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

ong posterio

ciliary artery

Mark Superior Rectus muscle with tissue marking dye.



Turn on Laser (blue arrow).



- Superior rectus m.
- Superior oblique m.

Right eye, viewed from behind

Short posteric

iliary arteries

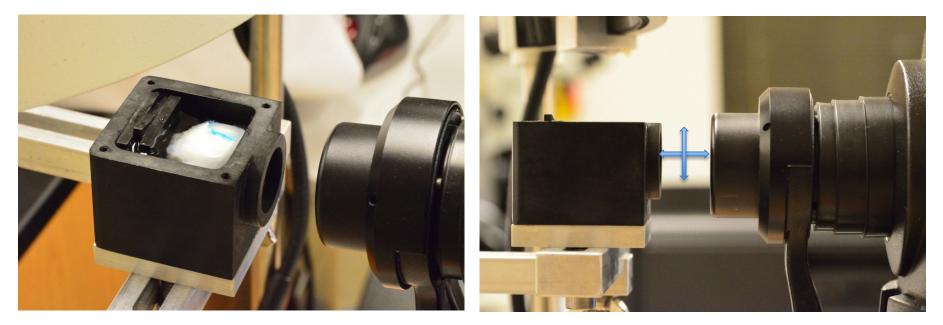
OCT head is oriented in position by moving the entire unit in the 2 axes with respect to the base (green arrow) then raising the height (y) of unit by rotating the joystick cw/ccw (blue arrows). Focusing the image is accomplished by rotating the knob (orange arrow). The black lever is in position R (\*).

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

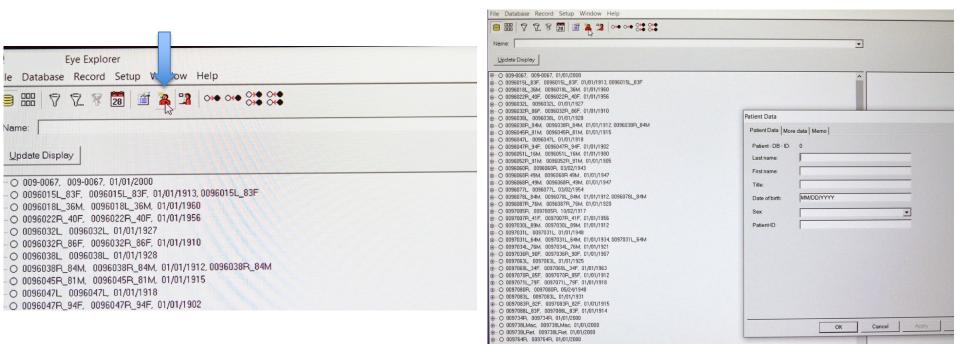
# Select ICON Program on desktop.

### A patient list will appear on left column.

	18 Window Help	- a x
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IR&OCT ART	💷 🔎 Type here to search 🛛 🗄 😑 💼 🏦 🔛 😭 🛃	

### 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.

File Database Record Setup Window Help		File Database Record Setup Window Help	
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Name:		Name:	·
Update Display		Update Display	
₩→         069-0057, 009-0057, 01/01/2000           ₩→         0095015L, 83F, 0095015L, 35A, 01/01/1913, 00950115L, 83F           ₩→         0095015L, 35A, 0095015L, 35A, 01/01/1913, 00950115L, 83F           ₩→         0095022F, 40F, 0095022F, 40F, 01/01/1950           ₩→         0095022F, 40F, 0095022F, 40F, 01/01/1950           ₩→         0095022F, 40F, 01/01/1927           ₩→         0095022F, 40F, 01/01/1927           ₩→         0095032F, 10/01/1927           ₩→         0095032F, 10/01/1927           ₩→         0095032F, 10/01/1927           ₩→         0095032F, 10/01/1928           ₩→         0095032F, 10/01/1928           ₩→         0095032F, 10/01/1928           ₩→         0095032F, 10/01/1928           ₩→         0095051L, 15M, 0095051L, 15M, 01/01/1917           ₩→         0095052F, 291M, 01/01/1917           ₩→         0095056F, 00950560F, 03/02/1934           ₩→         0095056F, 291M, 01/01/1917           ₩→         0095056F, 49M, 0095056F, 49M, 01/01/1947           ₩→         00950762L, 84M, 00950578L, 54M, 01/01/1947           ₩→         00957051L, 54M, 00957031L, 54M, 01/01/1947           ₩→         00957051L, 54M, 00957031L, 54M, 01/01/1947           ₩→         00957051L, 54M, 00977031L, 54M, 01/01/1947	Patient Data Patient Data Patient Data Patient Data Patient Data Patient Data More data   Memo   Patient DB - ID: 0 Last name: [vest5 First name: [vest5 First name: [vest5 First name: [vest5 Title: Date of birth: 1/1/1936 Sex Formale Patient-ID: QK Cancel Apply.	■         0.090087, 009-0057, 0101/2000           ●         0.0990115_03F, 0006015_03F, 0101/1913, 0006015_083F           ■         0.0990115_03F, 0006015_03F, 03F, 010/1913           ■         0.0990115_03F, 00060132, 010/1927           ■         0.0990115_03F, 0060032, 010/1927           ■         0.09900115_03F, 0060032, 010/1927           ■         0.0990032, 0060032, 010/1927           ■         0.0990032, 0060032, 010/1927           ■         0.0990032, 0060032, 010/1928           ■         0.0990032, 006033, 010/1928           ■         0.0990034, 0090034, 010/1928           ■         0.0990047, 0090474, 0090474, 010/1930           ■         0.0990051_016M, 00900571, 010/1931           ■         0.09900567, 010/1932           ■         0.09900571, 010/1932           ■         0.09900571, 010/1934           ■         0.09900571, 010/1947           ■         0.09900571, 010/1947           ■         0.09900571, 010/1947           ■         0.09900571, 010/1947           ■         0.09900571, 010/1947           ■         0.09900571, 010/1948           ■         0.0997051, 0097031, 010/1948           ■         0.0997051, 0097031, 00/19734           ■	Examination Data Examination Data Examination Data Diagnosis Patient test5, test5, 01/01/1936 DaterTime: 09/28/2020 Device Type: Spectralis HRA+ OCT Operator: messinger Study: Training

# The following blank screen will appear.

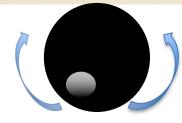


# Touch yellow button to start camera.

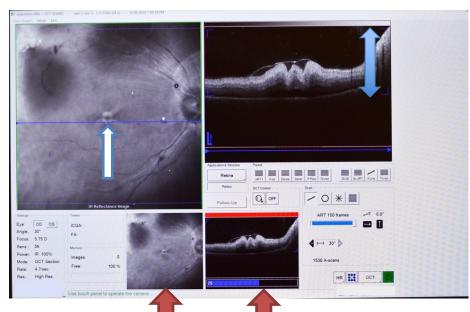


Press section and press IR +OCT. Other default settings are blue. Allow laser to acquire fundus image and OCT image. Rotate black round button to adjust intensity and press the same button to average image 9-100 frames (red arrows).





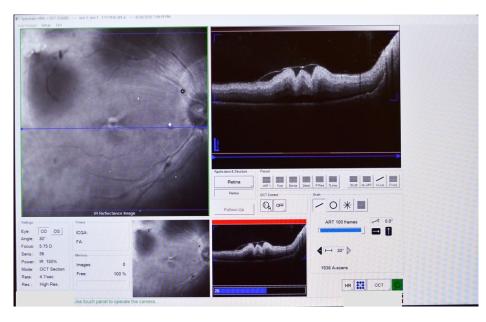
If unit is oriented correctly from slides 4-5, the fundus image should be in focus and the OCT image is in the top 1/3<sup>rd</sup> of the display (double blue arrow). On the left image, using the cursor to move the blue line and center the fovea (white arrow). Other default buttons are Retina, EDI (extended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

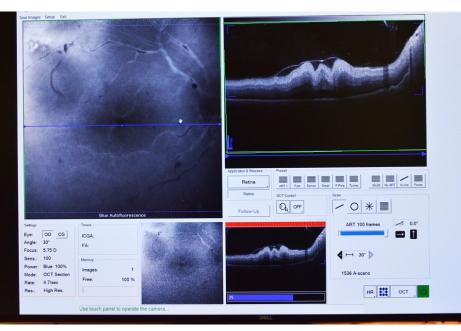
### Position after the switch



Display will also change select the autofluorescence mode.



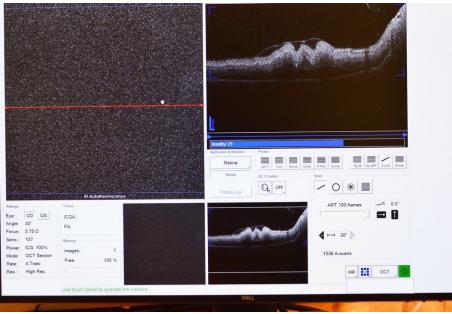
### Recheck alignment, press ACQUIRE.



### 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.

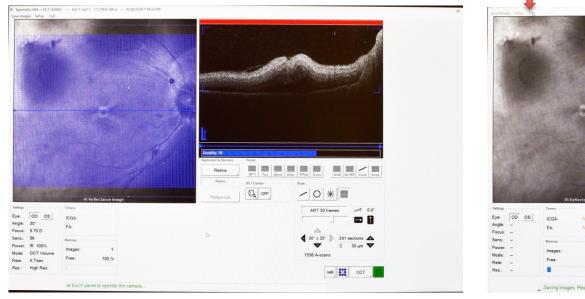


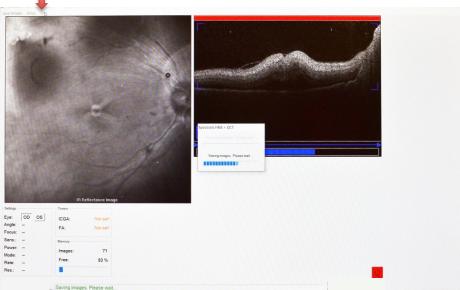
Match all the settings on this page by toggling all the appropriate buttons.

ART 1       Fast       Dense       Detail       P.Pole       7Lines       30x20       No ART       4.Line       Forea         Retina       OCT Control       Scan       Image: Control in the second sec	plication & Structure	Preset	
Follow-Up			
$ART 50 \text{ frames} \rightarrow 0.0^{\circ}$ $ART 50 \text{ frames} \rightarrow 0.0^{\circ}$ $\Rightarrow 0.0^{\circ}$	Retina	OCT Control	Scan
$4 30^{\circ} \times 25^{\circ} 241 \text{ sections} $ $\boxed{241 \text{ sections}} $ $\boxed{2} 30 \ \mu\text{m} $	Follow-Up	OFF OFF	_ 0 * ■
$\begin{array}{c} & & \\ \hline & \\ \hline & \\ \hline & \\ \hline \end{array} \end{array} \begin{array}{c} & \\ \hline \\ \hline$			ART 50 frames - 0.0°
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1536 A-scans			1536 A-scans
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Near infrared reflectance fundus view is now covered in blue Bscan lines. Recheck that OCT Bscan 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).

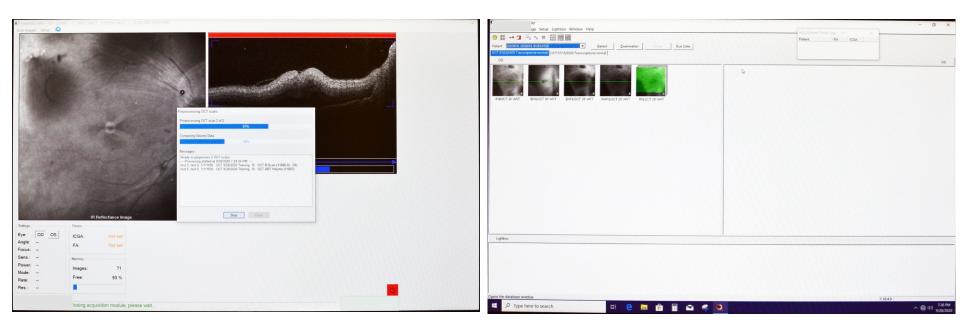




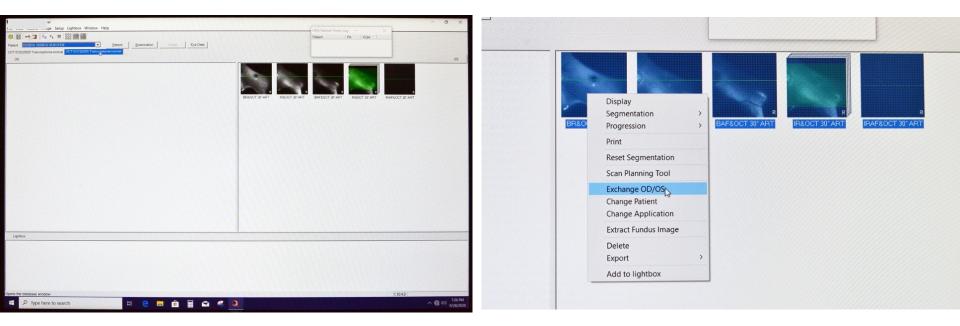
This eye shows postmortem retinal detachment, a common artifact.

# 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.

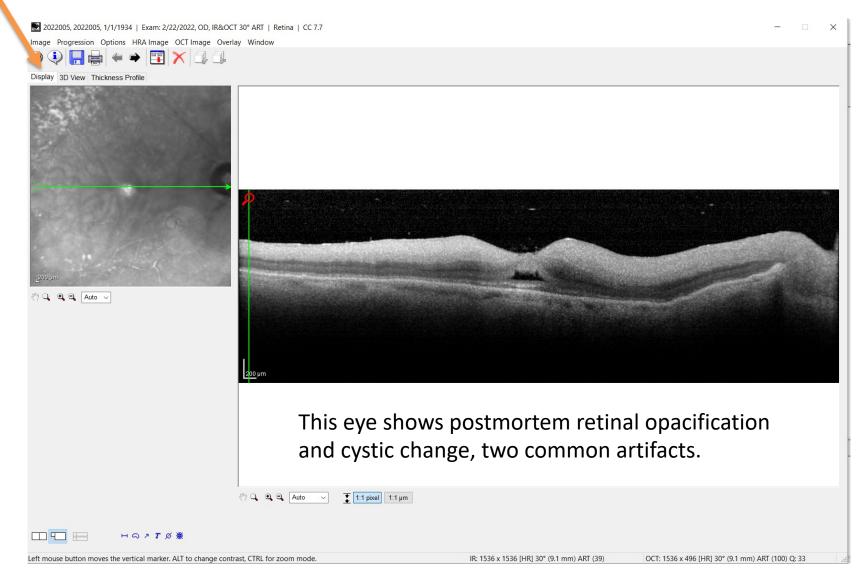


### Select>Window>Database

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



Browse to choose a predetermined folder created on the desktop for file transfers. Select OK Folder contains .E2E files to be copied on external hard drive and archived.

	^	● 2020018, 2020018, 01/01/1936		
	Export Options Destination Export folder. Use file name prefix Patient Last name: 2020018	×	¢	Eye Explorer
	First name: 2020018 Patient ID: Date of birth: 01/01/1936 Anonymize data Last Name	First Name		E2] files Date created: 6/24/2020 3:50 PM Size: 656 MB
	Fixed:     Patient-ID  Location Institute: UAB	PatientHD     First letter only		Files: 20200012, 20200022
IS 72M, 01/01/1941 1941	ОК	Cancel		

# Part 2 Imaging with a scanning laser ophthalmoscope

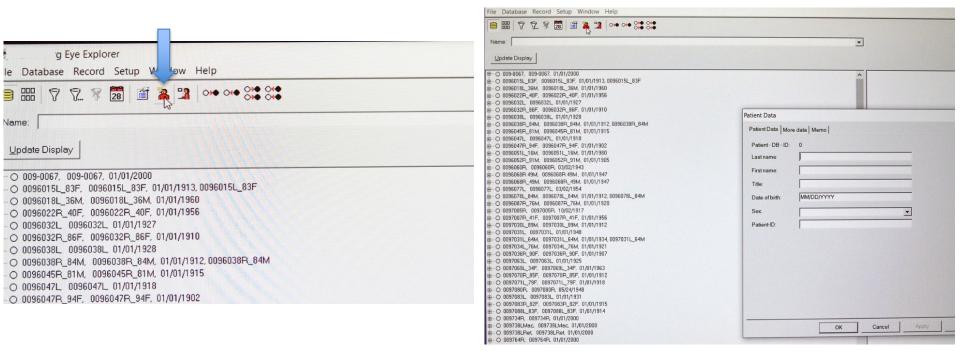
488 nm quantitative autofluorescence, 786 nm autofluorescence, Multicolor (Mcolor)

# 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

ile Database Record Setup Window Help			File Database Record Setup Window Help	
▤ \$\$\$ \$7 \$7. \$7 \$2 \$\$ \$\$\$ \$\$ \$\$			😑 ⅲ 🐬 况 🛠 🛅 🖆 🏝 🧏 아• ∾• 💝 💥	
Name: Update Display	•		Name: Update Display	•
<ul> <li>C 0095067.</li> <li>C 009507.</li> <li>C 009707.</li> <li>C 00970</li></ul>	Eye Data         OD         C-Curve [nm];         Refraction (dpt];         O_Ainder [dpt];         O_Ainder [dpt];         O_Hinder [dpt];         Pups iste [nm];         IOP [mmHg];         VFieldWear;         Ocrrective Lens;         None         CAUTION: Measurements end classification re entered correctly and the patient wars no co.	OS 77 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	<ul> <li>P. ○ 009-0067, 009-0067, 01/01/2000</li> <li>P. ○ 0096015L, 83F, 0096015L, 35F, 001/01/1913, 0096015L, 83F</li> <li>P. ○ 0096012L, 83F, 0096015L, 83F, 001/01/1910</li> <li>P. ○ 0096022F, 43F, 0098012CF, 43F, 01/01/1910</li> <li>P. ○ 0096022F, 43F, 0098022F, 43F, 01/01/1910</li> <li>P. ○ 0096023F, 43F, 0095032F, 43F, 01/01/1910</li> <li>P. ○ 0096023F, 43F, 0095032F, 43F, 01/01/1910</li> <li>P. ○ 0096023F, 43F, 0095032F, 43F, 01/01/1912</li> <li>P. ○ 0096023F, 43F, 0095032F, 43F, 01/01/1912</li> <li>P. ○ 0096023F, 01/01/1920</li> <li>P. ○ 0096023F, 0096047F, 94F, 01/01/1912</li> <li>P. ○ 0096023F, 0096047F, 94F, 01/01/1912</li> <li>P. ○ 0096047F, 94F, 0096047F, 94F, 01/01/1912</li> <li>P. ○ 0096047F, 94F, 0096047F, 94F, 01/01/1912</li> <li>P. ○ 0096057F, 0096047F, 94F, 01/01/1920</li> <li>P. ○ 0096077L, 0096077L, 0072/1947</li> <li>P. ○ 0096077L, 0072/1947</li> <li>P. ○ 0095077L, 00977L, 84F, 01/01/1942</li> <li>P. ○ 0095077L, 00977L, 1072, 1071/1942</li> <li>P. ○ 0095078F, 097708F, 45F, 01/01/1942</li> <li>P. ○ 0095078F, 097708F, 45F, 01/01/1942</li> <li>P. ○ 0095078F, 097708F, 097708F, 01/01/1942</li> <li>P. ○ 0095078F, 097708F, 097708F, 01/01/1942</li> <li>P. ○ 0097071L, 64M, 01/01/1947</li> <li>P. ○ 0097071L, 64M, 01/01/1942</li> <li>P. ○ 0097071L, 64M, 01/01/1942</li> <li>P. ○ 0097078L, 45F, 009738L, 45F, 01/01/1942</li> <li>P. ○ 0097078L, 45F, 009738L, 45F, 001/1942</li> <li>P. ○ 009708L, 009708L, 047/04774</li> <li>P. ○ 009708L, 047, 009738L, 45F, 001/1942</li> <li>P. ○ 009708L, 097708L, 00978L, 85F, 001</li></ul>	Eye Data         OD       OS         CCurve (mm)       77         77       77         Refraction (dpf)       0         Oyinder (degree of the provide source)       0         Pughi size to the provide source of the CCurve values are correct.       Peasurements will only be accurate if the CCurve values are correct.         VieldWate       Continue       Change C-Curve         VieldWate       Continue       Change C-Curve         OK       Cencel       OK

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

/01/1960 /01/1956		01/1960 /01/1956
/01/1910		/01/1910
1/01/1912, 0096038R_84M 1/01/1915		1/01/1912.0096038P_84M 1/01/1915
/01/1902		/01/1902 Eye Data
/01/1980 11/01/1905	CAUTION	/01/1980 1/01/1905 OD OS
1/01/1947 )1/01/1947	Clinical Mode	1/01/1947 C-Curve [mm]: 22 7.7 11/01/1947 Refraction (dpt): 0 0
1/01/1912.0096078L_84M 01/01/1920	Start Clinical Mode	1/01/1912.0096078L_84M Cylinder [dpt]: 0 0
1/01/1956 1/01/1912	- Study Mode	Axis [deg]: U U 1/01/1956 Pupil size [mm]: 0 0 1/01/1912
1/01/1934, 0097031L_64M 1/01/1921	Investigational software function. Limited to investigational use by United States, EU and other national law.	IOP[mmHg]:         0         0           1/01/1934.0097031L_64M         VFieldMean:         0         0
1/01/1907	Enter Study Password:	1/01/1907 VFieldVer: 0 0
/01/1963 11/01/1912 /01/1918	Start Study Mode	/01/1963     Corrective Lens:     None       1/01/1912     Vol/1918     CAUTION: Measurements and classification results will only be accurate if the C-Curve is entered correctly and the patient wears no corrective lenses during the examination.
01/01/1915 1/01/1914		1/01/1915 /01/1914 OK Cancel
/2000 2000		/2000
1/01/1910		1/01/1910
1/01/1913 01/01/1934		1/01/1913 01/01/1934

### Keep the C-curve @7.7 mm. Press OK.

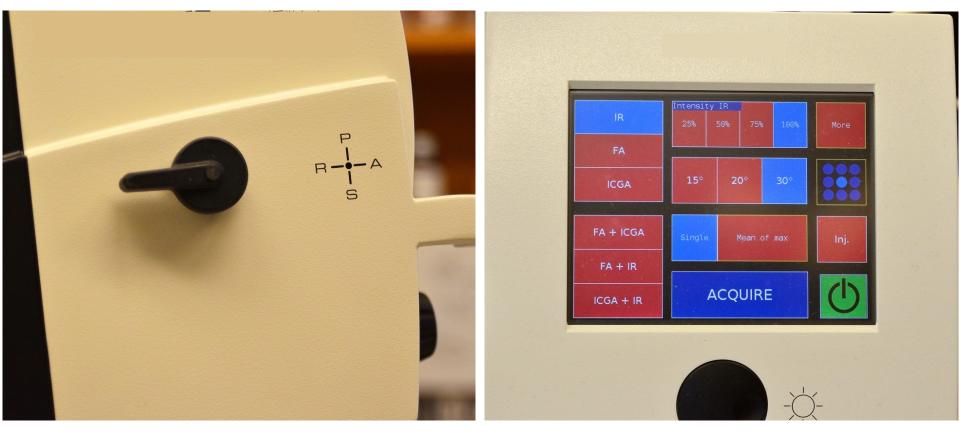
# Select continue to verify the C-curve

### /01/1960 iesto, iesto, 01/01/1000 /01/1956 /01/1910 1/01/1912, 0096038R\_84M 1/01/1915 23 Eye Data /01/1902 /01/1980 OD OS 1/01/1905 7.7 7.7 C-Curve [mm]: 1/01/1947 1/01/1947 Refraction [dpt]: 1/01/1912, 0096078L\_84M Cylinder X Eye Data )1/01/1920 Axis [dec Please verify that both C-Curve values are correct. 1/01/1956 Pupil size Measurements will only be accurate if the C-Curve values 1/01/1912 are correct. IOP [mmh 1/01/1934, 0097031L\_64M VFieldMe 1/01/1921 Change C-Curve Continue 1/01/1907 VFieldVa -/01/1963 Corrective \* 1/01/1912 /01/1918 CAUTION: Measurements and classification results will only be accurate if the C-Curve is entered correctly and the patient wears no corrective lenses during the examination. 11/01/1915 OK Cancel /01/1914 /2000 2000 1/01/1910

# 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 Multicolor 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).

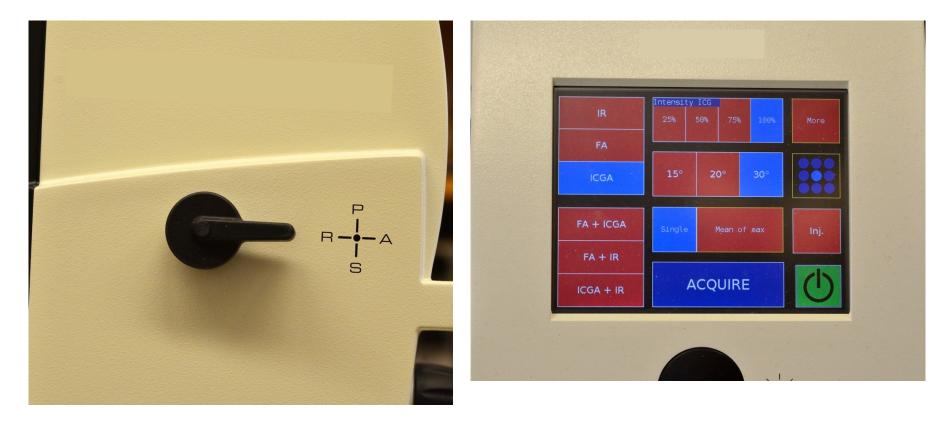


Image will appear as shown below.



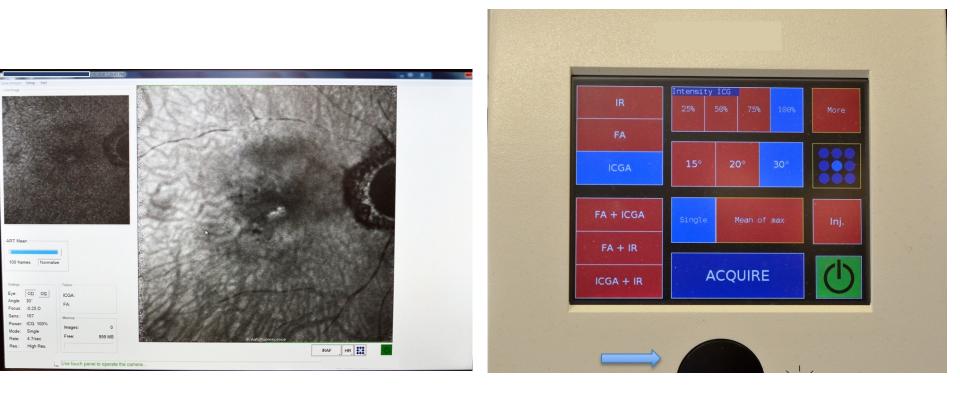
### Move selector knob from R to A

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



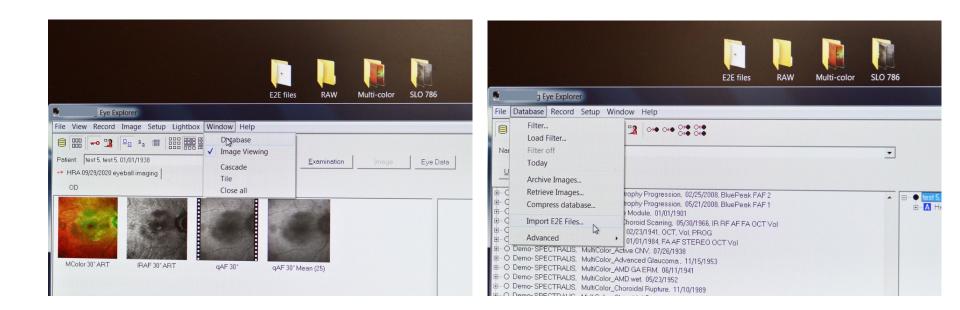
### Image will appear as below.

# Press black disc for averaging then select ACQUIRE.



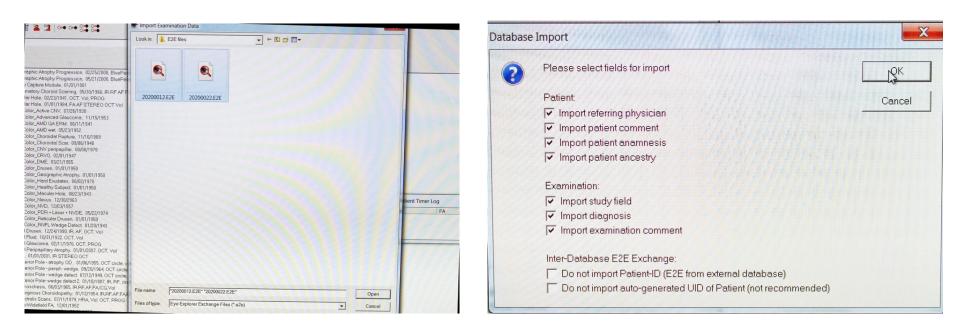
### **Choose Window > Database**

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



### Select open

# Check marks are the default, then select OK.

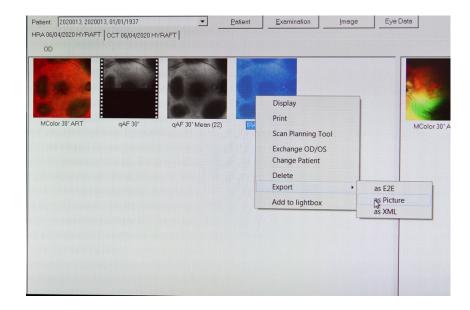


The patient now has 2 tabs, one showing the images acquired from the HRA (blue arrow).

And the other tab shows the images acquired from the HRA+OCT (red arrow).

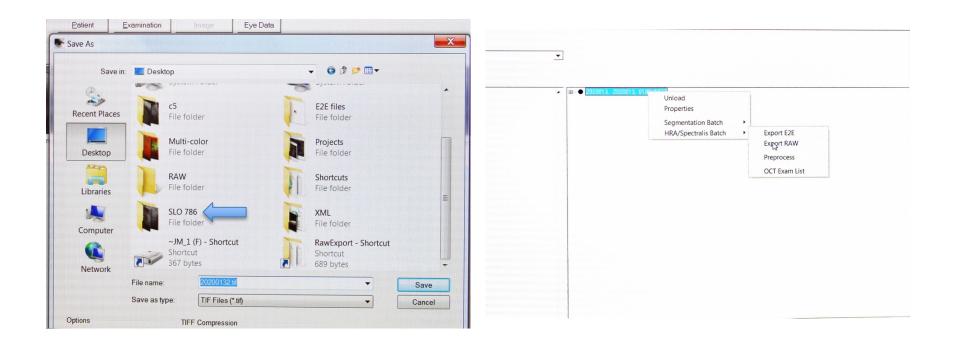


### Right-Click 786 (ICGA) image and export picture to a file labeled SLO 786 on the desktop.



### **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.

Include in library	Share with  Burn New folder									
ites	Name	Date modified	Туре	Size						
ctop	2020013_2_6204.vol	9/29/2020 2:43 PM	VOL File	683,871 KB		E2E files RA	W Multi-color SLO	786		
Inloads	2020013_2_6199.vol	9/29/2020 2:42 PM	VOL File	668,451 KB						
ent Places	2020013_2_6200_0.vol	9/29/2020 2:42 PM	VOL File	5,391 KB						
and the second	2020013_2_6201_0.vol	9/29/2020 2:42 PM	VOL File	5,391 KB						
ies	2020013_2_6202_0.vol	9/29/2020 2:42 PM	VOL File	5,391 KB			and the second	RAW		
uments	2020013_2_6203_0.vol	9/29/2020 2:42 PM	VOL File	5,391 KB	er 🕨 Windows (C:) 🕨	RawExport	✓ ♦ Search Ra…		e with ▼ Burn New folder	
ic	2020013_2_6196_0.vol	9/29/2020 2:42 PM	VOL File	5,390 KB	n Burn New fo	lder				Rathernation
ures	2020013_2_6197_0.vol	9/29/2020 2:42 PM	VOL File	5,390 KB	-	Name	Date mo	A Favorites	Name	Date
BOS	2020013_2_6198_0.vol	9/29/2020 2:42 PM	VOL File	5,390 KB		2020013_2_6204.vol	9/29/20	20 Desktop	2020013_2_6195_0.vol	9/29,
manager and a second second second	2020013_2_6195_0.vol	9/29/2020 2:42 PM	VOL File	5,390 KB		2020013_2_6199.vol	9/29/20	No Dominouus	2020013_2_6196_0.vol	9/29,
outer	2020013_2_0133_0.401	5/25/2020 2:12 1 11	TOLING			2020013_2_6200_0.vol	9/29/20		2020013_2_6197_0.vol	9/29,
						2020013_2_6201_0.vol	9/29/20	20 🙀 🛄 Libraries	2020013_2_6198_0.vol 2020013_2_6199.vol	9/29,
dows (C:)						2020013_2_6202_0.vol	9/29/20		2020013_2_6199.Vol	9/29/ 9/29/
nive (D:)					=	2020013_2_6203_0.vol	9/29/20	20 Ausic	2020013_2_0200_0.vol	9/29/
FA (Q:)						2020013_2_6196_0.vol	9/29/20	20	2020013_2_6202_0.vol	9/29/
						2020013_2_6197_0.vol	9/29/20	- VIGCO3	2020013_2_6203_0.vol	9/29/
ork						2020013_2_6198_0.vol	9/29/20.		2020013_2_6204.vol	9/29/
OIK						2020013_2_6195_0.vol	9/29/20	20 Scomputer		
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