Supplementary Material 2 for Messinger, J. D., Brinkman, M., Kimble, J. A., Berlin, A., Freund, K. B., Grossman, G. H., Ach, T., Curcio, C. A. *Ex vivo multimodal imaging of human donor eyes for research in age-related macular degeneration*

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

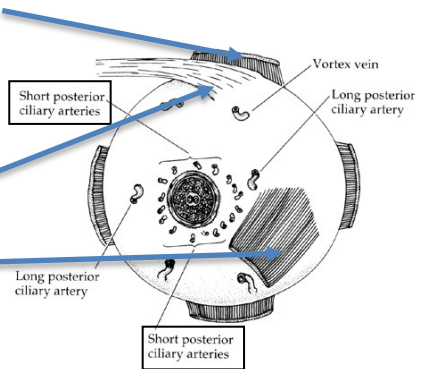Mark Superior Rectus muscle with tissue marking dye.

Turn on Laser (blue arrow).

Superior rectus m.

Superior oblique m.
Inferior oblique m.

Vortex vein
Short posterior ciliary arteries
Long posterior ciliary artery
Long posterior ciliary artery
Short posterior ciliary arteries

Right eye, viewed from behind

OCT head is oriented in position by moving the entire unit in the 2 axes with respect to the base (green arrow) then raising the height (y) of unit by rotating the joystick cw/ccw (blue arrows). Focusing the image is accomplished by rotating the knob (orange arrow). The black lever is in position R (*).
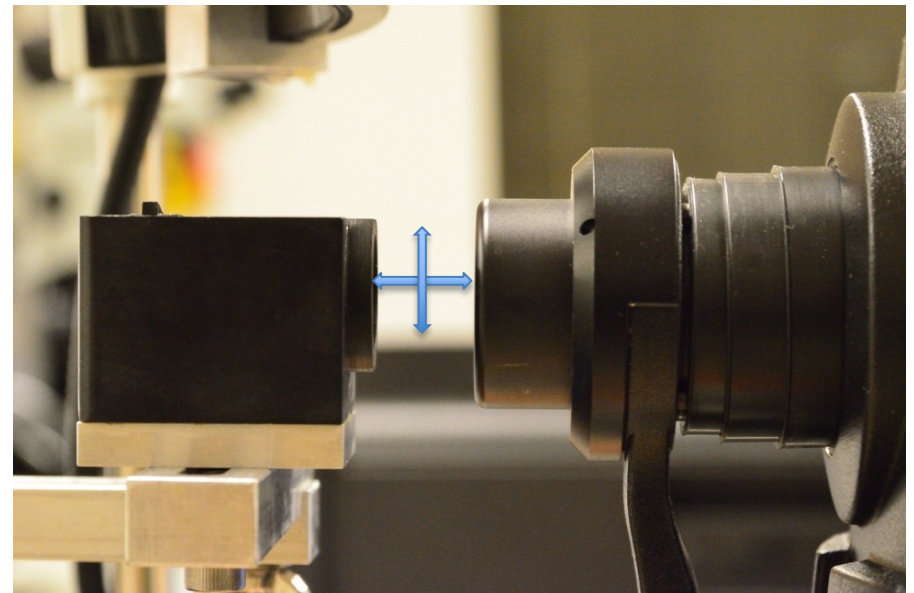
After the head is in position for fundus and OCT images, lock down the unit by securing the thumb screw (purple arrow).

Insert eye in holder, shimmed from the posterior, oriented with tissue mark facing up.
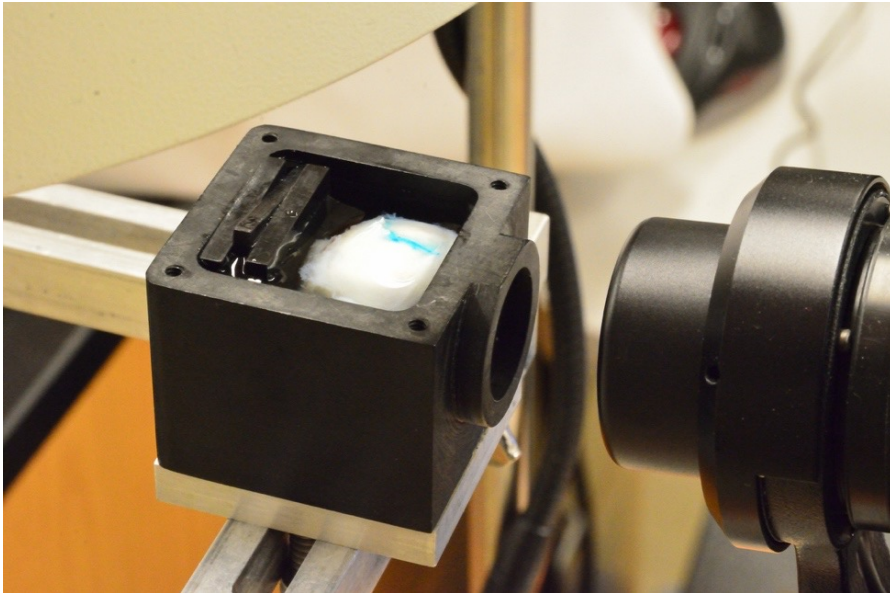
Approximate distance from front of holder is ~25 mm.

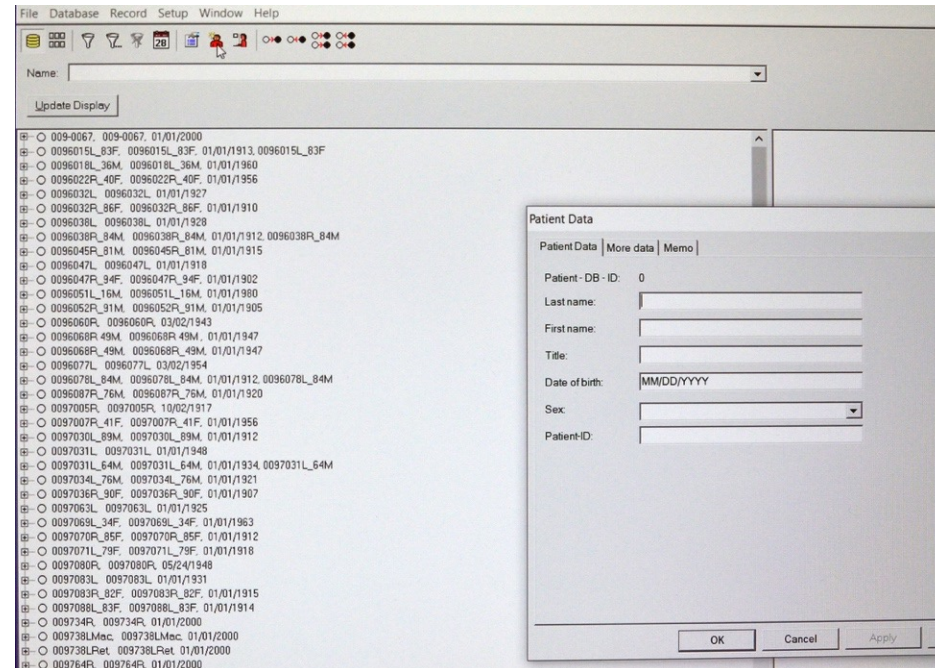For information about the eye holder contact jeffreymessinger@uabmc.edu
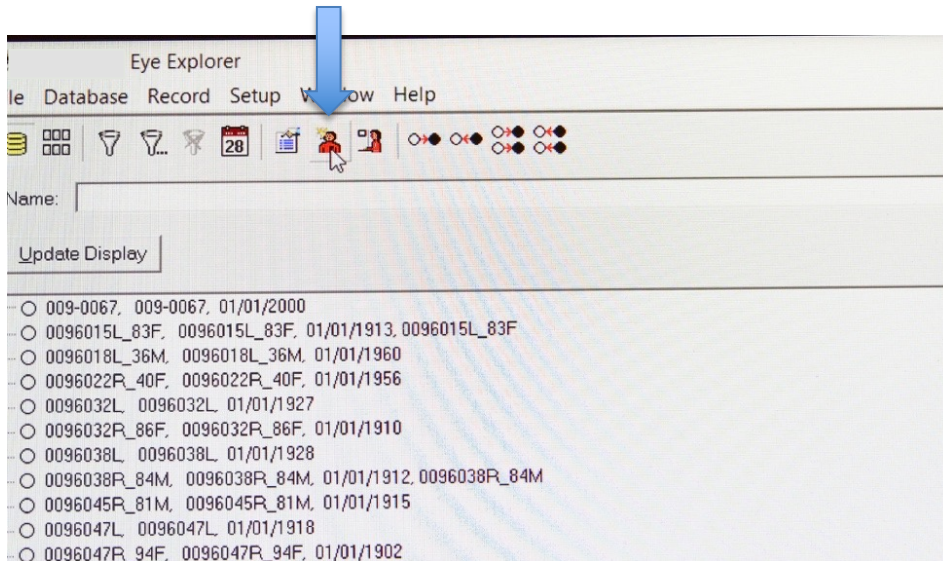
Select ICON Program on desktop.

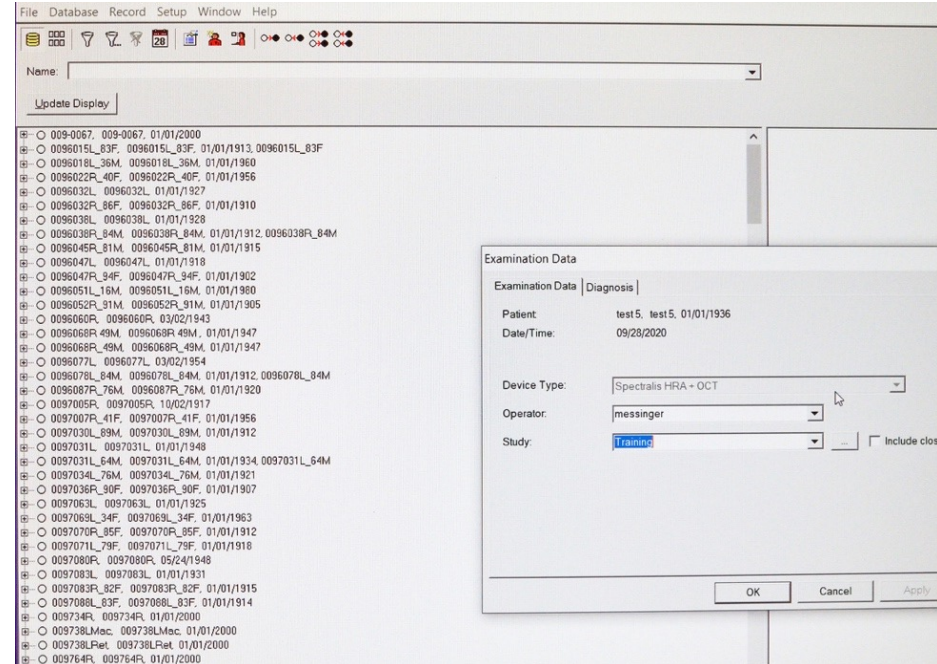**A patient list will appear on left column.**
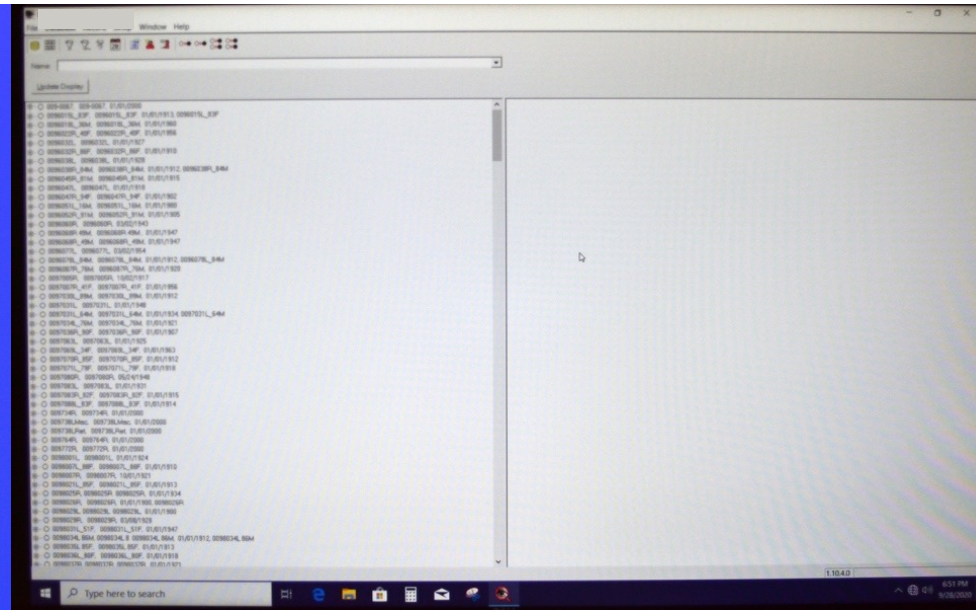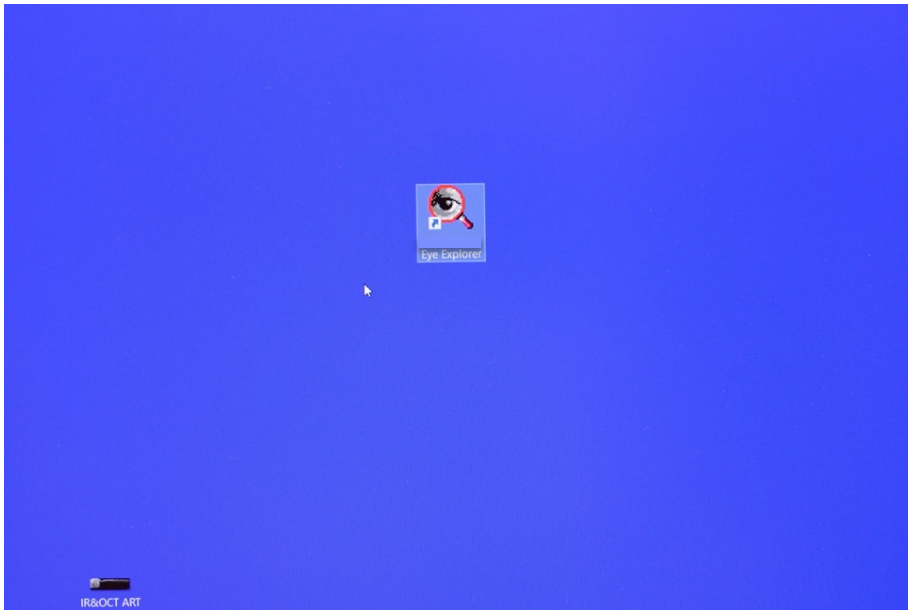
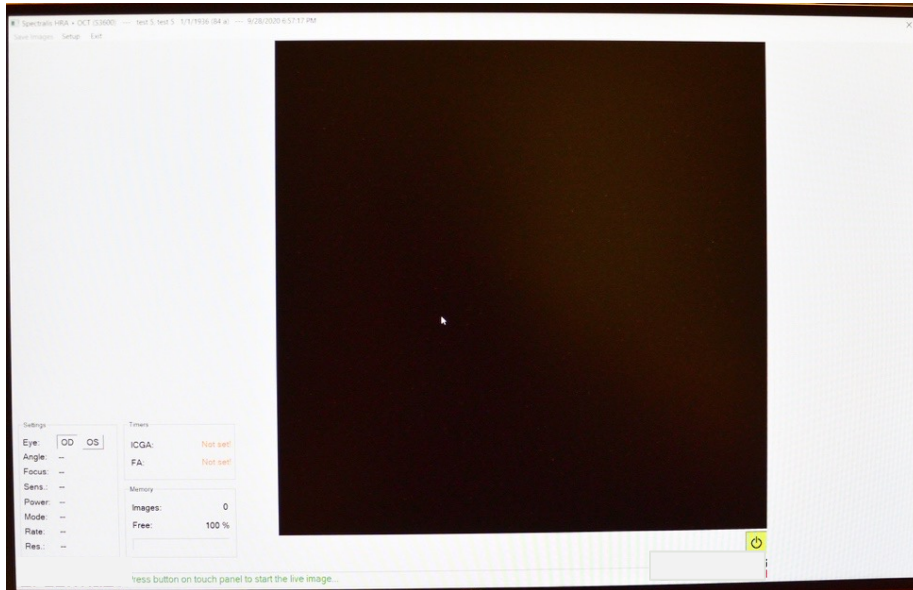**Select the 'New Patient' Icon.**   **Complete Donor data info as needed. Select OK.**

Our numbering system for individual eyes is: YYYYNNNL,R_agesex

For example, 2022027L_79M

Data entry is continued on the following window. Press OK

Select operator and study from drop down menu. This information appears in the meta-data.
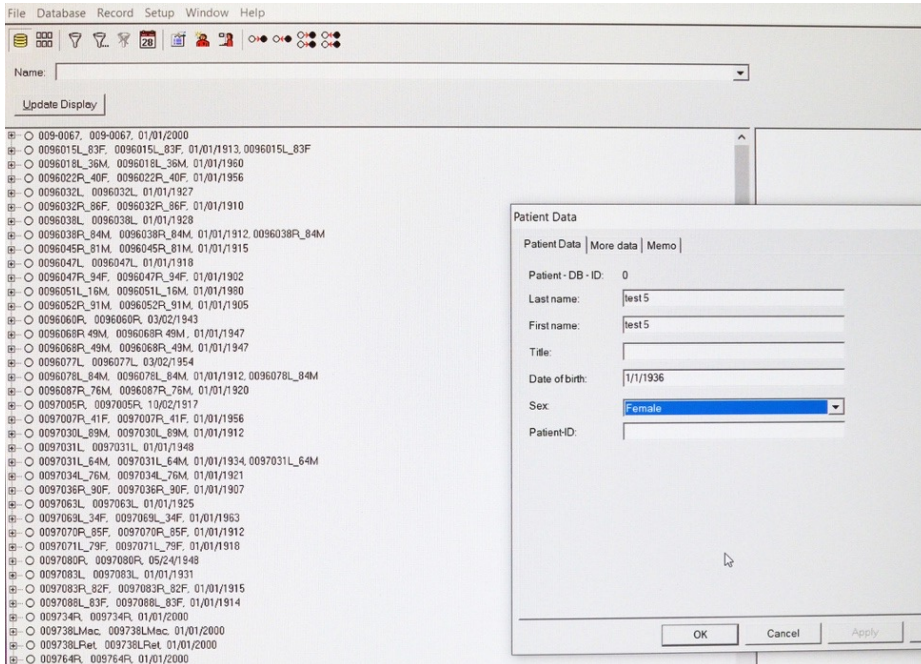
The following blank screen will appear.

Touch yellow button to start camera.

Press section and press IR +OCT.

Other default settings are blue. Allow laser to acquire fundus image and OCT image. Rotate black round button to adjust intensity and press the same button to average image 9-100 frames (red arrows).

If unit is oriented correctly from slides 4-5, the fundus image should be in focus and the OCT image is in the top 1/3rd of the display (double blue arrow). On the left image, using the cursor to move the blue line and center the fovea (white arrow). Other default buttons are Retina, EDI (extended depth imaging, off), line scan.

Press ACQUIRE.
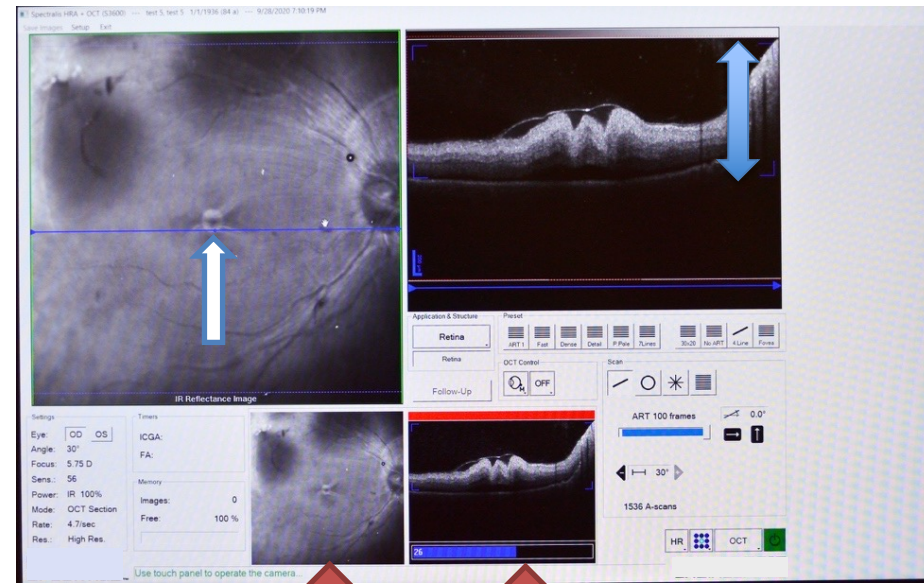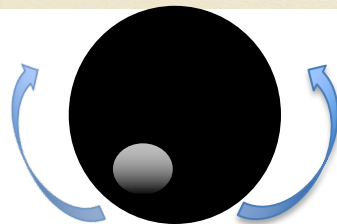
Press RF + OCT for the next acquisition.

Re-check position so image has not moved or degraded. Press black button for averaging.

Press ACQUIRE.
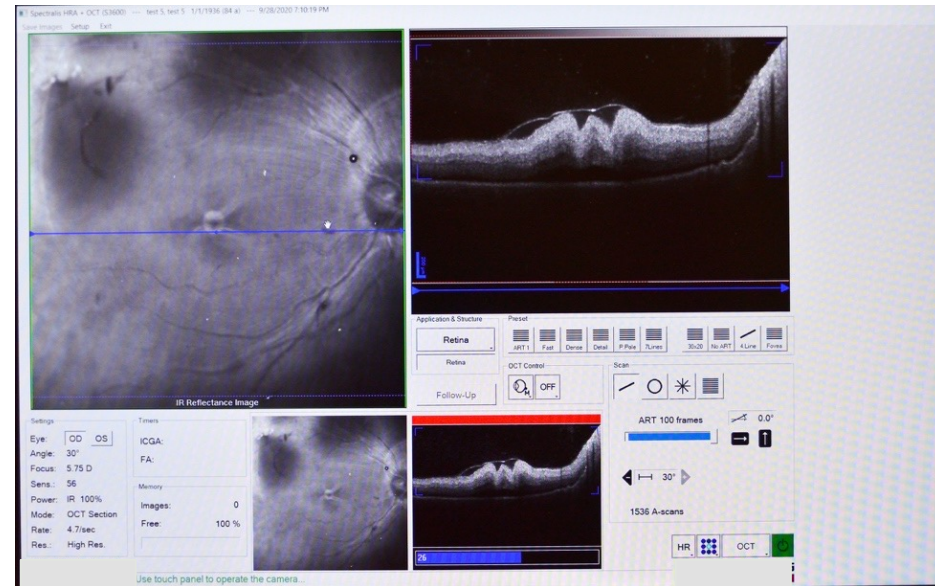
Switch the internal cube to A for autofluorescence imaging 488 nm and 787 nm.

Position after the switch
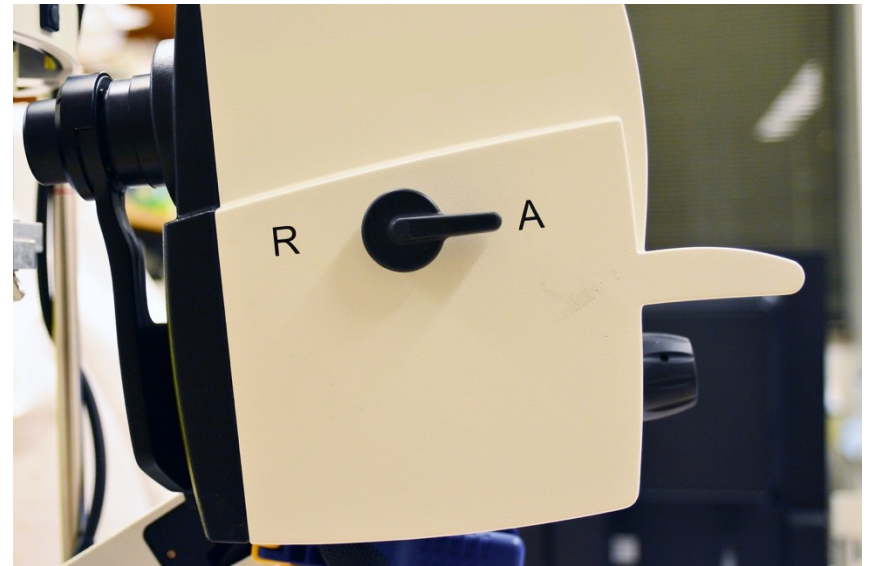
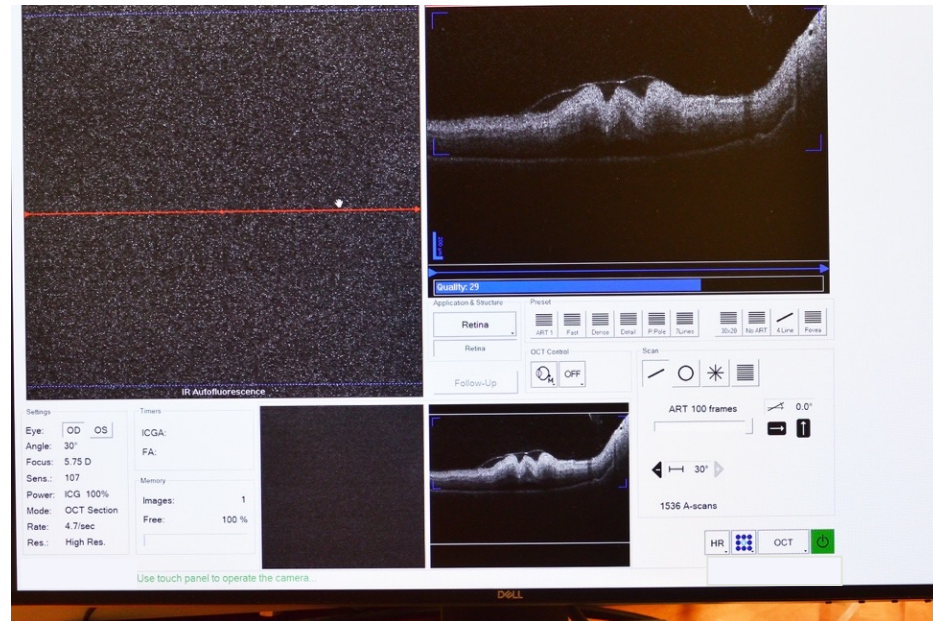**Display will also change select the autofluorescence mode.**
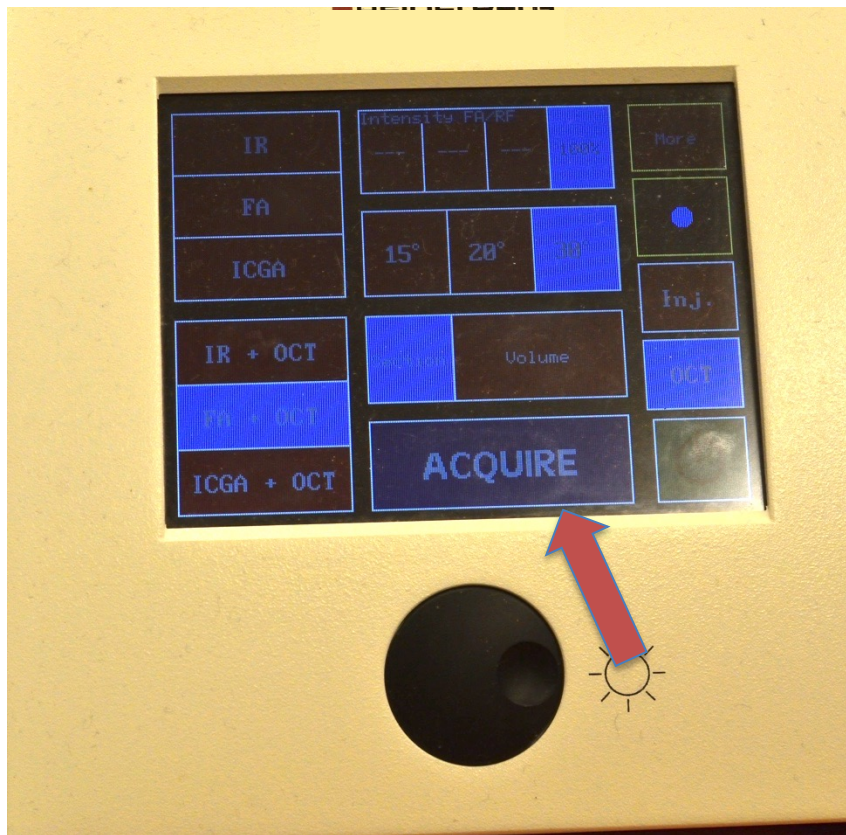
Recheck alignment, press ACQUIRE.
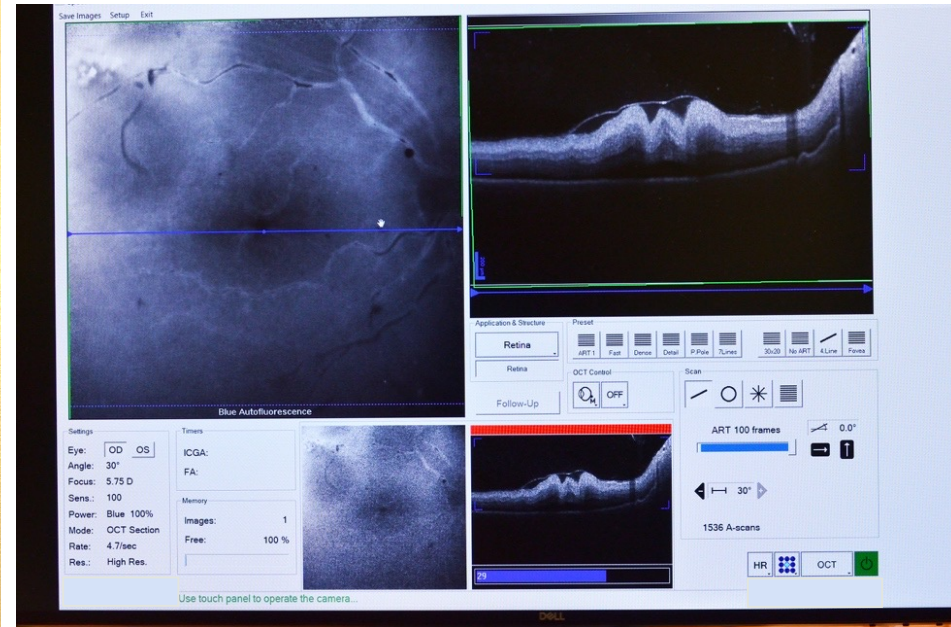
Select ICGA (indocyanine green)

Generally, the fundus image will not appear due to the internal cube attenuating the beam. Recheck position, press ACQUIRE. This modality is excellent for cases of RPE disruption, especially atrophy.

Switch the internal cube back to "R" for IR and Red free imaging for volume acquisition.

Position after the switch

Protocol to acquire an OCT volume

Select IR and the volume setting.

Match all the settings on this page by toggling all the appropriate buttons.
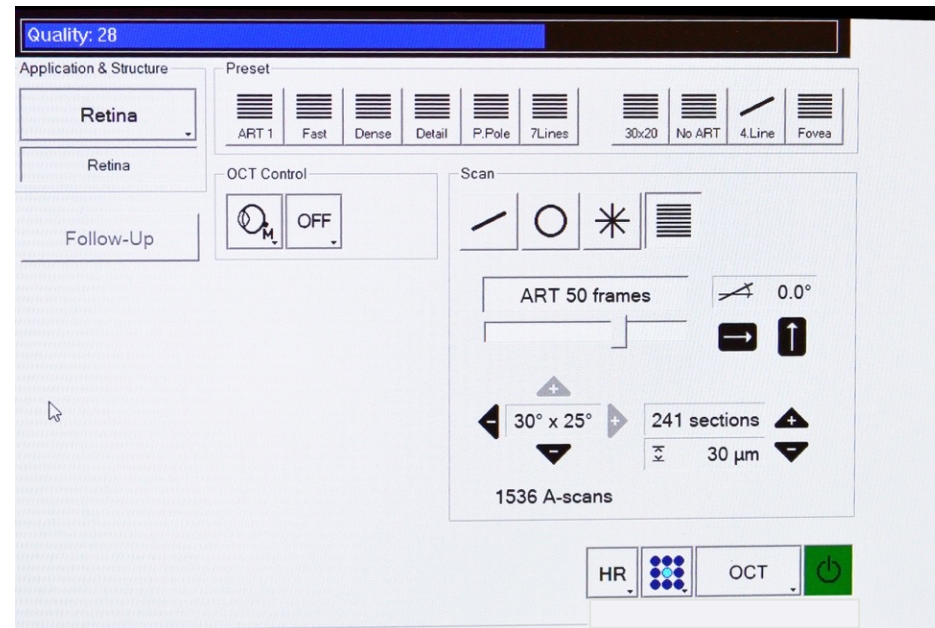
Near infrared reflectance fundus view is now covered in blue B-scan lines. Recheck that OCT B-scan position is in the upper third of the right window. Press ACQUIRE on the Control Module. The scan takes 5 minutes. Blue lines will delimit the distances in μm, shown in previous view.

Scan will start from bottom and proceed upward. Note red line progression. When completed, select EXIT Images will be saved (red arrow).
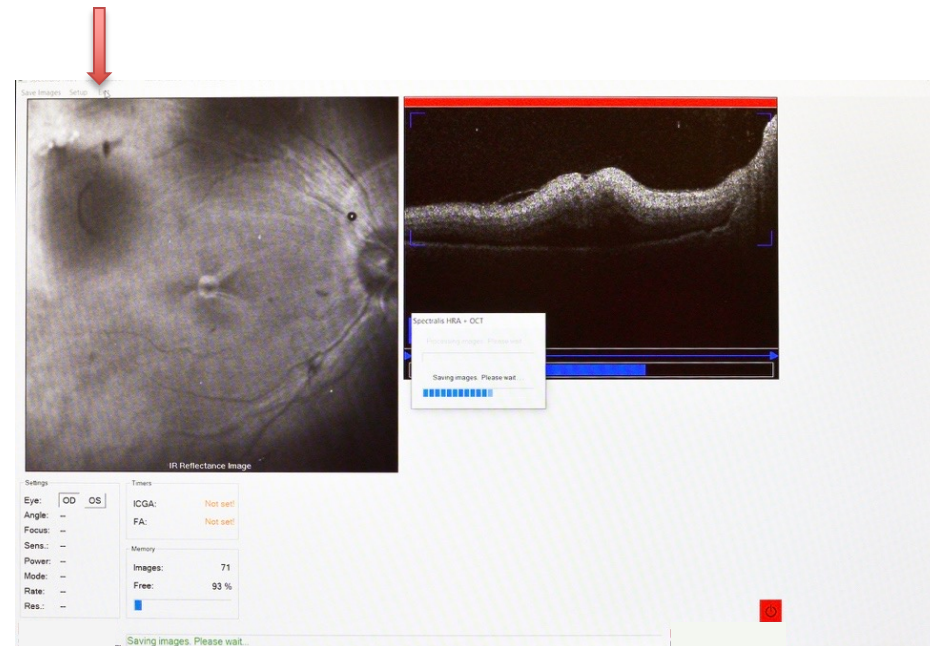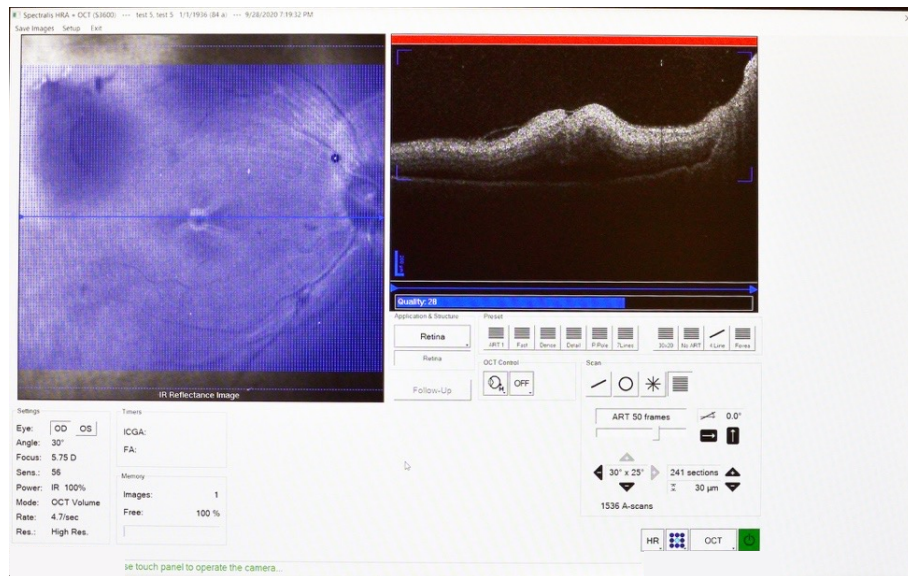
This eye shows postmortem retinal detachment, a common artifact.

Allow computer to preprocess all images acquired.

Processed images will appear on this screen.
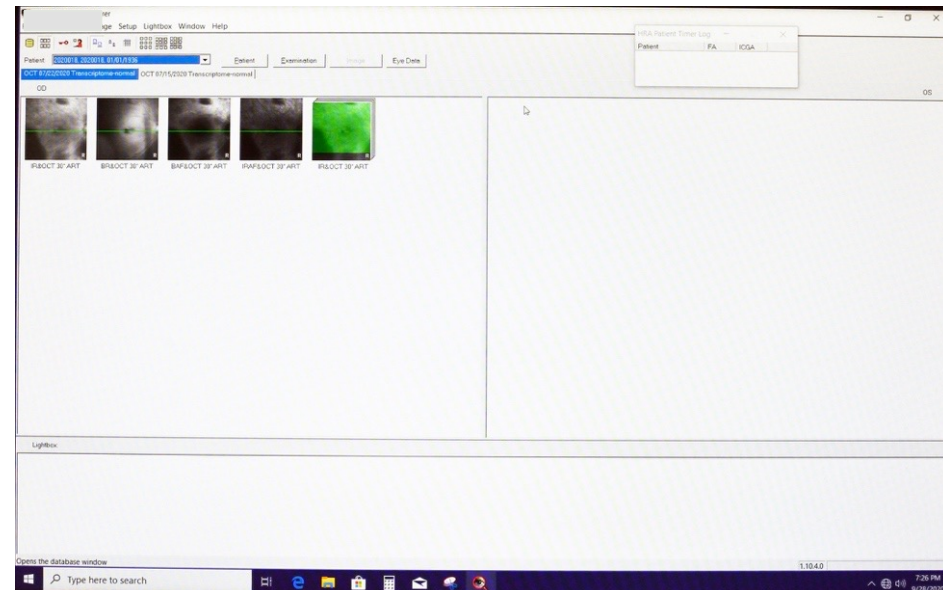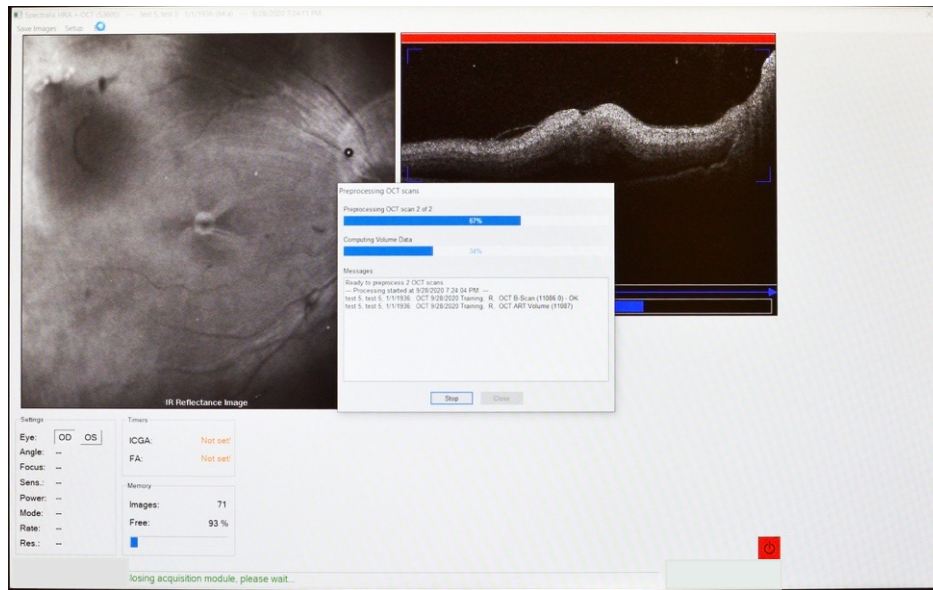
It is recommended to not change the position of the mounting bracket between imaging fellow eyes. If left eye is imaged first, the results will show in the OD Eye column --------->.

Right-Click, select all the images, then select 'Exchange OS/OD'. The images will shift to the OS column.
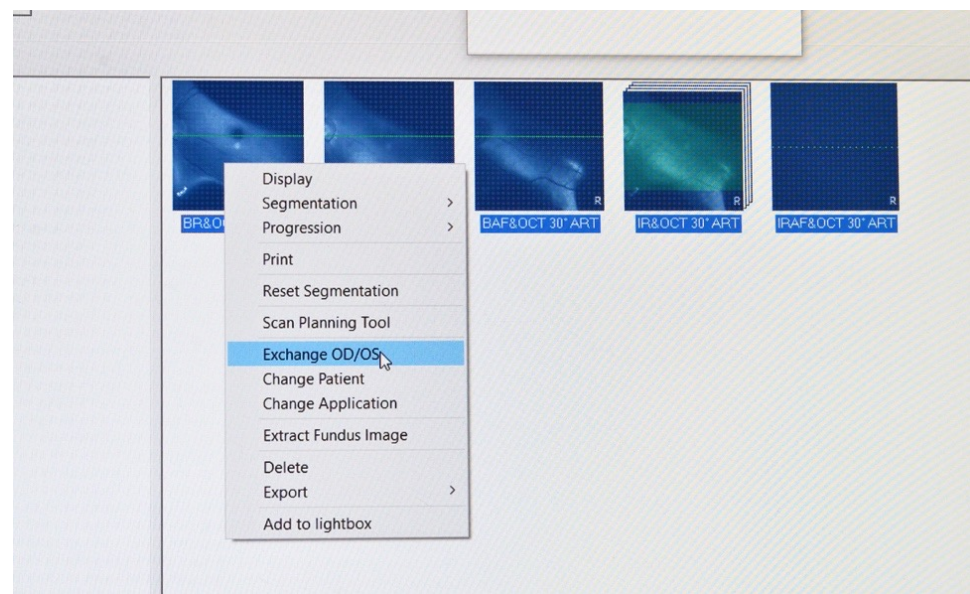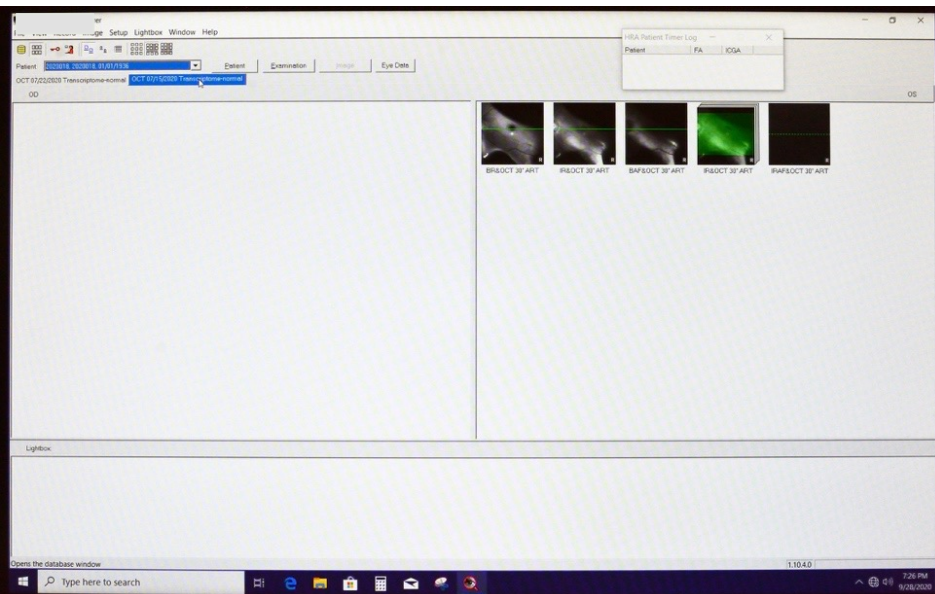
Select>Window>Database

Screen defaults to panel 6 with the addition of patient imaged in the right column. Right-Click on patient to the dropdown menu, choose bottom selection, and export .E2E file.
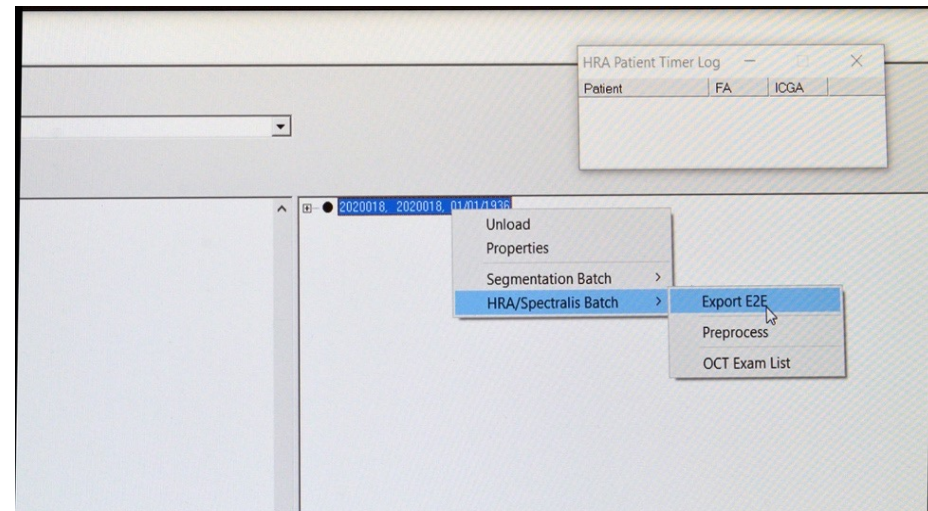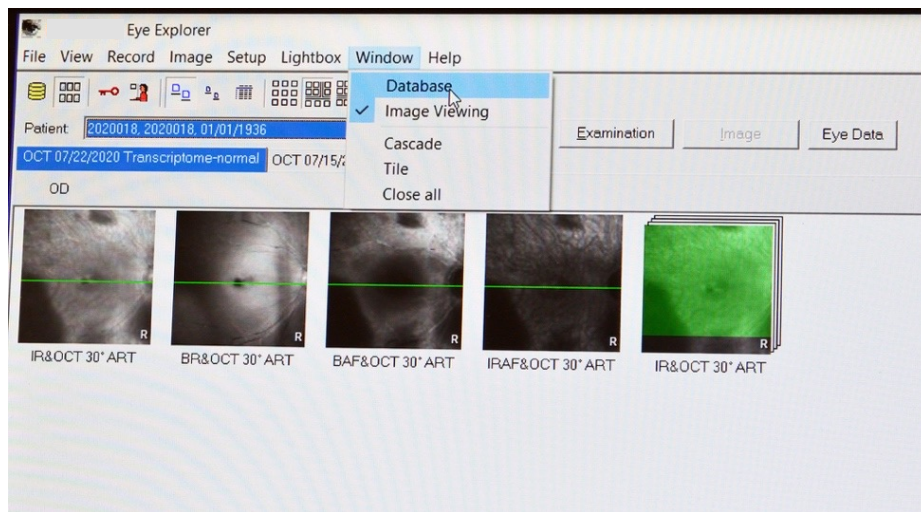
Double click on one of the icons to reveal the following formats DISPLAY MODE
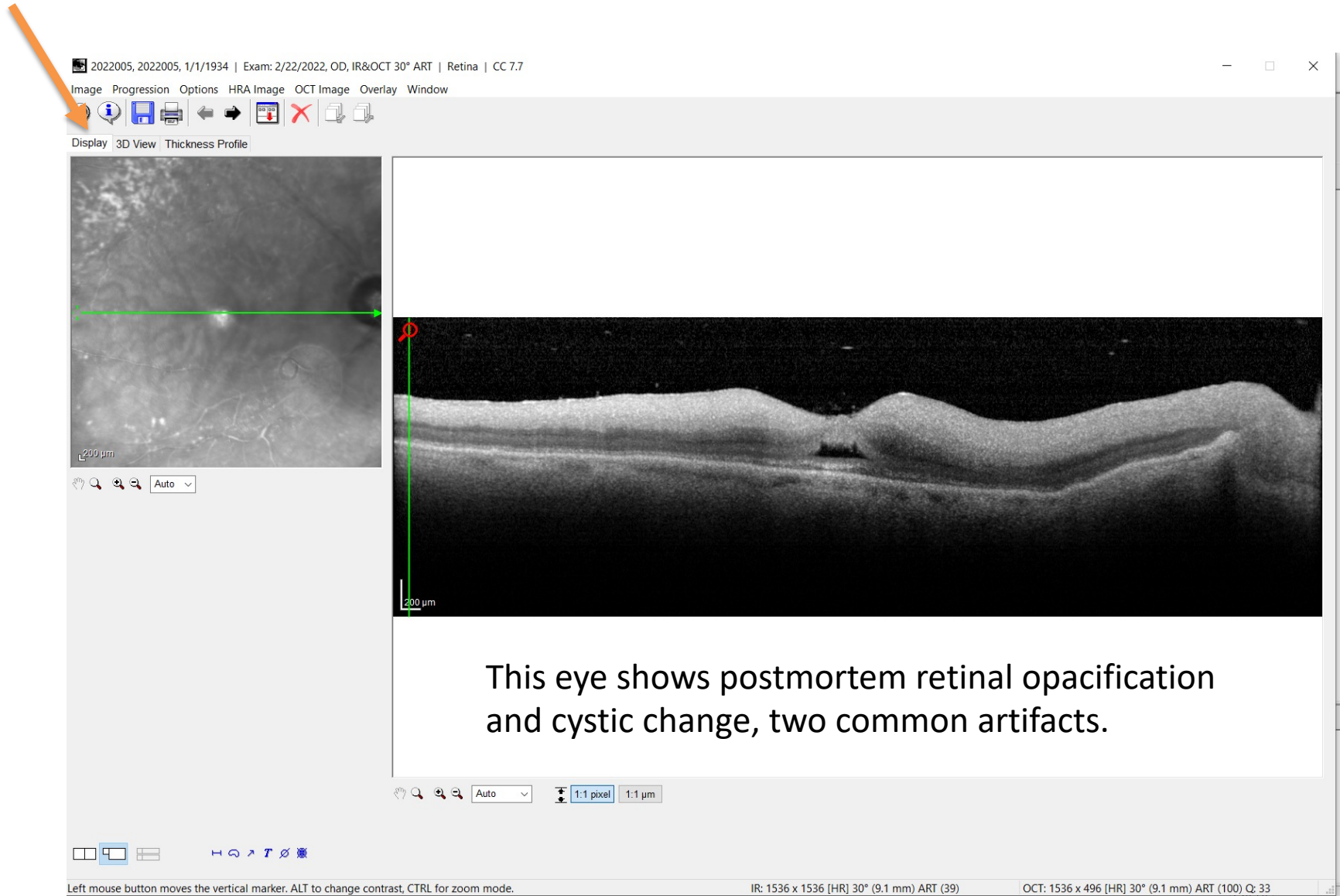
This eye shows postmortem retinal opacification and cystic change, two common artifacts.

Browse to choose a pre-determined folder created on the desktop for file transfers. Select OK

Folder contains .E2E files to be copied on external hard drive and archived.
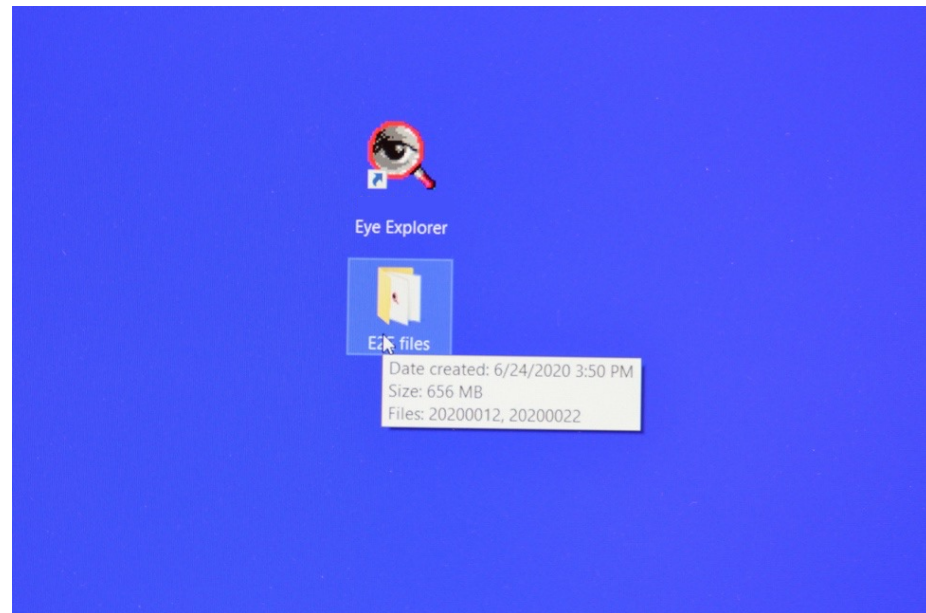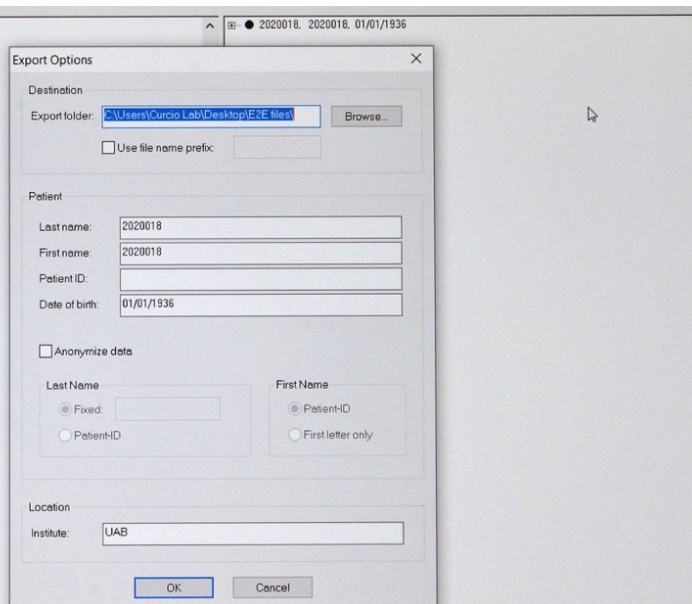
Part 2 Imaging with a scanning laser ophthalmoscope

488 nm quantitative autofluorescence, 786 nm autofluorescence, Multicolor (Mcolor)

Turn on computer and laser for OCT.

Select New Patient Icon   **Complete patient data as needed**
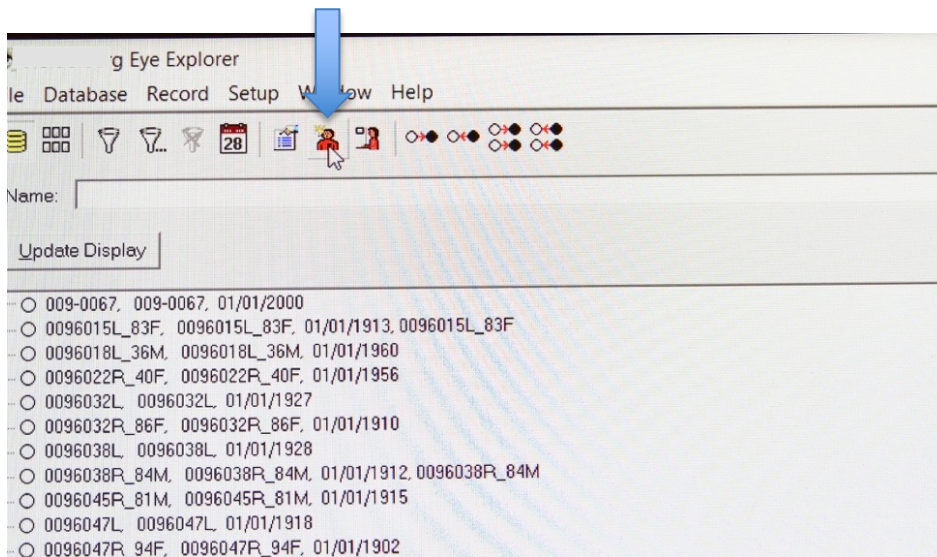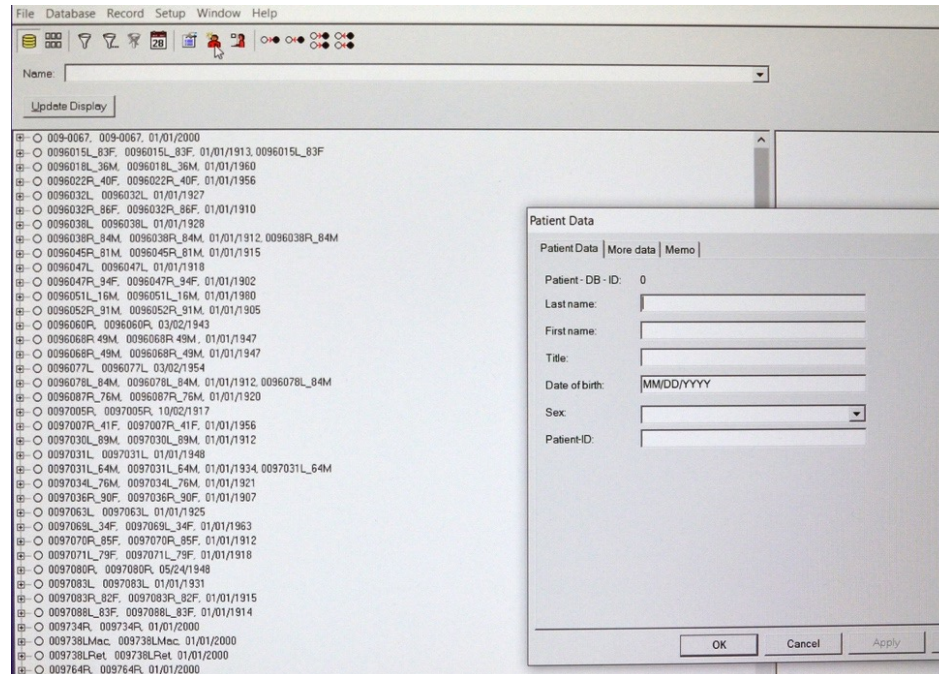
Keep eye data sheet the same press OK

C-Curve stays the same, press continue

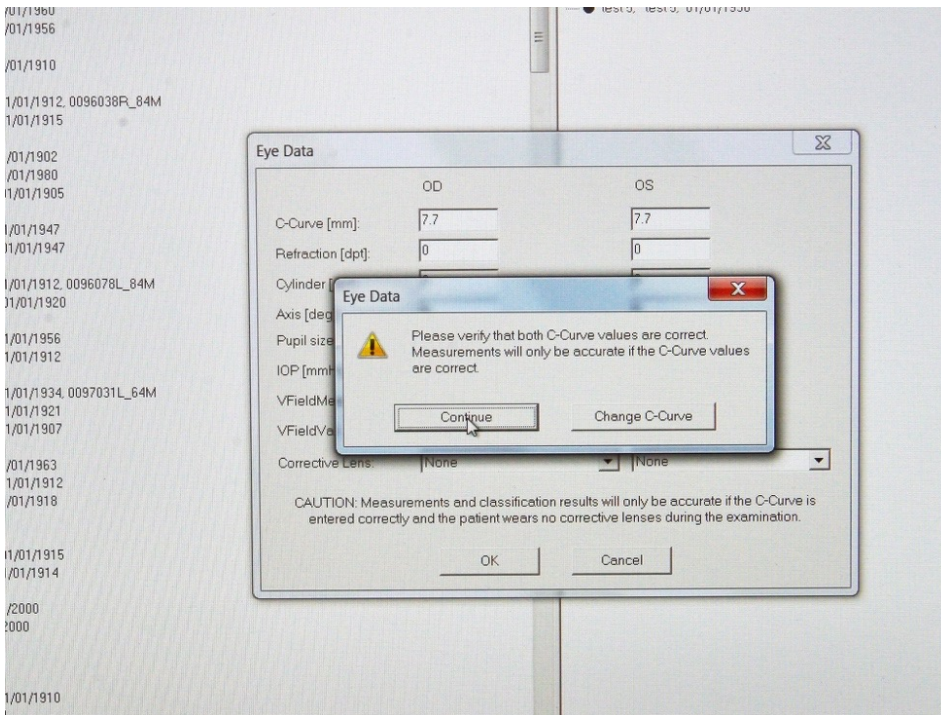**Select study mode and enter the password (if needed).**

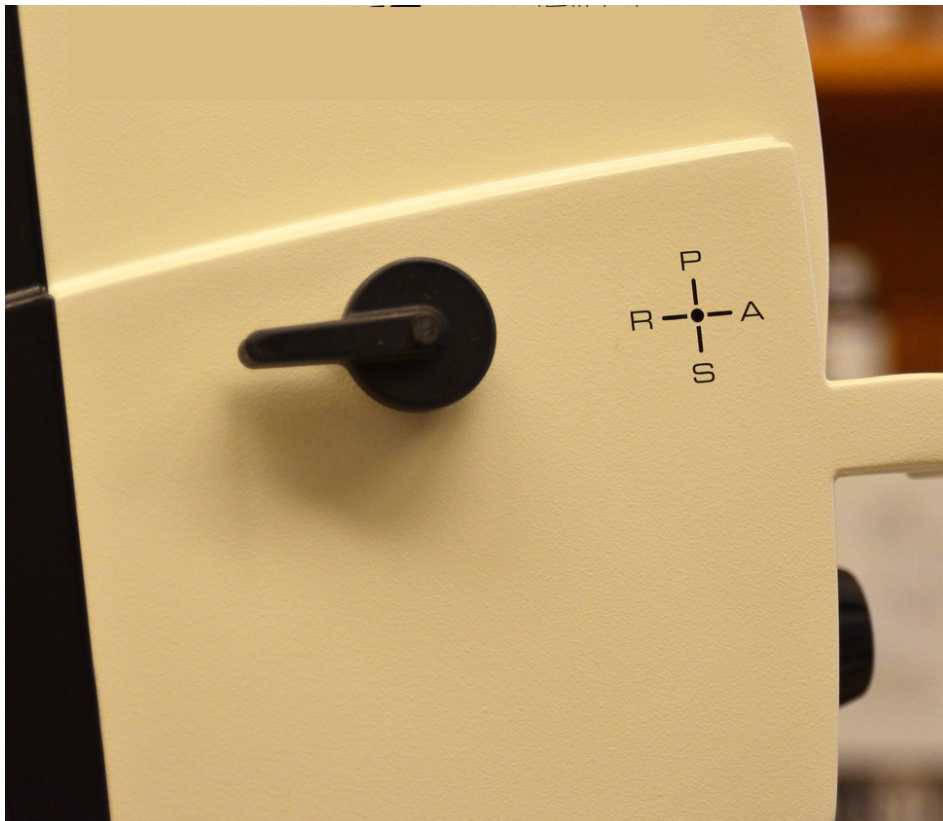**Keep the C-curve @7.7 mm. Press OK.**
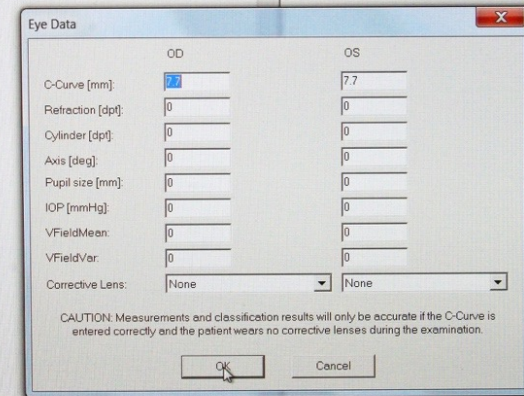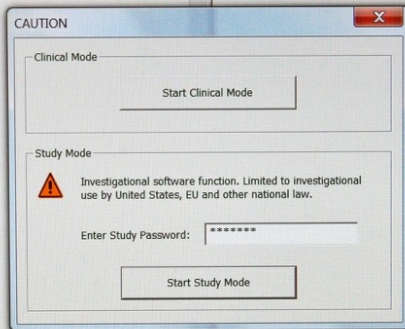
Select continue to verify the C-curve

Select yellow indicator to start camera.

Select to the R position to align and orient globe

Select the IR mode to focus and orient the sample first before imaging in the Multi-color and 786 nm modes.

Camera head is oriented in position by moving the entire unit in the 2 axes with respect to the base (green arrow) then raising the height (y) of unit (blue arrows). Focusing the image is accomplished by rotating the knob (orange arrow). The black lever is in position R (*). After fundus and OCT images are in position, lock down the unit by turning the thumb screw (purple arrow).
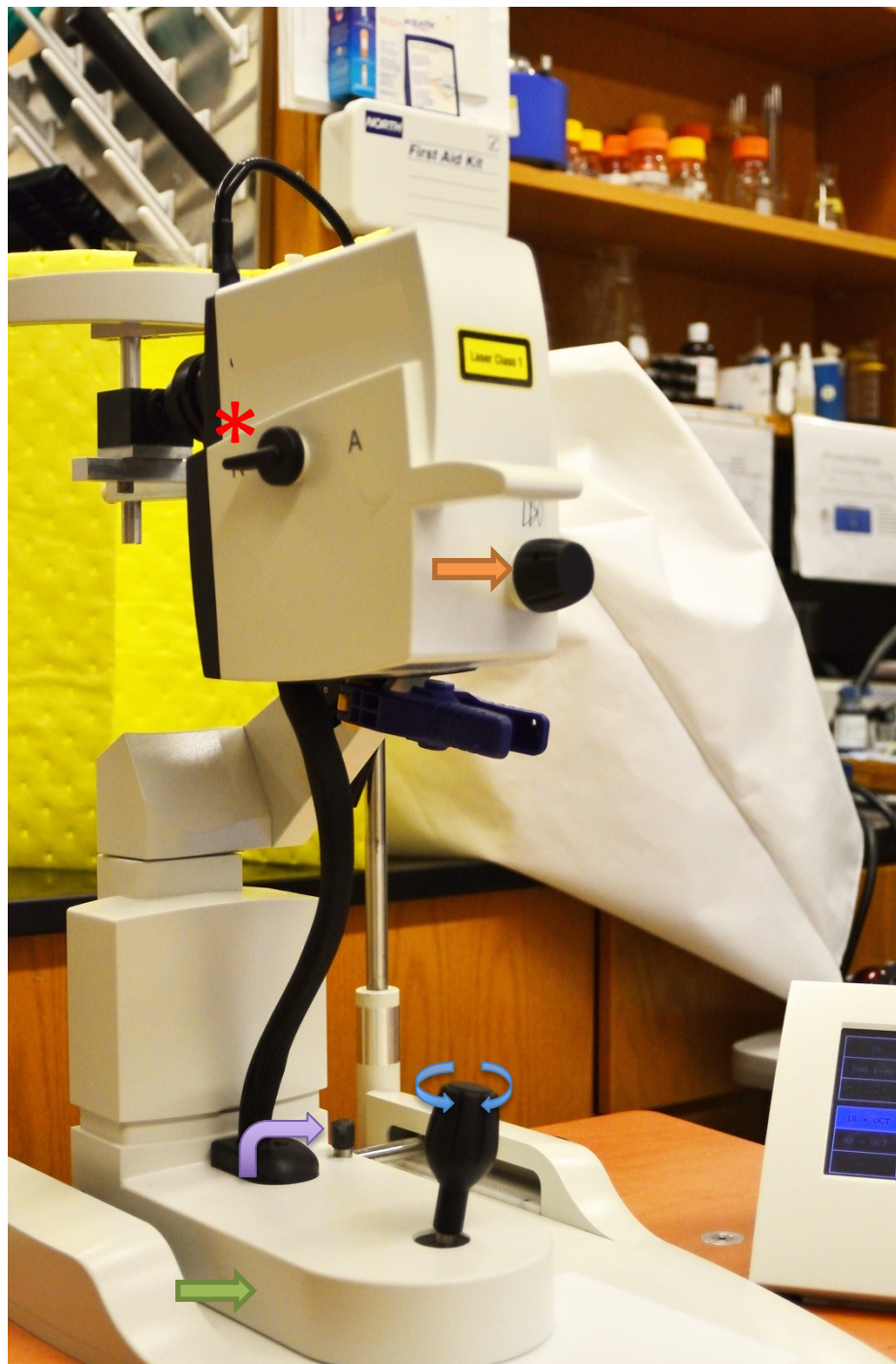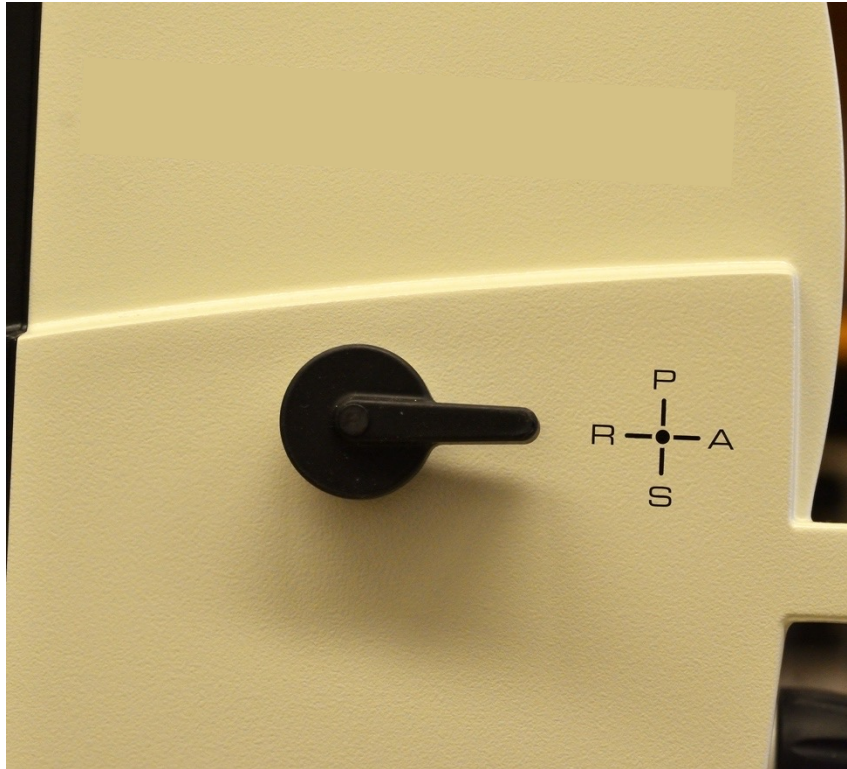
Image will appear as shown below.

Move selector knob from R to A

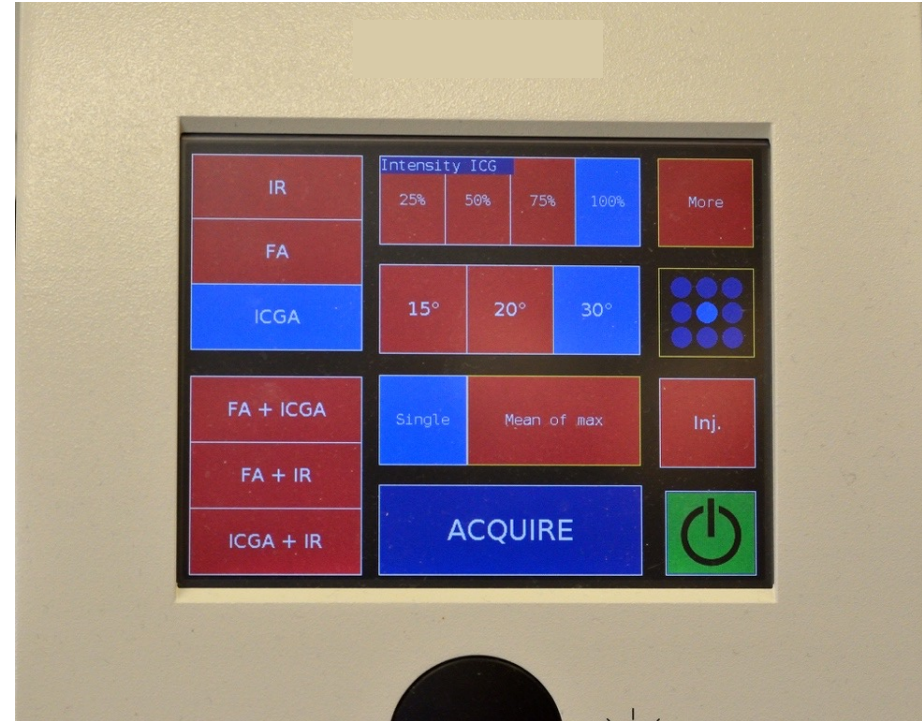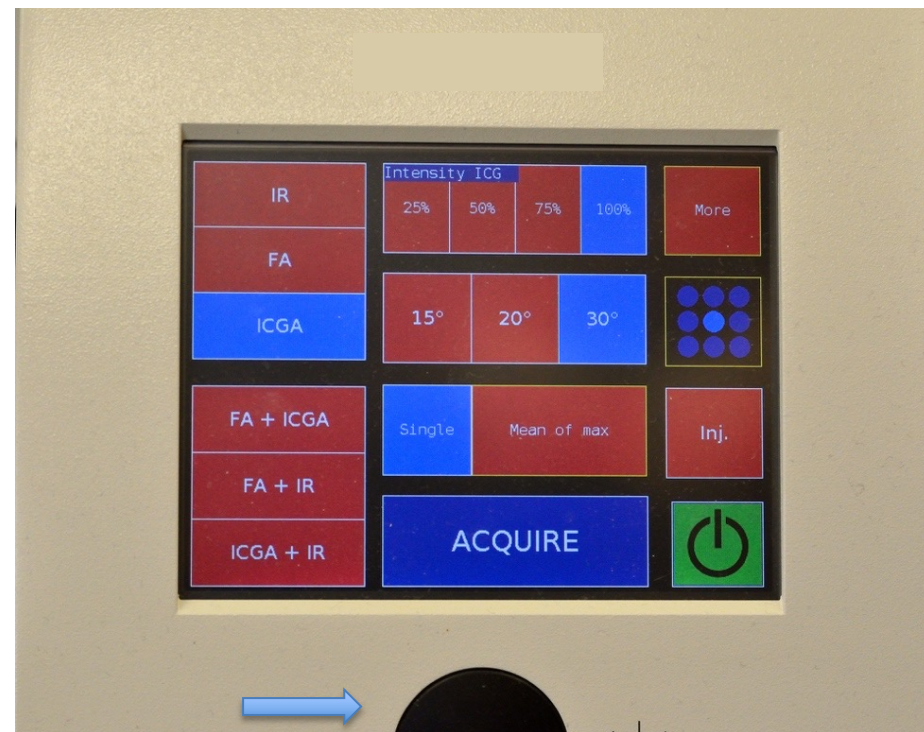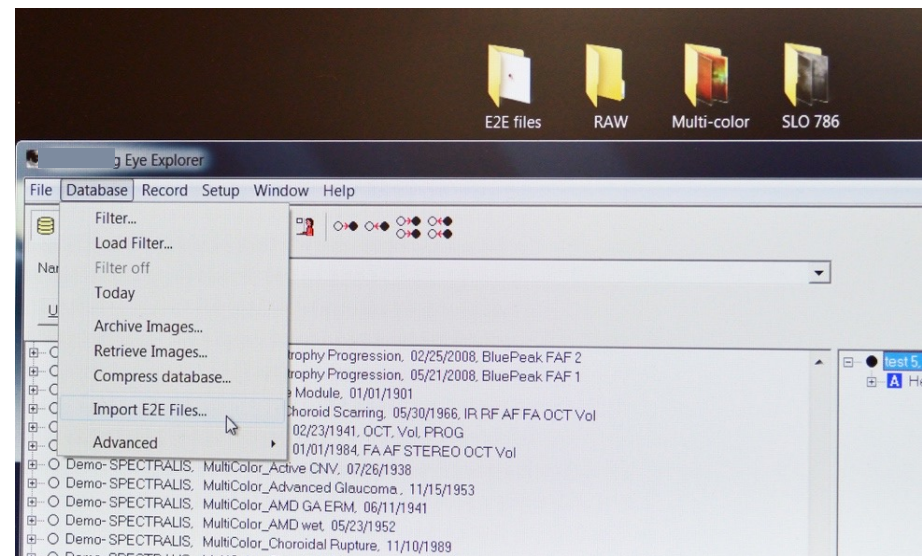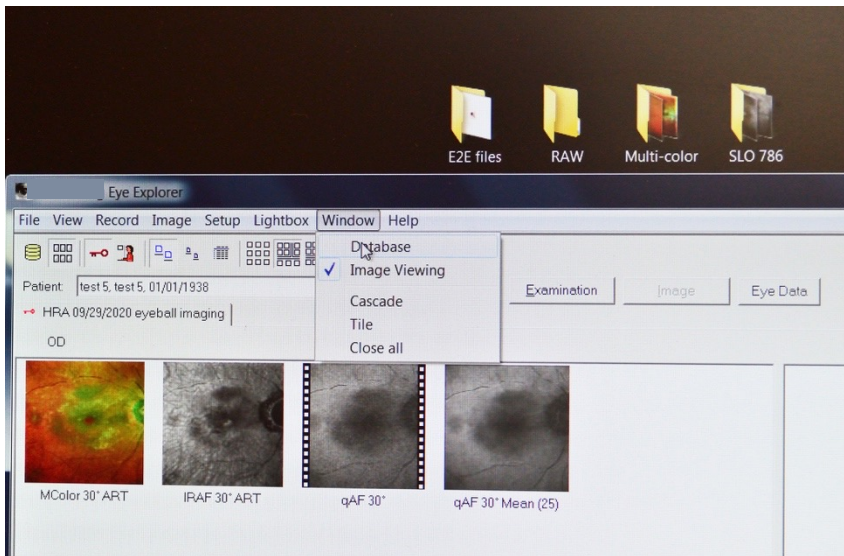**Select ICGA (fluoresces to 787 nm excitation) in blue, 100% intensity, 30° field of view, single phase imaging.**

Image will appear as below.

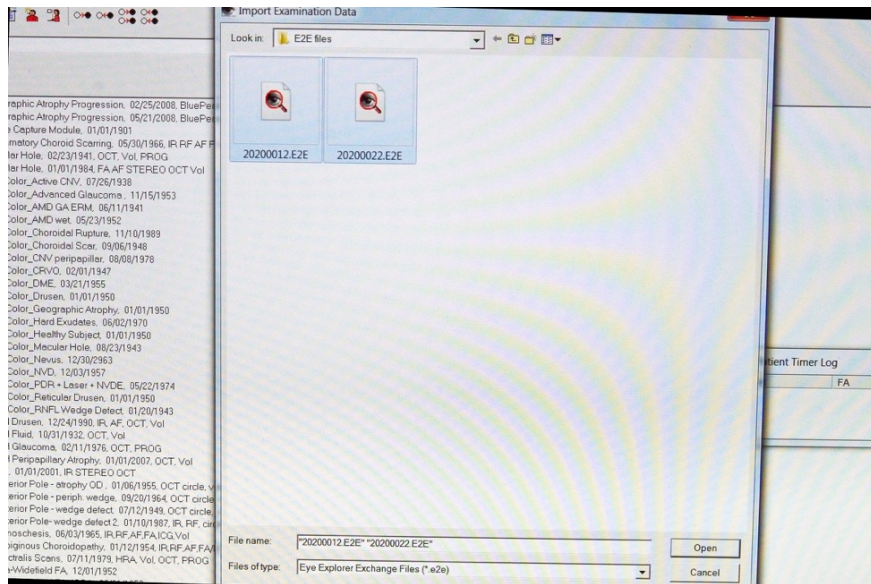**Press black disc for averaging then select ACQUIRE.**
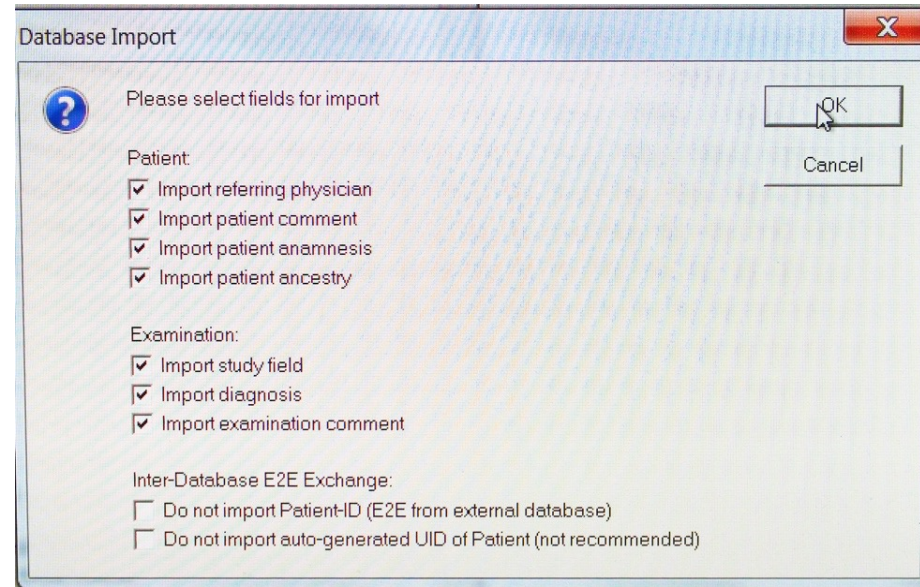
Choose Window > Database

Select import .E2E files from HRA stored on external hard drive uploaded to desktop.
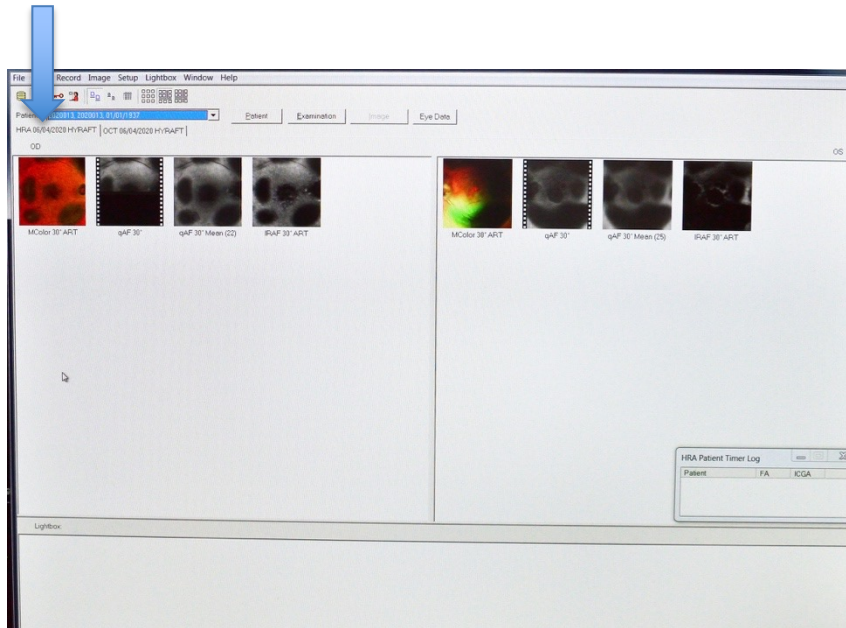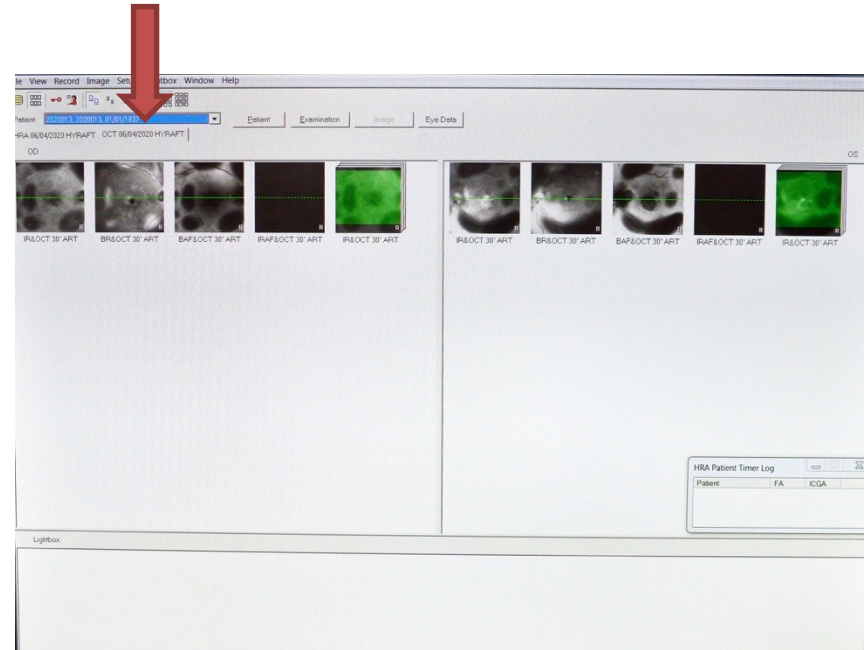
Select open

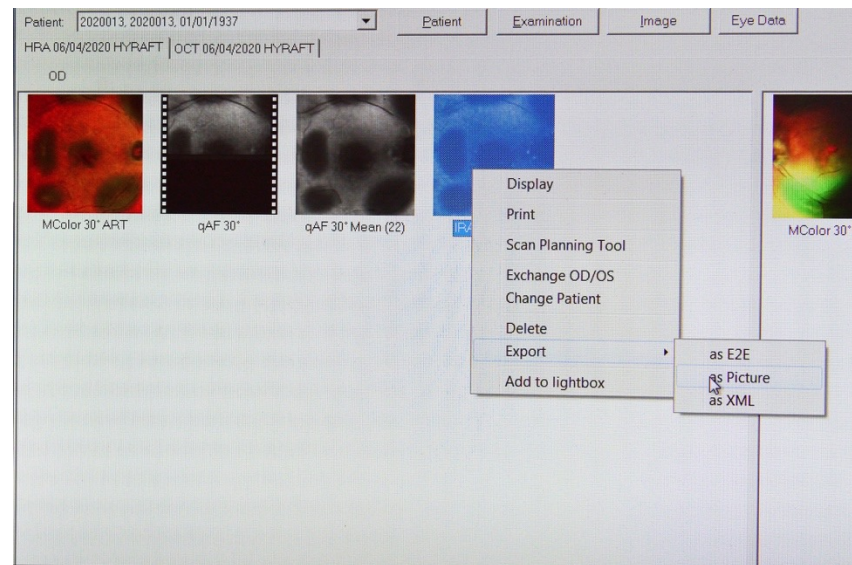**Check marks are the default, then select OK.**

The patient now has 2 tabs, one showing the images acquired from the HRA (blue arrow).

And the other tab shows the images acquired from the HRA+OCT (red arrow).
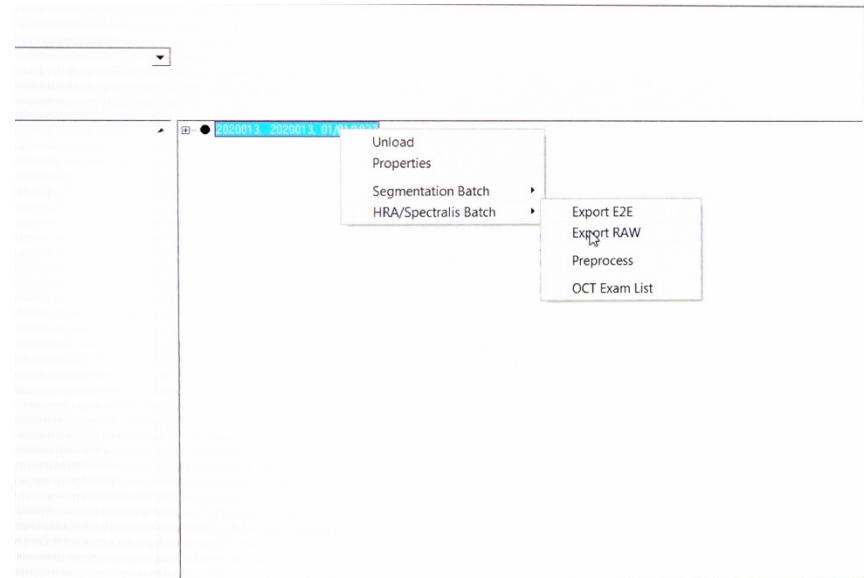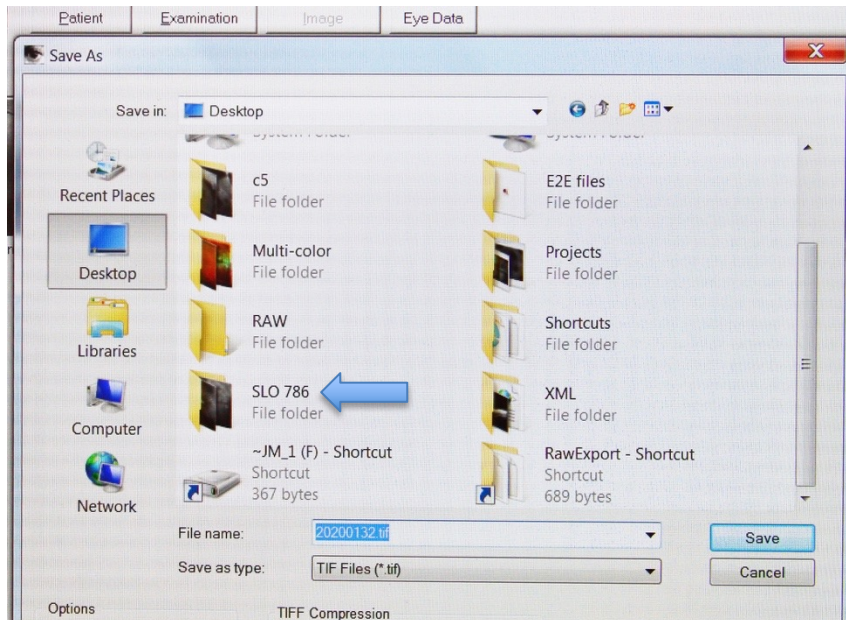
Right-Click 786 (ICGA) image and export picture to a file labeled SLO 786 on the desktop.

Example of saving to SLO 786

Select patient, Right-Click to export images as RAW to a folder on the desktop.

Images from HRA

Copy and paste from RAWEXPORT to a folder labeled RAW to be transferred to archive computer, for review.