Human Donor Eye Protocol
DeAngelis Lab
Moran Eye Center

Precautions:

Always use standard precautions when working with tissue or blood. Samples from human donors have typically not been tested for HIV, hepatitis or other pathogens. Wear a lab coat, goggles and gloves and any other protective equipment as needed for protection. Change gloves frequently and always put on clean gloves before handling equipment (camera, computer, etc.). Disinfect all areas with ethanol.

Supplies Required:

Petri dishes
4 mm disposable punches
6 mm disposable
8 mm disposable punches
Dissection scissors
Dissection tweezers/forceps
Razor blades
Kimwipes or gauze
Dewar of liquid nitrogen
Punch tubes
RNALater

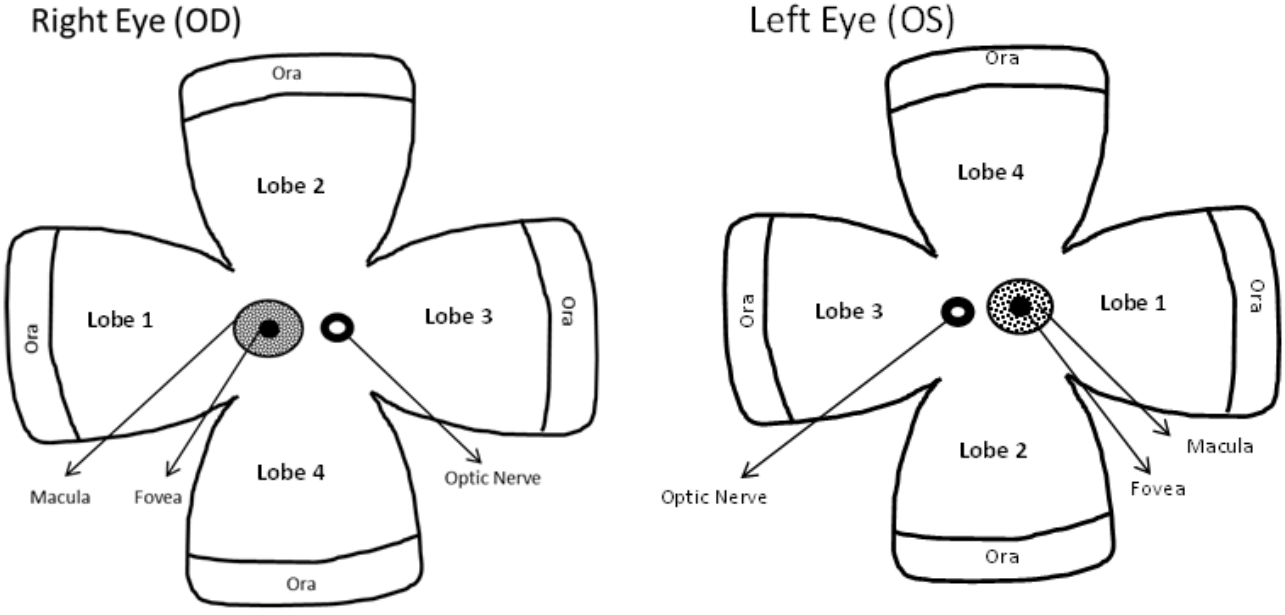
Receiving and Processing Samples

The eyes must be received and in the lab no more than 6 hours post mortem. Before processing the tissue, log the samples into our logbook and label each sample and all the accompanying paperwork with the DeAngelis number (D###-YY, where D stands for DeAngelis, ### is the numerical order of the sample and YY is the last two digits of the year). Record in the log sheet what was received (example: 2 purple blood tubes, OS and OD), other information as available on the label. Place the blood in an appropriate rack in the blood extraction hood (keep at RT) and proceed with processing the eyes. If a marble top tube is received, spin the tube at 2500 RPM for 15 minutes. Make 6, 250ul aliquots then 1.5 ml aliquots of the remaining serum, and store at -80^0 C in the "donated eye serums" box. If more than one purple top tube is available, process the second for plasma. Spin the tube at 2900 RPM for 5 minutes. Make 6, 250ul aliquots then 1.5 ml aliquots of the remaining plasma.

Dissection of Eyes

1. If the eyes are not labeled OS (left) and OD (right), determine which is which. This can be best done by identifying the inferior oblique muscle on the posterior (back) surface of the eye and lining it up beneath the blue horizontal cilliary vessel. If the muscle is on the left when beneath the cilliary vessel, it is the OS eye; if the muscle is on the right, it is the OD eye. Process one eye at a time. Never discard any tissue.
2. NOTE: All samples, except for the adipose, vitreous and sclera, are put into tubes containing RNALater – 100 uL/tube for 8 mm punches, 600 uL/tube for all others. These cannot be flash frozen!! They should sit at 4^0 C for at least 24 hours to allow the RNALater to permeate the tissue and can then be directly moved into the -80^0 freezer.
3. Remove any excess optic nerve stalk, and orbital fat from the exterior and put into appropriately labeled tubes.
4. Measure the eye's diameter's using the calipers: axial, nasal-temporal, superior-inferior and record these measurements on the log sheet.
5. Place the eye in a petri dish and gently make an incision with a fresh razor blade just beneath the limbus. The eye may be placed on a folded kimwipe or a piece of gauze to keep it more stable.
6. Use the dissection scissors to cut around the entire limbus and then remove the anterior segment (cornea, lens and iris). Separate and place the lens, cornea and iris each into the appropriate tubes. Note on the log sheet if the lens was artificial.
7. Place globe in the cuvette to take OCT images, following OCT protocol.
8. Make 4 equal cuts between the attachments points of the eye muscles, laying the eye into 4 equal "lobes". Carefully trim away and collect as much vitreous as possible without disturbing the retina and put into the appropriate tube. Keep the tube out to collect any remaining vitreous as the dissection proceeds.
9. Carefully, with the eye sclera-side down, move the petri dish to the camera, change gloves and collect photographs of the eye with the digital camera. Focus on the macular region and take photos at 1.25X, then 0.7X. If there are any abnormal or unusual features or morphology take photographs of these as well at appropriate magnification levels. Save all photos with the lab assigned number, OD or OS, the date and the magnification (example: D###-YY_OD_05-10-2012_0.7X) on the lab network server. (Z:\Post-Mortem Eye Tissue\Photos).
10. Use a 4 mm punch and collect the optic nerve and place in appropriate tube (may already contain the excess optic nerve stalk).

11. With an 8 mm punch centered over the fovea, collect the macula retina, followed by a 6mm punch of the remaining RPE/choroid. – collect the retinal and RPE/choroid layers separately. After removal of the retina, the 6mm RPE punch must be taken quickly and RPE tissue must immediately be placed in RNAlater to prevent degradation.
12. Carefully cut off the ora (anterior to the ora serata, about 2 mm posterior of the darkly pigmented cilliary body) from each lobe and place in the appropriately labeled tube (ora).
13. Use a razor blade to make an "X" to separate the lobes. Starting with lobe #1 (the lobe containing the macular region), collect the retinal and RPE/choroid layers from each lobe separately in clockwise order. See OS and OD diagrams below.
14. Collect the sclera in a single tube and flash freeze. Note on the log sheet the time all samples have been processed/frozen.
15. Follow the steps 3 – 12 and process the 2nd eye.
16. Quickly transfer the flash frozen tubes from the liquid nitrogen and place in the appropriate boxes in the -80^{0}C freezer. Do not let the tubes thaw!!
17. Place rack with samples in RNALater in the fridge.
18. Make any notes as needed on the logsheet. Disinfect the bench with ethanol and clean up the work area. Rinse dirty instruments in water before placing them in the container (filled with EtOH) for soaking.
19. At least 24 hours after processing the tissue, transfer any samples in RNALater to the -80^0 freezer.



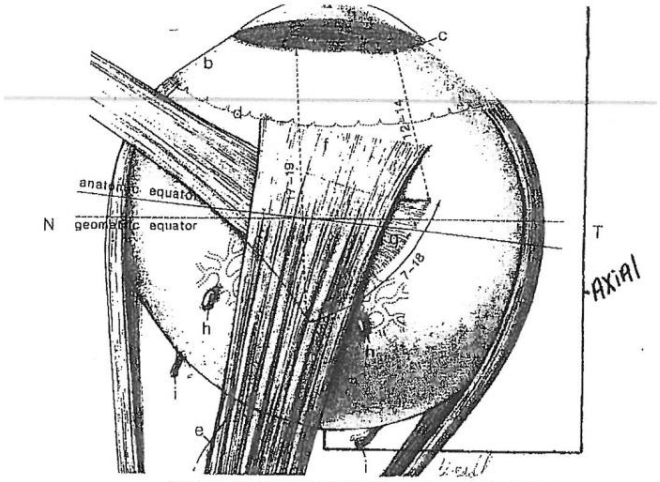


Figure 2-4. Drawing of the upper half of the eye. T-temporal; N-nasal. The contrasting degree of curvature of the cornea (a) and sclera (b) are evident. At the limbus (c), where they

S-I equatorial -

Eye Measurement

AXIAL — front (ant.) to back

NT eq. — Nasal to Temporal

SI eq. — Superior to Inferior

* measure at largest diameter to closest mm !!

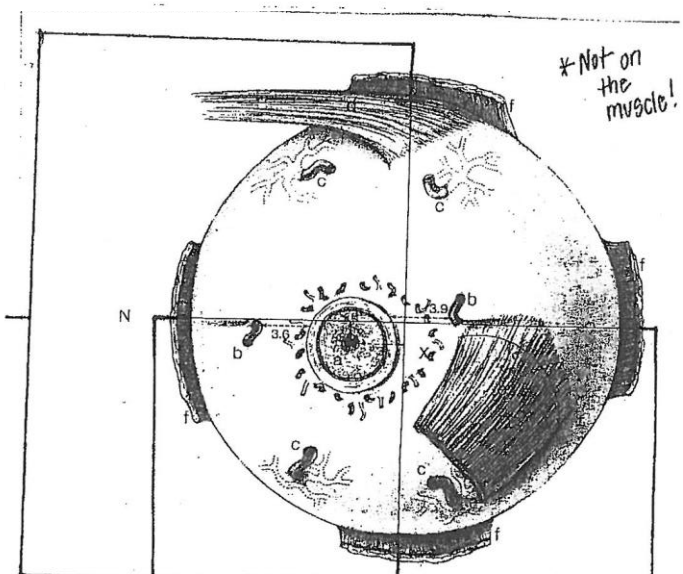


Figure 2-5. Drawing of the posterior eye. T-temporal; N-nasal. The optic nerve (a) wi its central vessels and surrounding meningeal sheaths is seen. Its center is located 3 mm. na and 1 mm. inferior to the posterior pole of the eye. Surrounding it are the short posterior ciliar arteries and nerves. The approximate position of the macula is at (x). Along the horizont meridian which bisects the eye are the long posterior ciliary arteries and nerves (b). The exits four vortex veins are shown, one for each quadrant (c). The curved, oblique insertions of t superior oblique (d) and inferior oblique (e) muscles are seen. The cut ends of the four rect muscles are at (f).

N-T equatorial

Human Donor Eyes Log Sheet

Date & Initials:_____

ULEB#:_____ DeAngelis Lab#:_____

Specimens Rec'd:_____

Age:_____ DOD & TOD:_____

Sex:_____ Enucleation:_____

COD:_____ Iris color:_____

OS globe measurements

Nasal-Temporal:_____

Superior-Inferior:_____

Axial:_____

OD globe measurements

Nasal-Temporal:_____

Superior-Inferior:_____

Axial:_____

Prior Medical History (including Alzheimer's, cardiovascular, cholesterol, blood pressure, etc if available):_____

_____

Eye History:_____

_____

_____

Smoking History:_____

_____

Photos (yes/no):_____ OCT images (yes/no):_____ Artificial Lens (yes/no):_____

Adipose Collected? Both OS only OD only no adipose collected

Notes:_____

_____

_____

_____

_____

_____

OD Tissue frozen/processed at _____ DNA extraction:_____

OS Tissue frozen/processed at _____ DNA quant:_____

QUICK VERSION

Dissection of Eyes

1. Trim off excess nerve stalk and any adipose
2. Measure the eye's diameter's axial, nasal-temporal, superior-inferior
3. Remove the anterior segment (cornea, lens and iris). Separate and place the lens, cornea and iris each into the appropriate tubes.
4. Take OCT images (see OCT protocol).
5. Flower eye.
6. Take photographs of the whole globe at 0.7X and 1.25X, also of any unusual features or morphology. Save all photos: D###-YY_OD_05-10-2012_0.7X on the lab flash drive.
7. Use a 4 mm punch and collect the optic nerve and place in optic nerve tube.
8. With an 8 mm punch centered over the fovea, collect the macula retina – followed by a 6mm punch of the RPE/choroid. Place samples in RNAlater.
9. Carefully cut off the ora from each lobe, place in the appropriate tube.
10. Use a razor blade to make an "X" to separate the lobes. Starting with lobe #1 (the lobe containing the macular region), collect the retinal and RPE/choroid layers from each lobe separately in clockwise order
11. Collect the sclera in a single tube and flash freeze.
12. Note on the log sheet the time all samples have been processed/frozen.
13. Transfer the flash frozen tubes from the liquid nitrogen to -80^{0}C freezer. Put tubes with RNALater in fridge.
14. Make any notes as needed on the log sheet. Disinfect, rinse dirty instruments and let soak in EtOH.