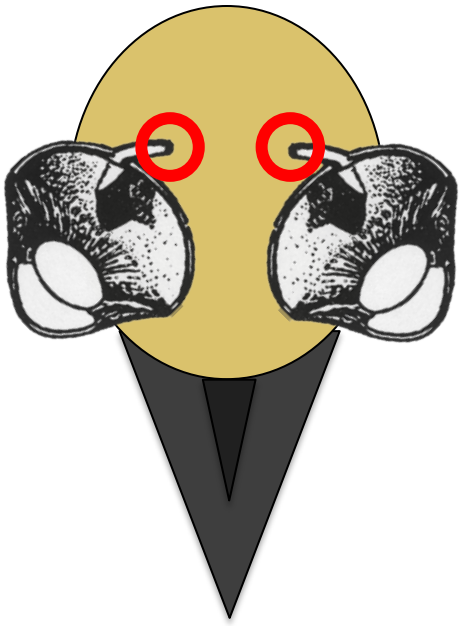
Figure 4 shows all palpebral conjunctival tissue removed, with some bulbar conjunctival tissue remaining (orange arrows). Continue to remove bulbar conjunctiva until most has been separated from the eye. You can begin to see the space around the eye into the orbital cavity (black outline). This is where you will insert your curved forceps to remove the eye.





Enucleation #3: Pulling out the eye

Once the eye has been freed from most of the conjunctiva, slide your curved forceps around the eye. Note in the figures (left) the location of the optic nerve circled in red. Since the optic nerve is an extension of the retina that sends visual information to the brain, pulling on the optic nerve also pulls on the retina. After sliding your forceps around the eye, grasp the optic nerve firmly to ensure that when pulled it is separated from the optic chiasm and does not pull the retina out from the back of the eye. Once gripped, gently pull the entire eye out of the orbit. You may have to readjust to separate/cut extraocular muscles or remaining conjunctiva from the eye.



Fixing the Eye/Head

- Proper Fixative:
  - 95% Ethanol 30mL
  - Formaldehyde 20mL
  - Glacial Acetic Acid 10mL
  - Distilled Water 30mL

Be sure to place the tissue in 10x the volume of fixative. Fix the tissue for about 4 days (eyes) to 2 weeks (heads) prior to shipping. Only once ready to ship, place the tissue in 10x the volume of 70% ethanol (tissue storage solution). Samples should be in tight-sealing containers (the screw-top glass jars work well). Placing the jars inside plastic bags will prevent any incidental leaking.

Be sure to label each sample for proper identification of the individual. Permanent marker used on the back of a dry skull is recommended, as well as labeling bottles.

Packing and Shipping the Samples

Pack super-padded so nothing will break if dropped or stepped on. Packing sample tight with bubble wrap inside foam coolers inside a box works really well. FedEx will probably be cheapest, and is very reliable.

Please ship to:
Esteban Fernandez-Juricic
Dept. of Biological Sciences, Purdue University
Lilly Hall G-347
915 W. State Street
West Lafayette, IN 47907

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