Messinger, J. D., Brinkmann, M., Kimble, J. A., Berlin, A. Freund, K, Grossman, G. H., Ach, T., Curcio, C. A. *Ex vivo* OCT-based multimodal imaging of human donor eyes for research in age-related macular degeneration.

# Supplemental Material 1

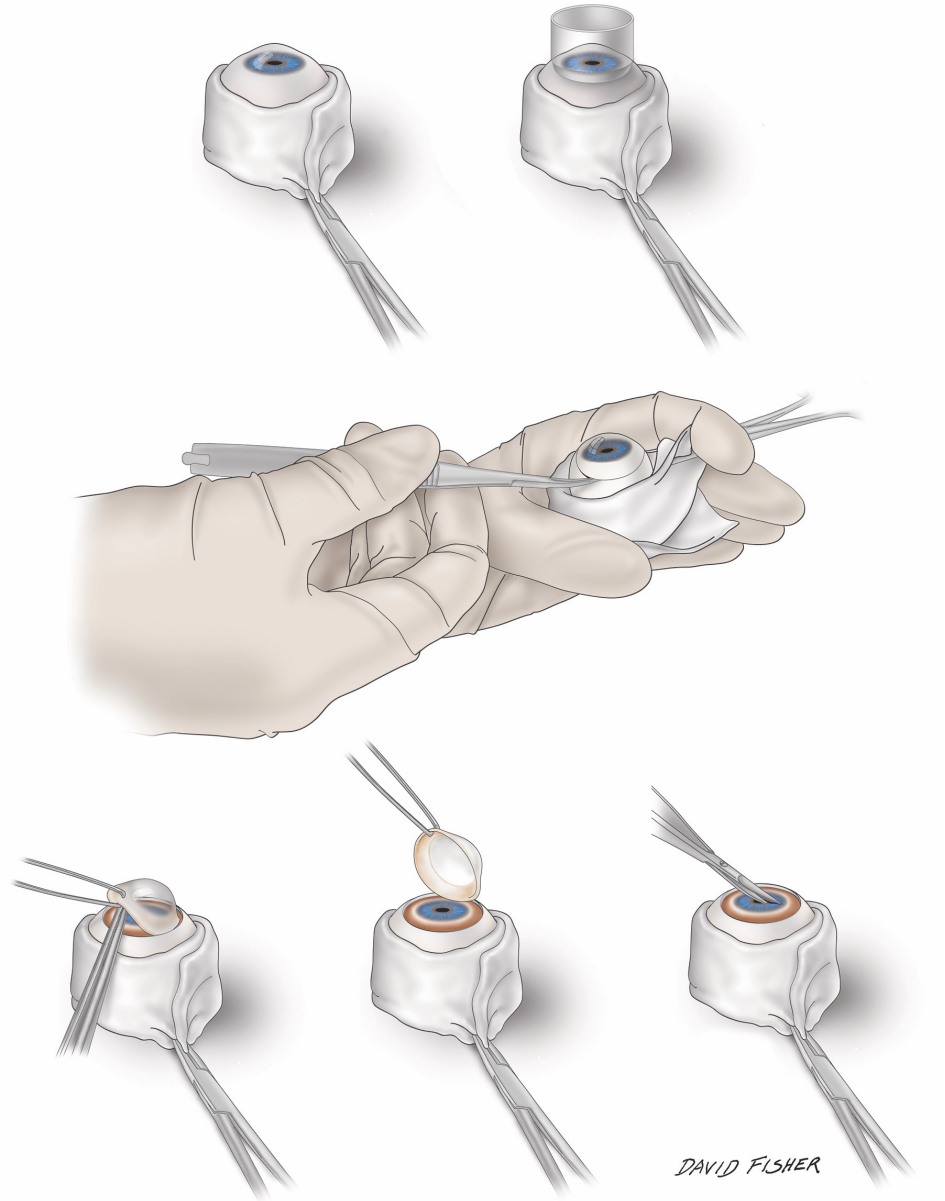
- This guide provides an overview for *ex vivo* imaging of the ocular fundus in human donor eyes, using clinical devices for optical coherence tomography (OCT) and multimodal imaging (MMI)
- Methods were used in these representative previous publications:
  - Litts, K. M., Messinger, J. D., Dellatorre, K., Yannuzzi, L. A., Freund, K. B., Curcio, C. A., 2015. Clinicopathological correlation of outer retinal tubulation in age-related macular degeneration. JAMA Ophthalmol 133, 609-612. PMID 25742505
  - Tan, A. C., Pilgrim, M., Fearn, S., Bertazzo, S., Tsolaki, E., Morrell, A., Li, M., Messinger, J. D., Dolz-Marco, R., Nittala, M. G., Lei, J., Sadda, S. R., Lengyel, I., Freund, K. B., Curcio, C. A., 2018. Calcified nodules in retinal drusen are associated with disease progression with age-related macular degeneration. Sci Transl Med 10, 466-477. PMID 30404862
  - Anderson, D. M. G., Messinger, J. D., Patterson, N. H., Rivera, E. S., Kotnala, A., Spraggins, J. M., Caprioli, R. M., Curcio, C. A., Schey, K. L., 2020. The molecular landscape of the human retina and supporting tissues by high resolution imaging mass spectrometry. J Am Soc Mass Spectrom 31, 2426-2436. PMID 32628476
  - Cao, D., Leong, B., Messinger, J. D., Kar, D., Ach, T., Yannuzzi, L. A., Freund, K. B., Curcio, C. A., 2021. Hyperreflective foci, OCT progression indicators in age-related macular degeneration, include transdifferentiated retinal pigment epithelium. Invest Ophthalmol Vis Sci 62, 34. PMID 34448806



**See also users' guide from the manufacturers of your camera and OCT device**

# Retrieving of donor eyes by The Eye Bank

- Excellent tissue quality is the first step of impactful discovery. To achieve this goal, our local Eye Bank has continuously optimized a comprehensive recovery process:
  - Minimizing death-to-preservation (d-to-p) time is key. Our research criterion d-to-p is $\leq$ 6 hours. In 2016-17 our mean d-to-p time was 3.9 hours.
  - Call center enhancements have led to death referrals received in ≤ 1 hour.
  - Donor Risk Assessment Interview asks AMD-, and glaucoma-specific questions.
  - Recovery is usually done in the hospital room, with preservation medium at hand.
  - Eyes are opened by consistent handling methods.
  - Eye bank staff communicates with investigators on timing, protocol, and delivery.




**Corneal excision from a human donor eye for immersion fixation of retina , by the eye bank**

Top left, excised donor eye is held in place by a sheath of gauze stabilized by a hemostat; top middle, 18 mm trephine is used to make a clean score of the cornea with a 2 mm rim of sclera; top right, the scored circle is finished by a cut with spring-loaded curved tipped scissors, while stabilizing the globe with the hemostat-clipped gauze; bottom left, the cornea is lifted off the sclera, exposing the iris (blue) and ciliary body (tan-brown); bottom middle, the cornea is lifted completely and handled independently; bottom right, iris is snipped perpendicular to the pupillary margin to facilitate penetration of the preservation into the vitreous chamber.

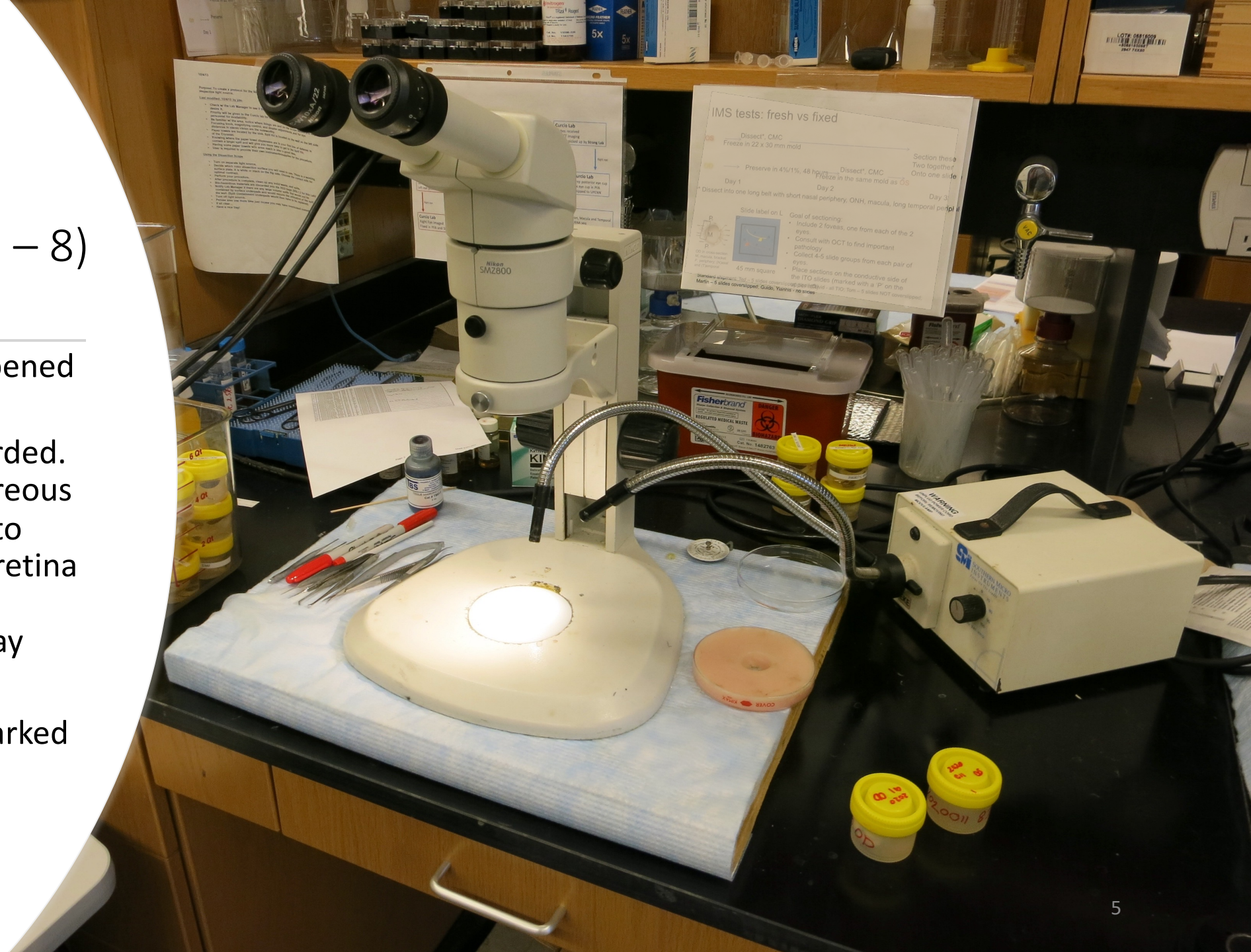
Supplemental material for: Messinger, J. D., Brinkmann, M., Kimble, J. A., Berlin, A. Grossman, G. H., Ach, T., Curcio, C. A.
*Ex vivo* OCT-based multimodal imaging of human donor eyes for research in age-related macular degeneration.

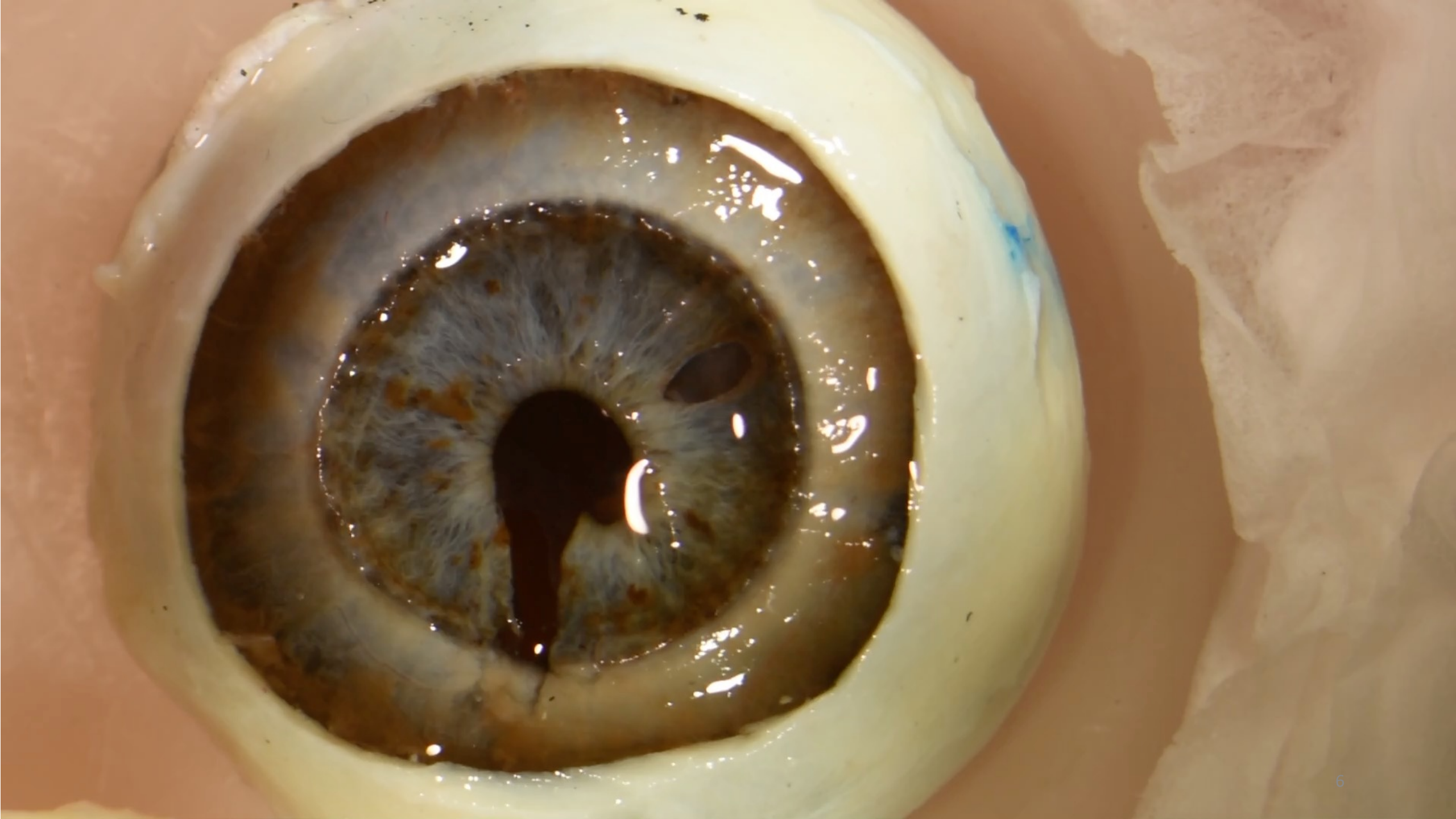
# Tissue recovery

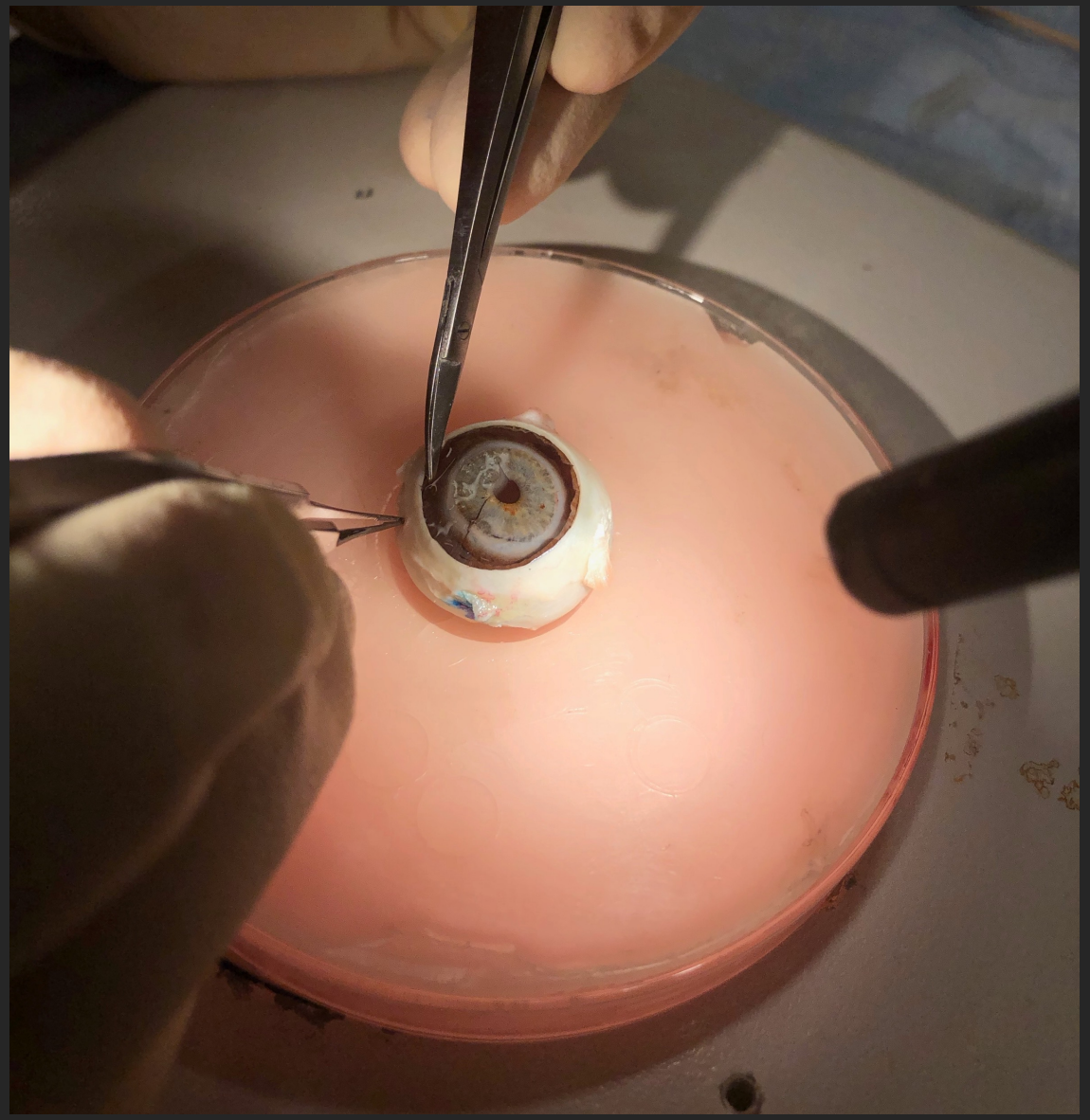
- Globes are recovered on-site, scored with an 18-mm trephine to facilitate corneal removal with scissors, and immersed in cold buffered 4% paraformaldehyde.
- They are held in cold fixative until delivered to the laboratory

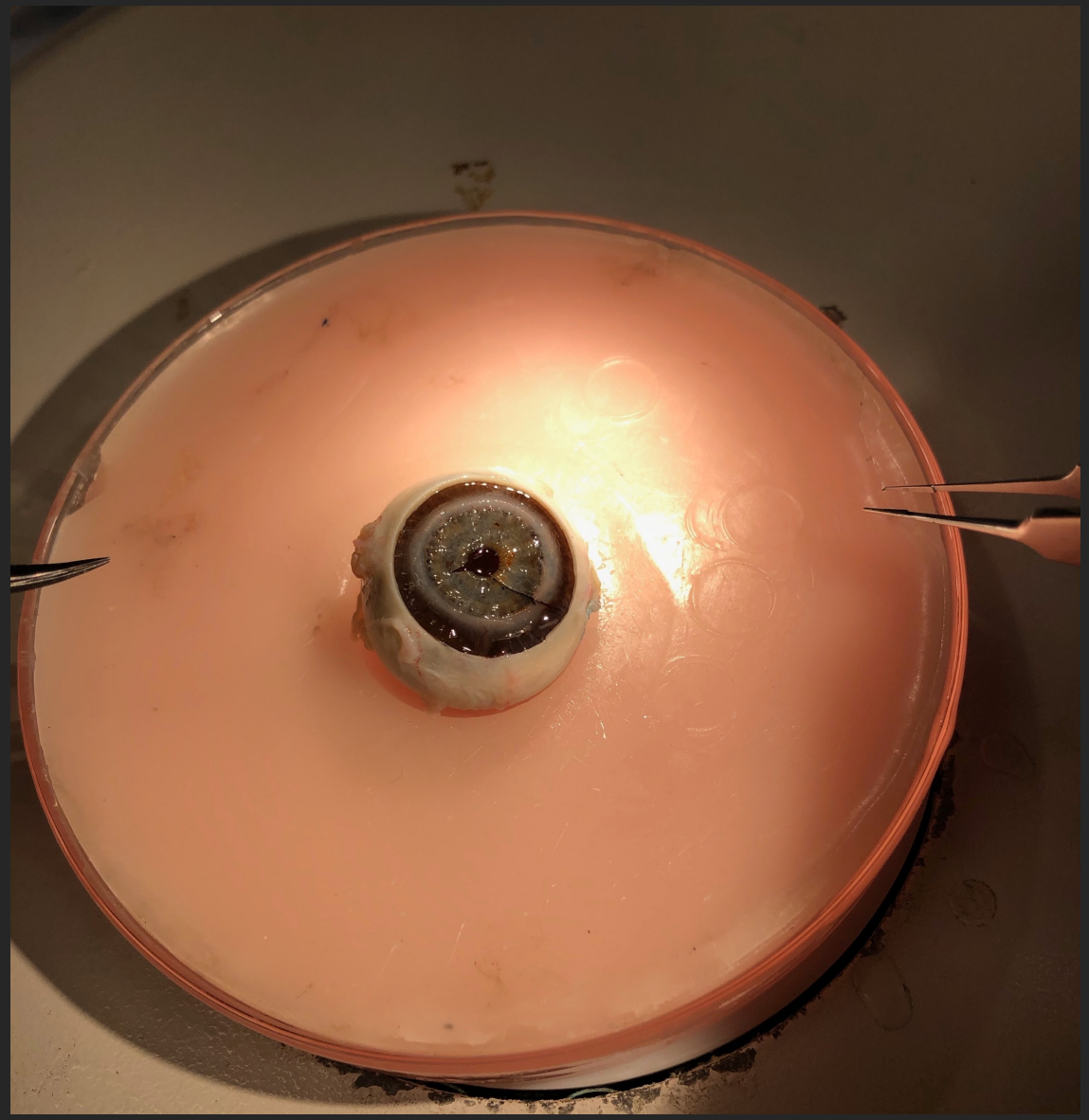
# Next steps (slides 6 – 8)

- The eyes are then fully opened to allow MMI (Figure 1).
- Liquified vitreous is discarded.
- The vitreous base and vitreous attached to it is retained to ensure adherence of the retina to the choroid and sclera. Pulling on the vitreous may detach the retina
- Superior pole of eye is marked with tissue marking dye.









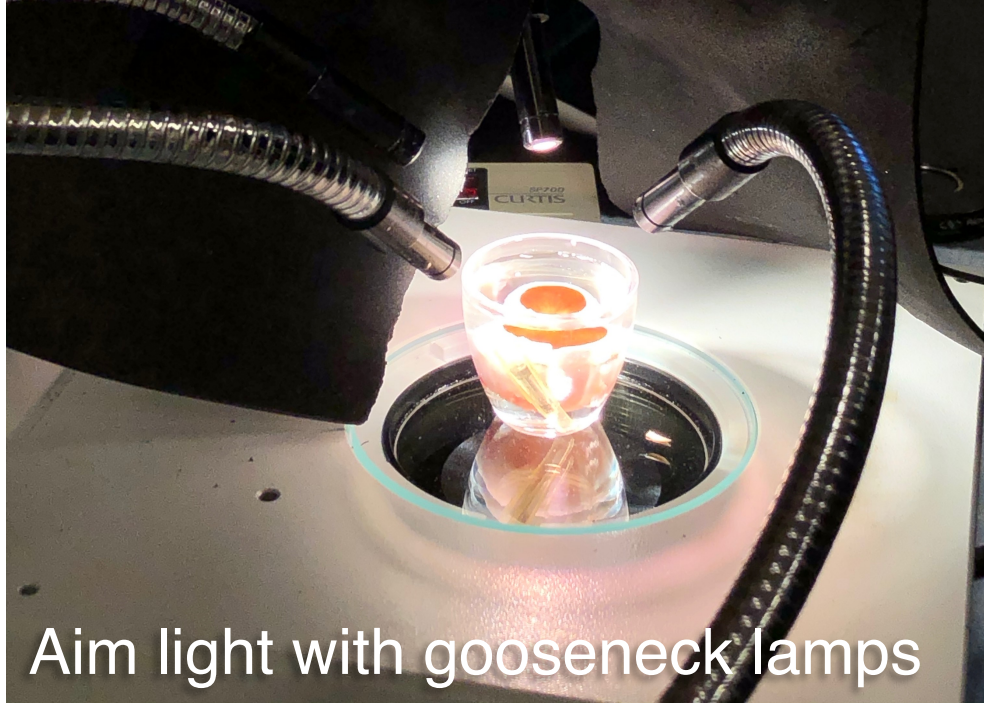
Laboratory: Eye is stabilized for dissection on dental wax previously indented with a 25mm heated  ball bearing

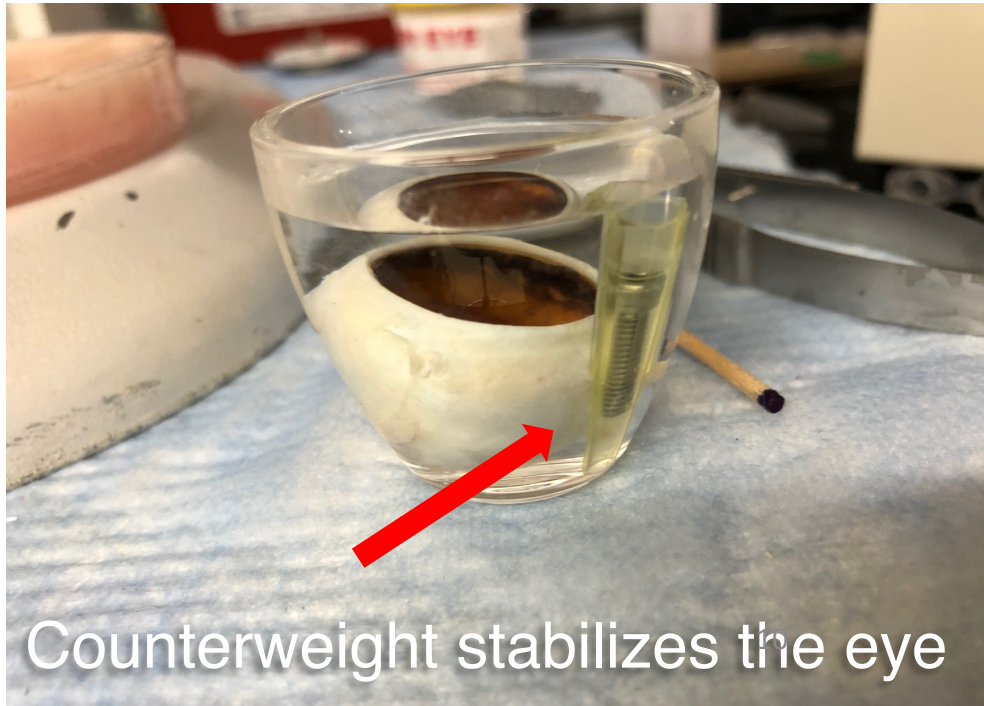
# Laboratory: eye under dissecting microscope

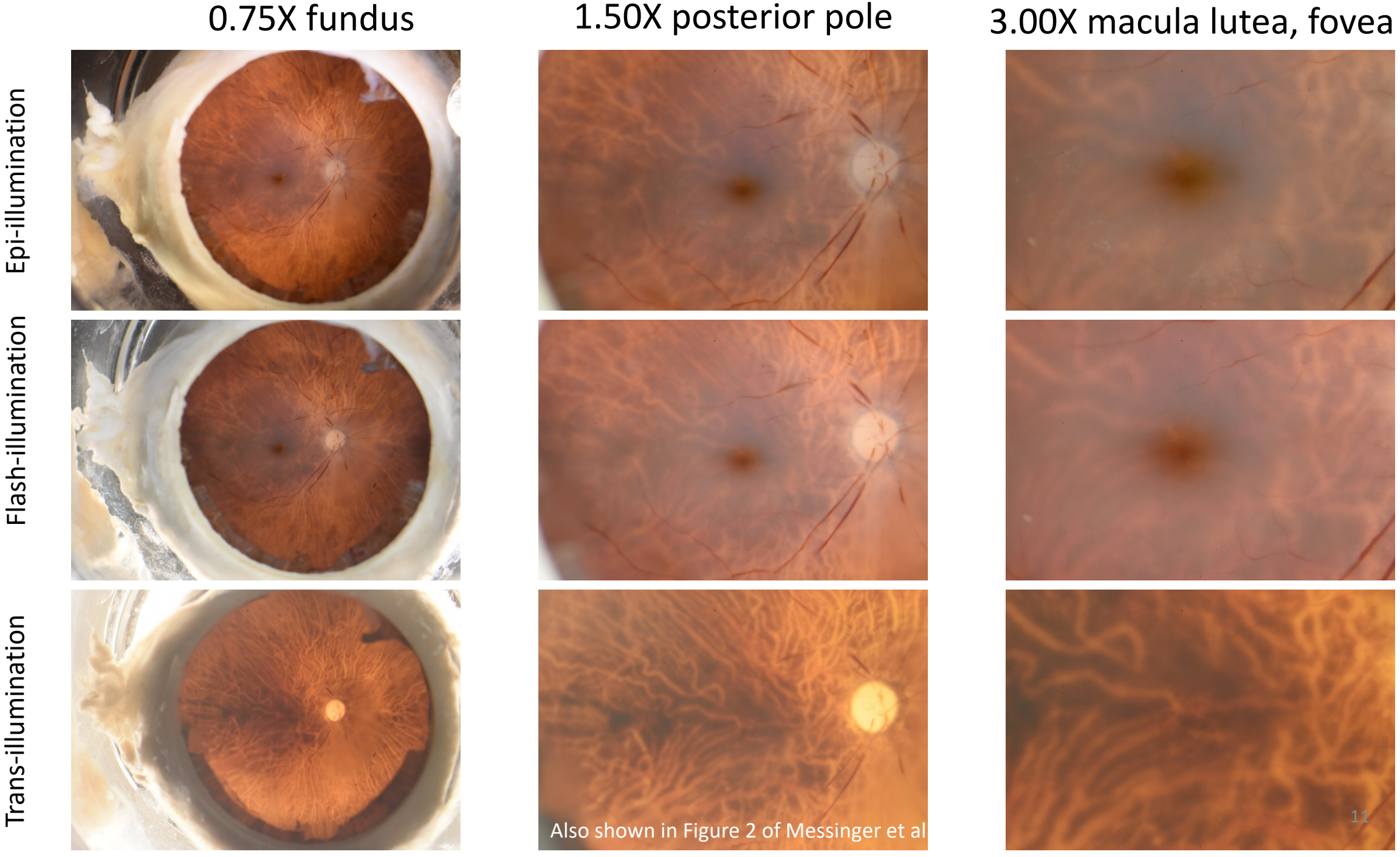
# Color fundus photography

- Eyes are placed in a quartz crucible filled with buffer.
- And placed under a camera for fundus pictures.
- Pictures are taken with different light sources (epi-, flash- and transillumination).


BKT

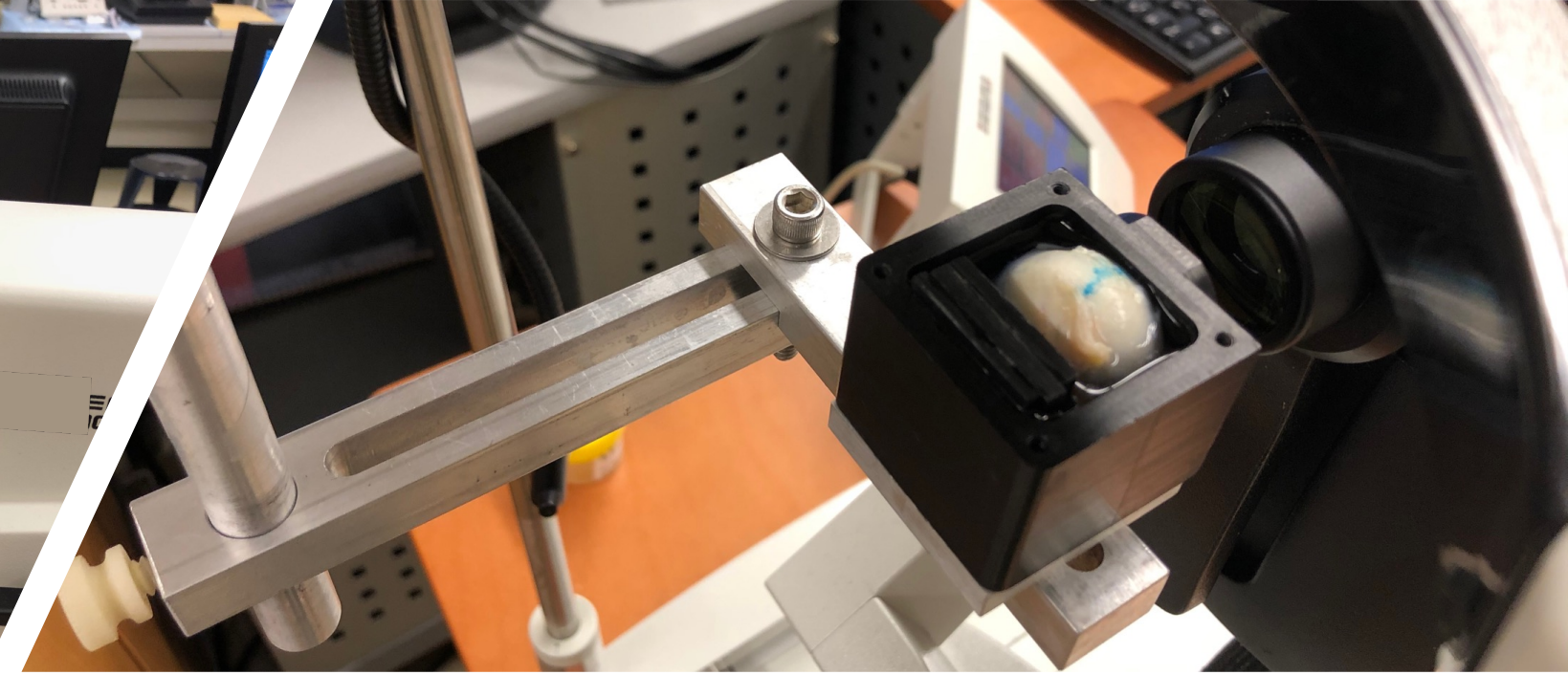

Aim light with gooseneck lamps

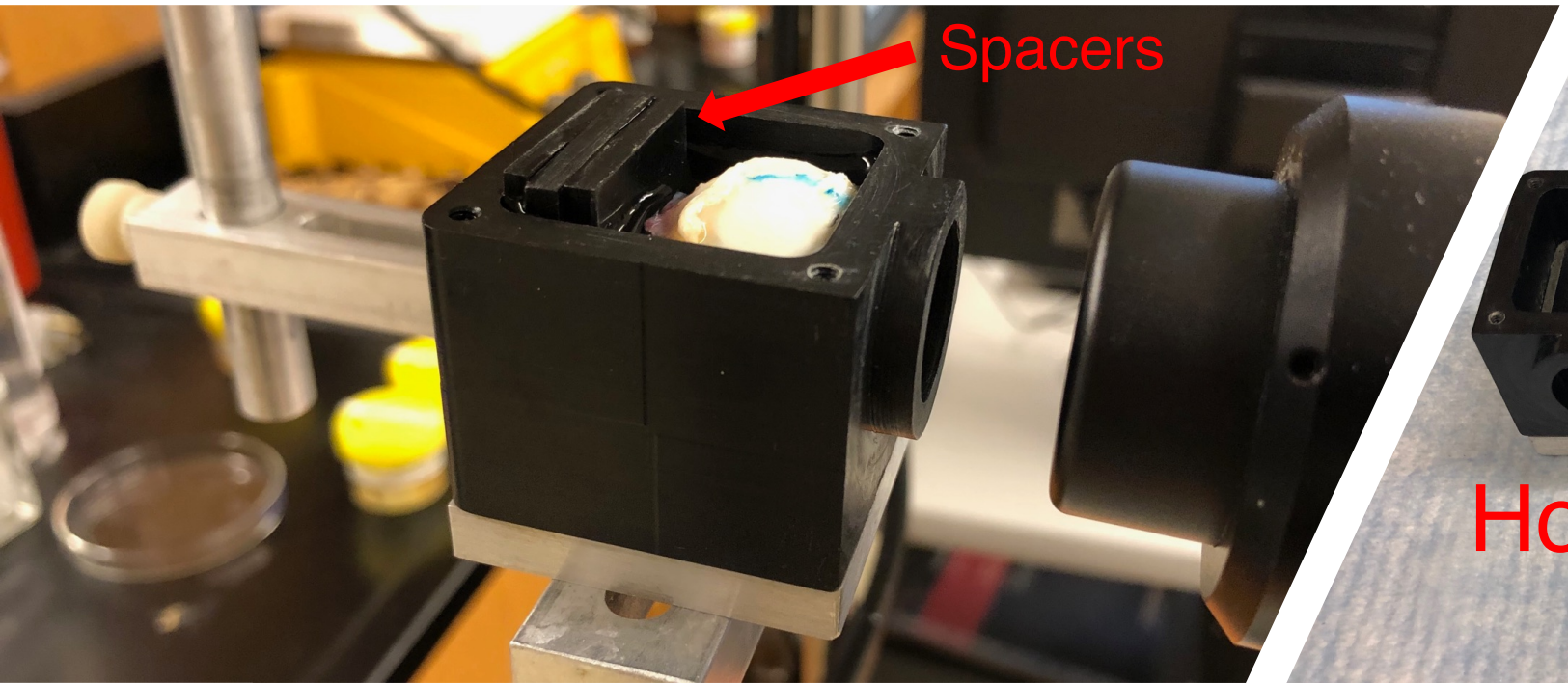

Counterweight stabilizes the eye

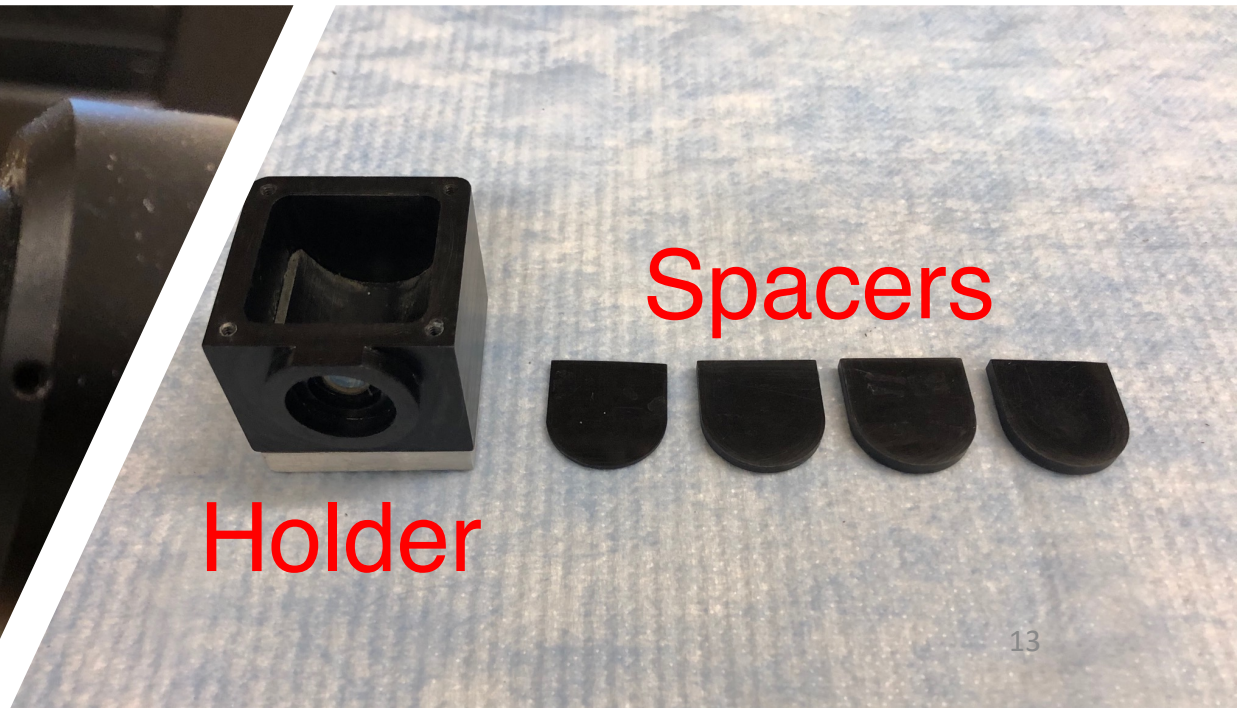

0.75X fundus
1.50X posterior pole
3.00X macula lutea, fovea
Epi-illumination
Flash-illumination
Trans-illumination
Also shown in Figure 2 of Messinger et al

# OCT

- Each eye is placed in a custom-made closed-chamber eye holder with a 60-diopter lens.
- It is filled with buffer and attached to the headrest of the OCT device, so that the eye “looks in” as would a patient.




Spacers

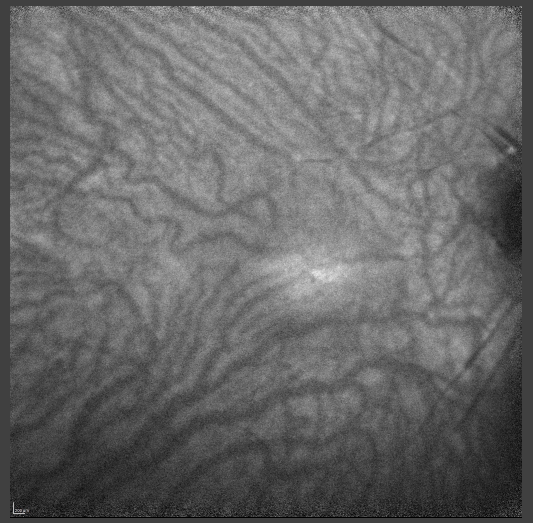

Spacers
Holder

# Which images are taken with the OCT device?

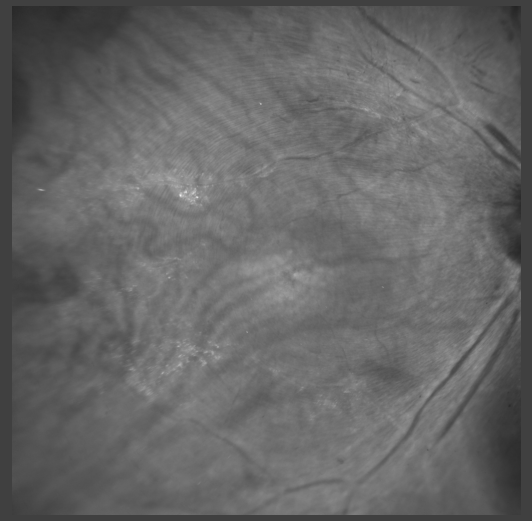
- Near-infrared reflectance scanning laser ophthalmoscopy
- Red-free reflectance scanning laser ophthalmoscopy
- 488 nm fundus autofluorescence
- 787 nm fundus autofluorescence
- Spectral domain OCT volume

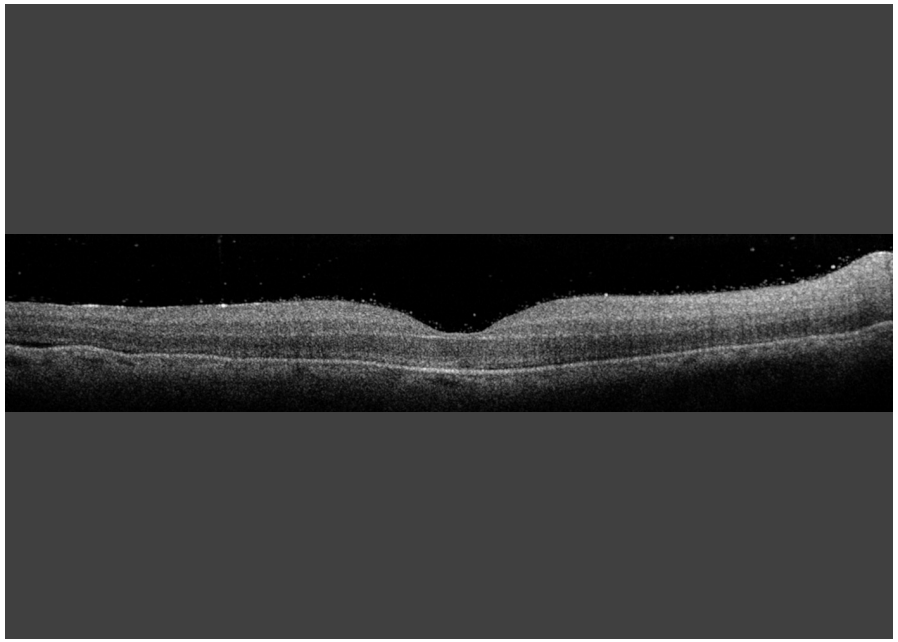
AF

(787)

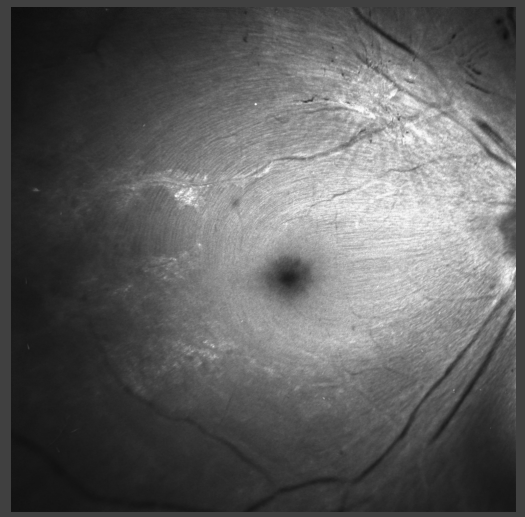


NIR



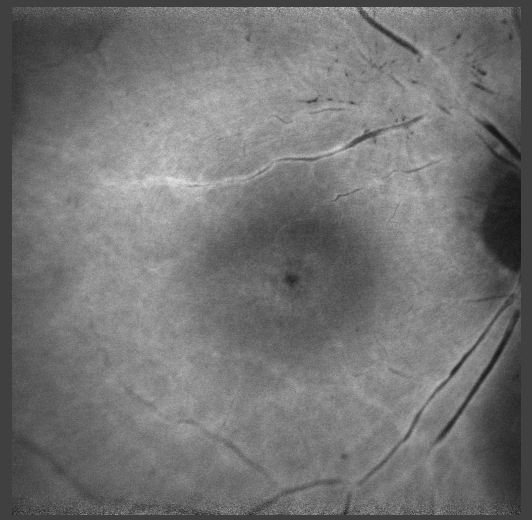


RF



AF

(488)



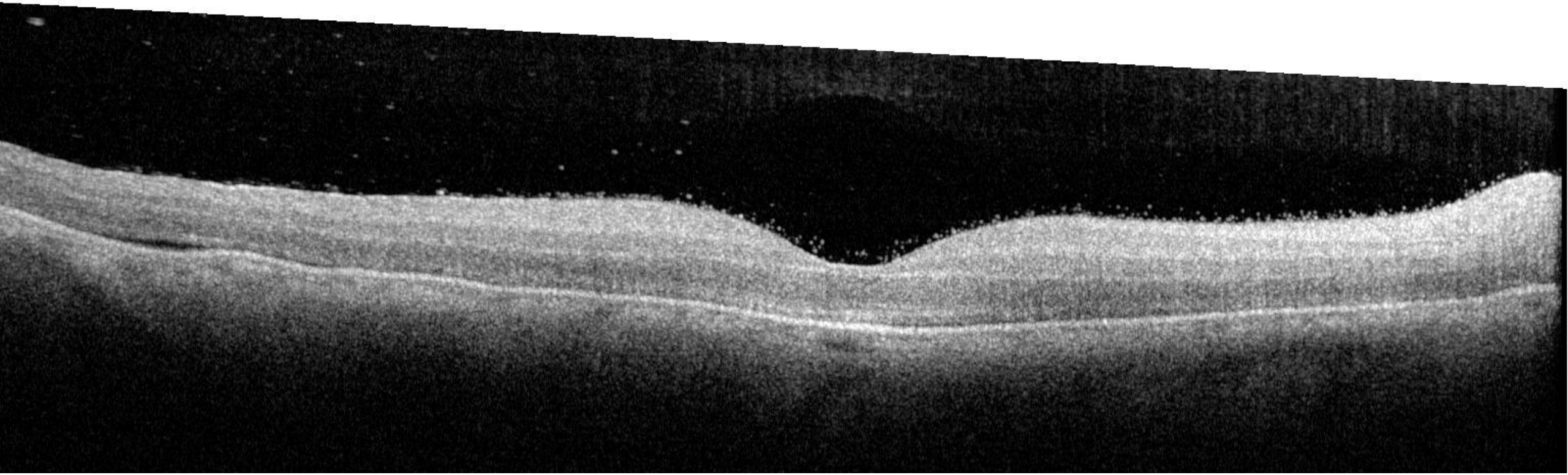
Also shown in Figure 2 of Messinger et al

# Capture mode for OCT

- Averaging rate = 50 frames
- Scan size = 30° x 25°
- # of sections = 241
- Distance between sections = 30 µm


Also shown in Supplementary Material 2 of Messinger et al

# Video showing OCT B-scans of a healthy right donor eye



Details in Figure 2 of Messinger et al