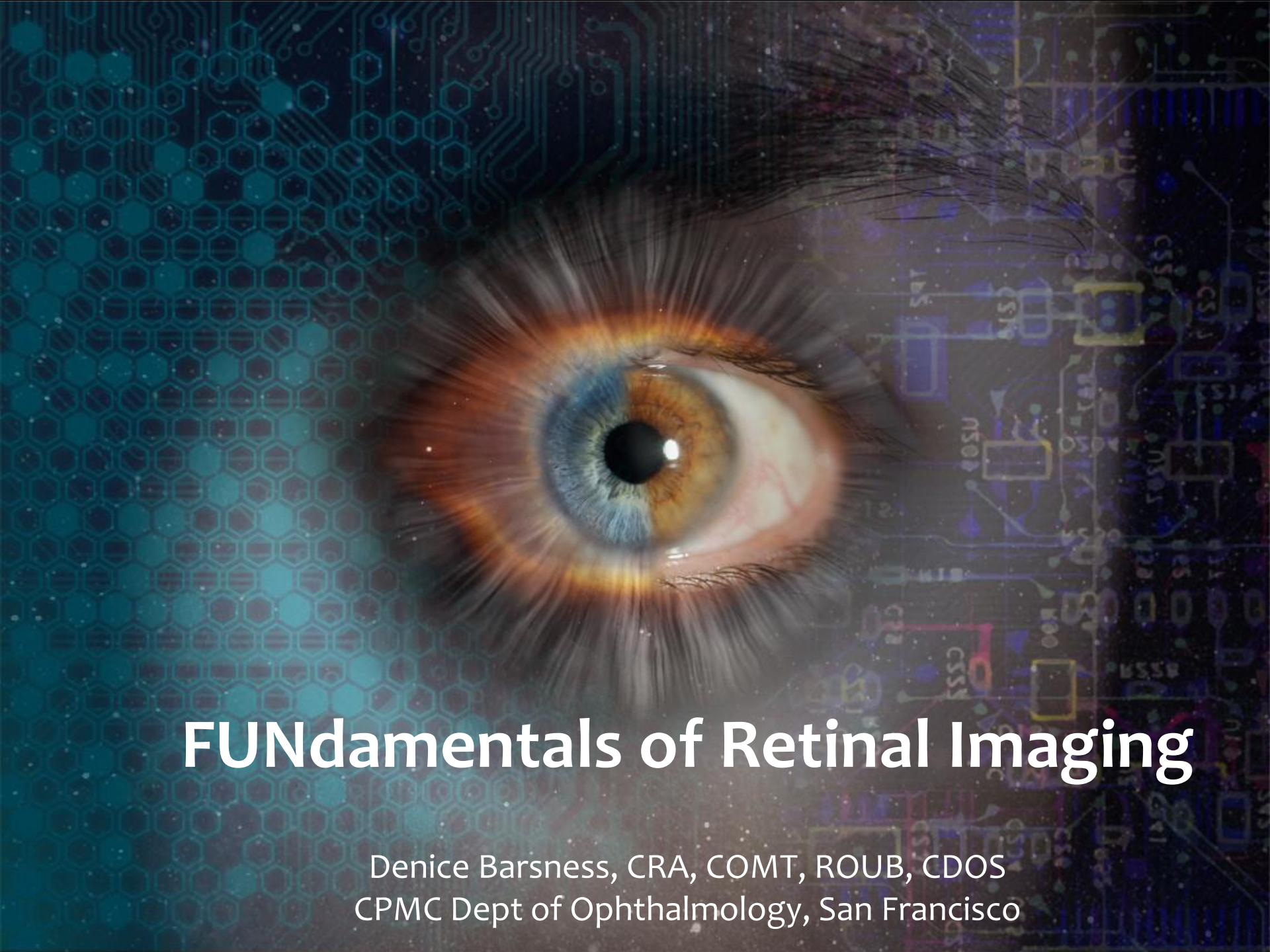
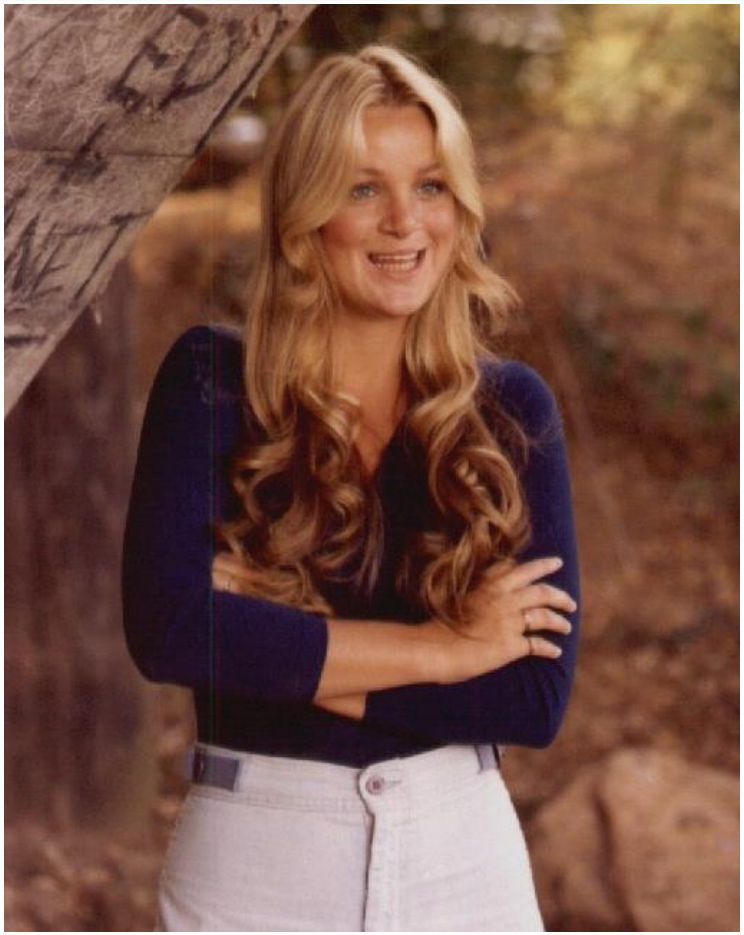


FUNdamentals of Retinal Imaging

Denice Barsness, CRA, COMT, ROUB, CDOS
CPMC Dept of Ophthalmology, San Francisco

Thank You OAO! My last Lecture before retiring….

Be careful what you know, you may have to teach it someday….



My first year in ophthalmic photography 1979…Clueless!!!!

President of the Ophthalmic Photographers' Society 1994

Disclaimers

- I am not a retinal physician and know my boundaries
- This presentation is the WHAT, not the WHY
- I have no financial stake in any fundus imaging company, alas….
- I have 45 minutes to cover a subject of which any one portion could be a lifetime of study… give me a break…
- Don't worry about notes- you will get the full lecture to look back and review

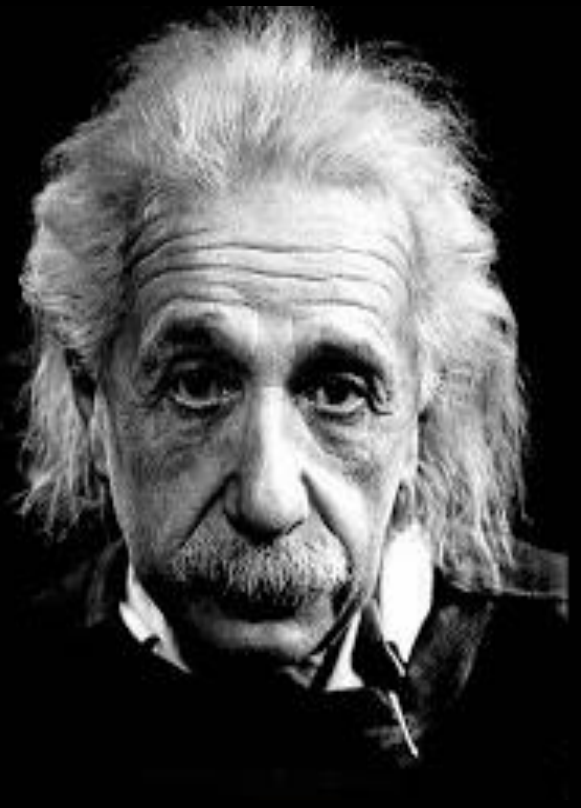
TRELLCHEM
TRELLCHEM
TRELLCHEM
SCOTT
AIR-PAK

This Lecture Will be Vendor Agnostic

Imaging devices have more similarities than differences – they all have basic components like a car- wheels, ignition, steering wheel, etc.

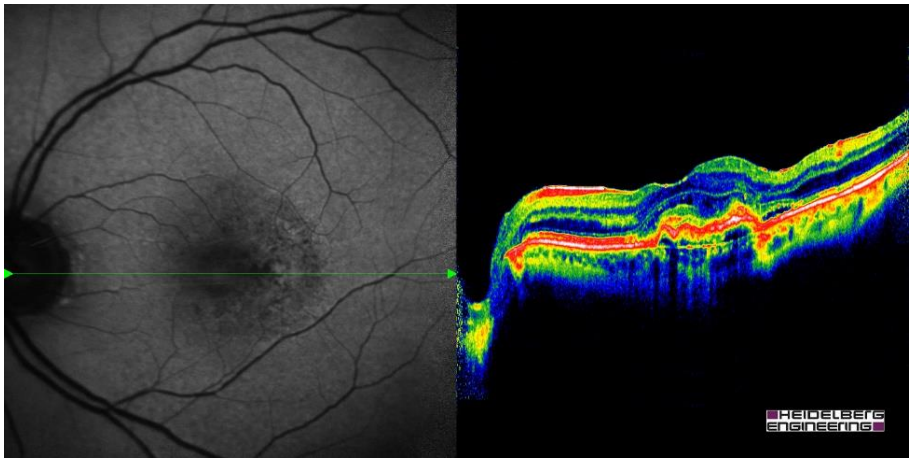
You don’t really understand something until you can explain it to your grandmother….

Albert Einstein



The Origins of Retinal Imaging

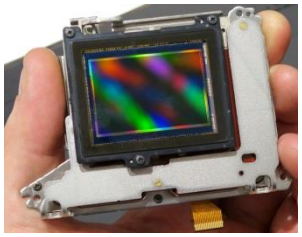
- **1850** Helmholtz invents the ophthalmoscope allowing first view of living retina
- 1854 first color fundus illustration by van Trigt
- 1910 Retina and choroid examined using sodium fluorescein
- 1953 Electronic flash technology applied to fundus photography
- **1959** two medical students from Indiana, Novotny and Alvis, use exciter/barrier filters to perform angiography on cat retina
- 1970 First ICG of Owl Monkey
- **1987** First digital angiography system introduced by Topcon & OIS
- **1993** Zeiss introduces Time Domain OCT1 technology to clinical world
- **2006** Spectral Domain OCT technology immerges
-



Imaging vs. Photography

- Light, silver, chemicals

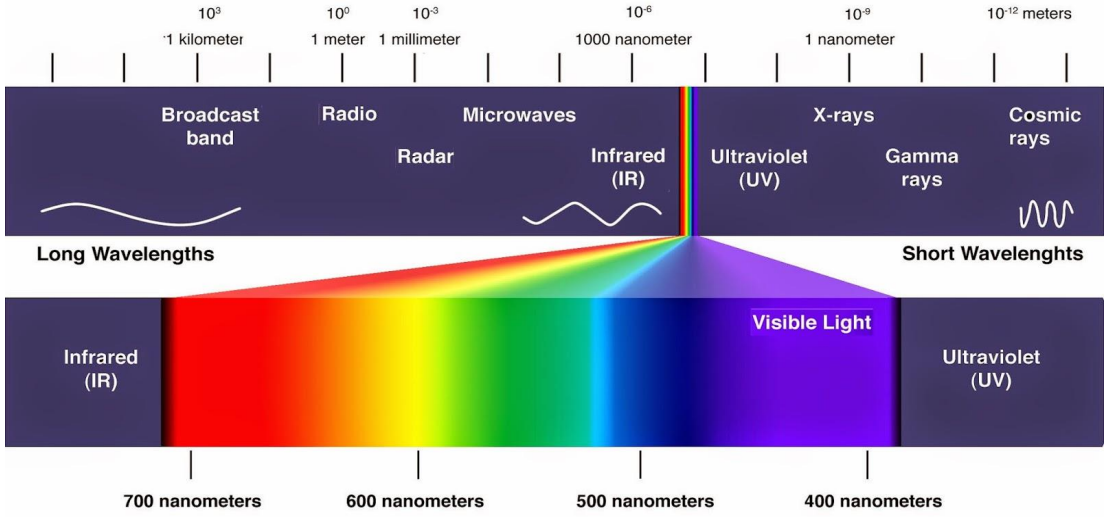
- Light, computer chip, pixels



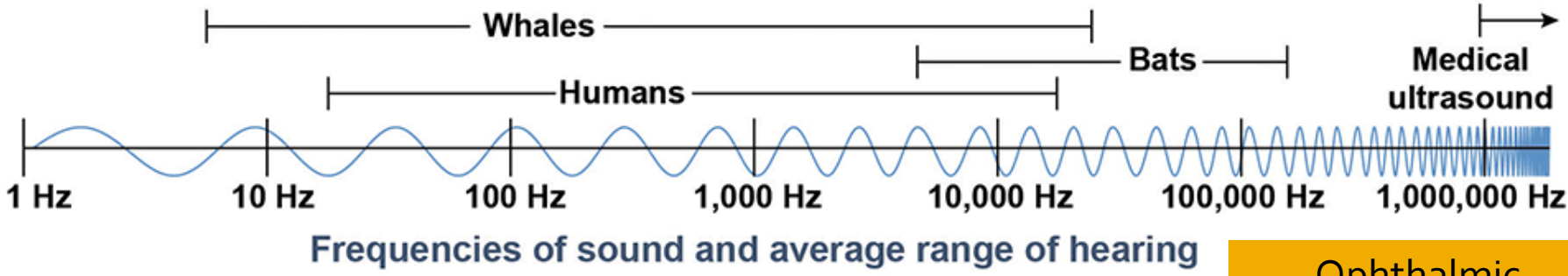
Introduction: Retinal Imaging

- Advances in retinal imaging modalities allow us to visualize posterior segment findings to a previously unmet degree.
- The clinical utility of these imaging techniques is constantly expanding, with new developments in machinery allowing for improvements in patient care. Better fields of view, image clarity/detail and modes of acquisition not only help during diagnosis of retinal disorders but also during screenings and follow-ups
- Well-captured retinal images allow us to identify disease severity and stage, compare follow-up visit images to documented findings, track progression of disease between visits, and screen for pathology early.
- Additionally and importantly, the capture of clear retinal images allows for unambiguous communication between physicians and contributes to the education of trainees.

Wavelengths & Soundwaves



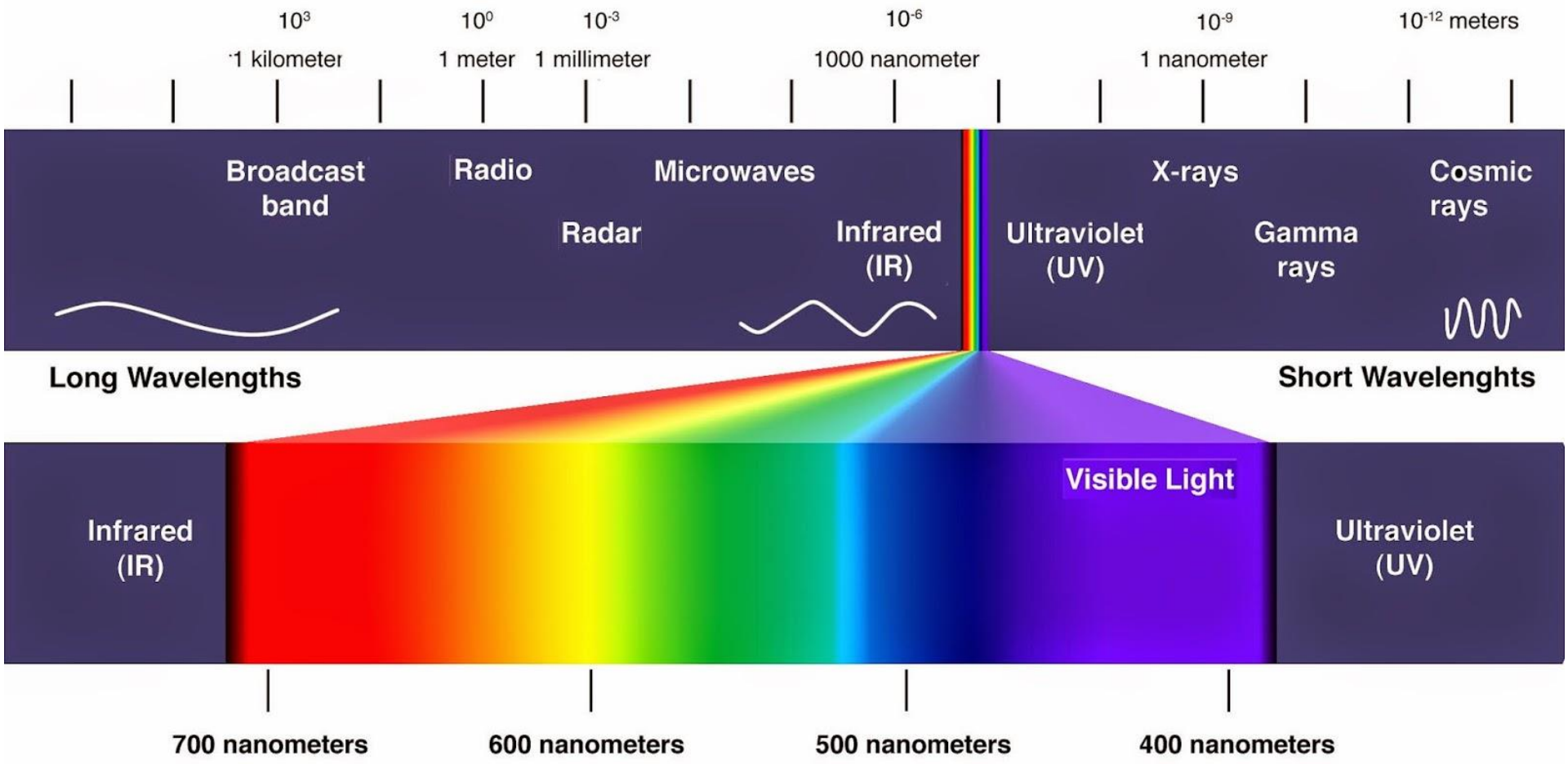
Light is the range of frequencies of electromagnetic wavelengths that stimulate the retina
Shortest is 400 nm all of the way up to near infrared or 800-900nm



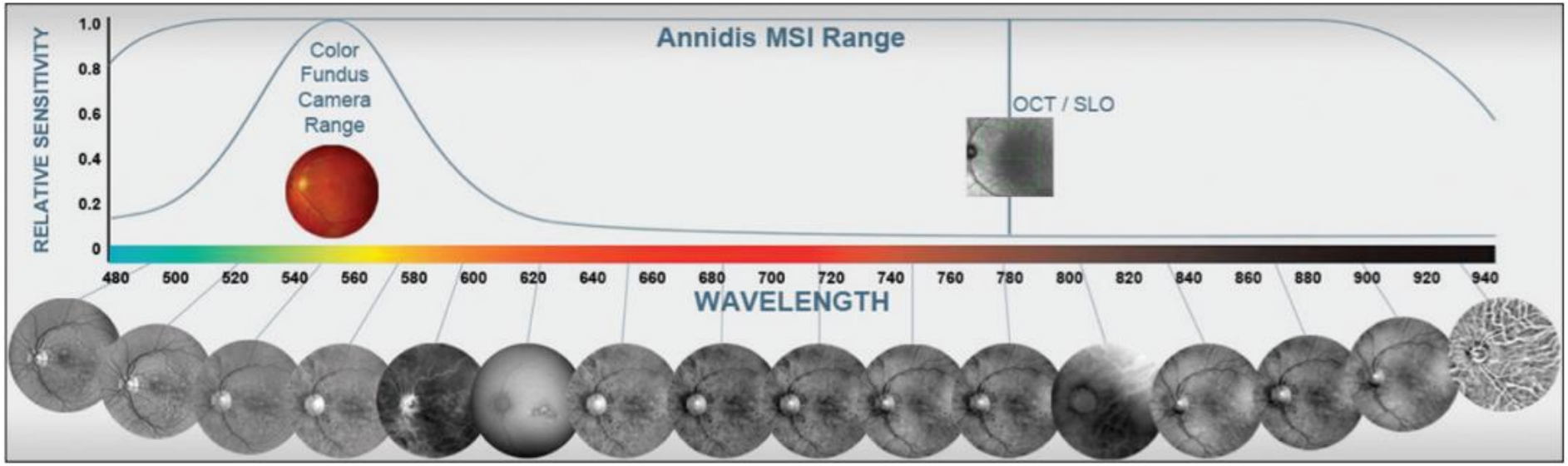
Frequencies of sound and average range of hearing

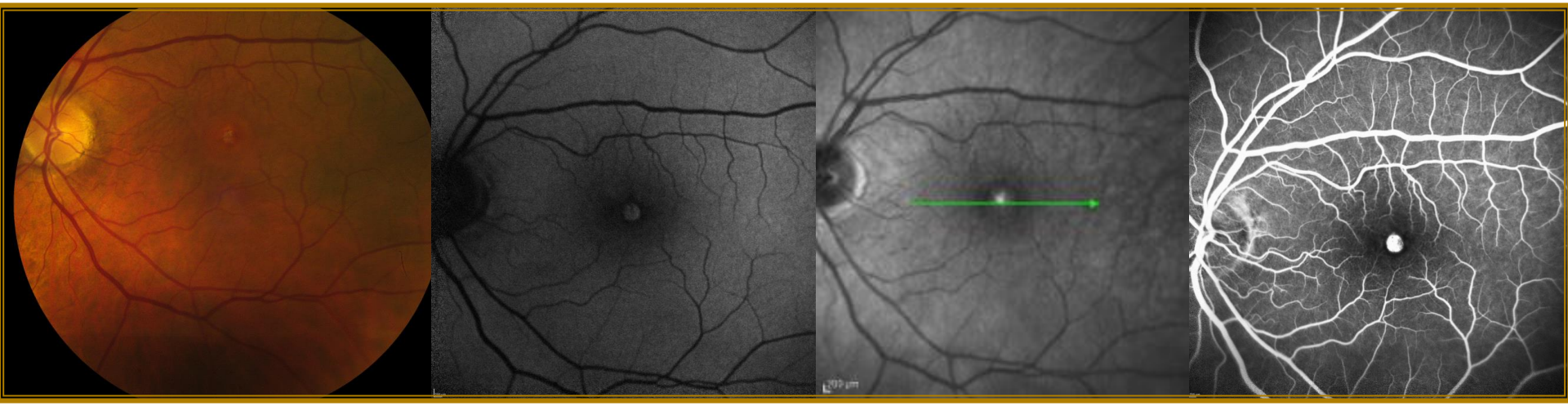
Ophthalmic Diagnostic US: 10-20Mhz

Wavelengths/ Visible Spectrum

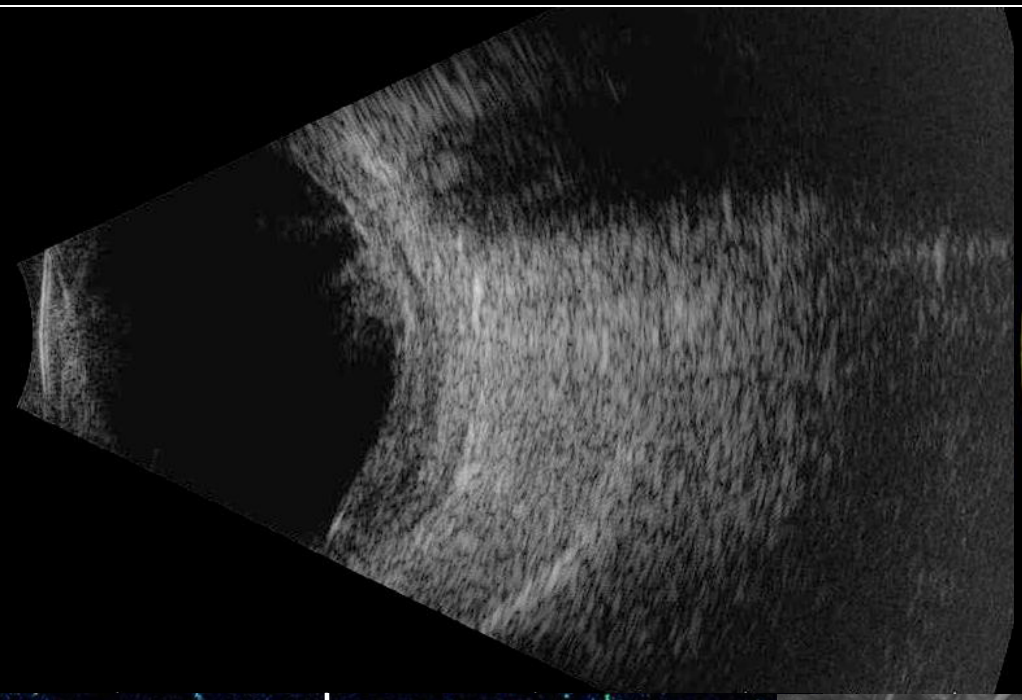


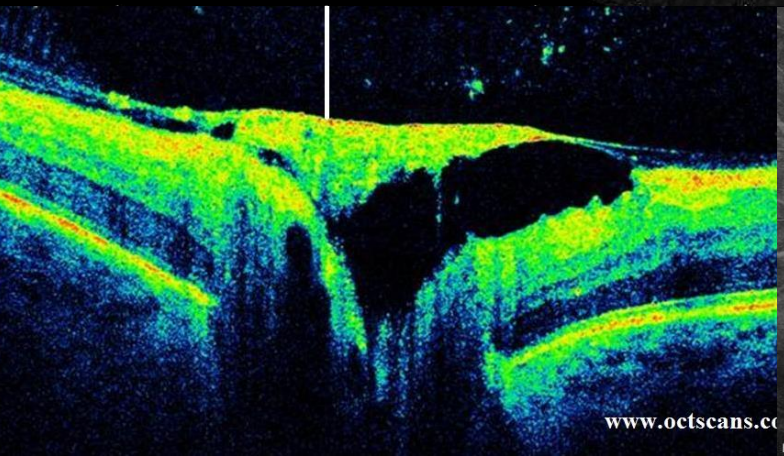
Multispectral Imaging

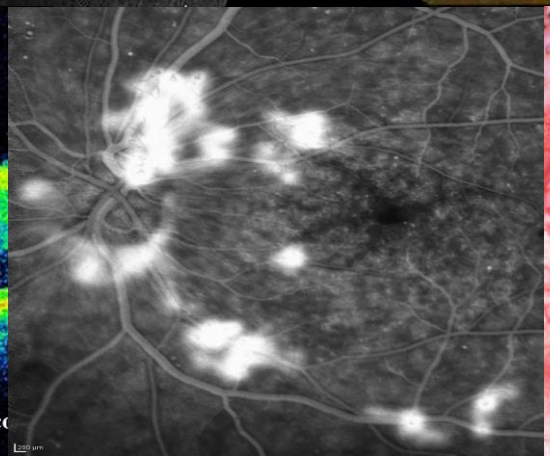




Mulitmodal Imaging







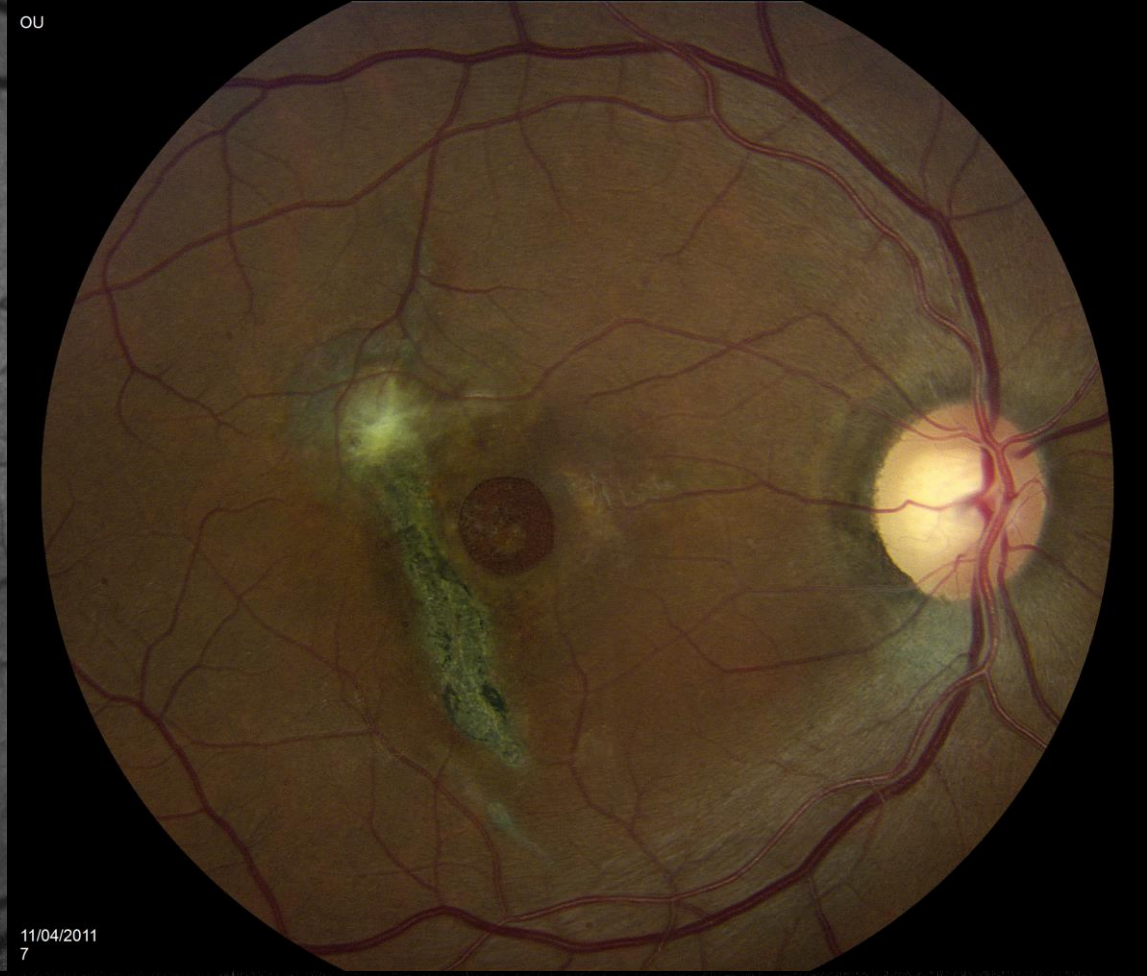
Invasive or Non Invasive?

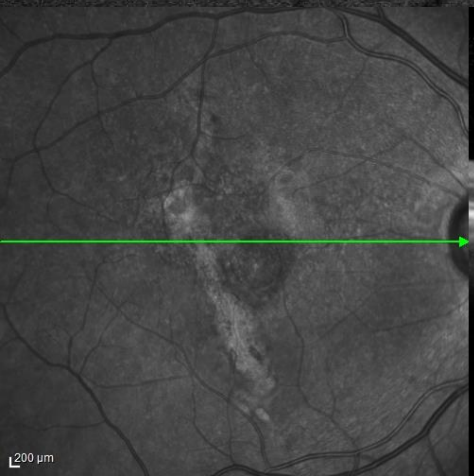
- Technically, dilating a pupil for imaging is invasive and actually governed by defined regulations/ protocols by each state
- You can see the AAO OMIC position on "dilation"
- Speaks to a Mydriatic or Non Mydriatic imaging device

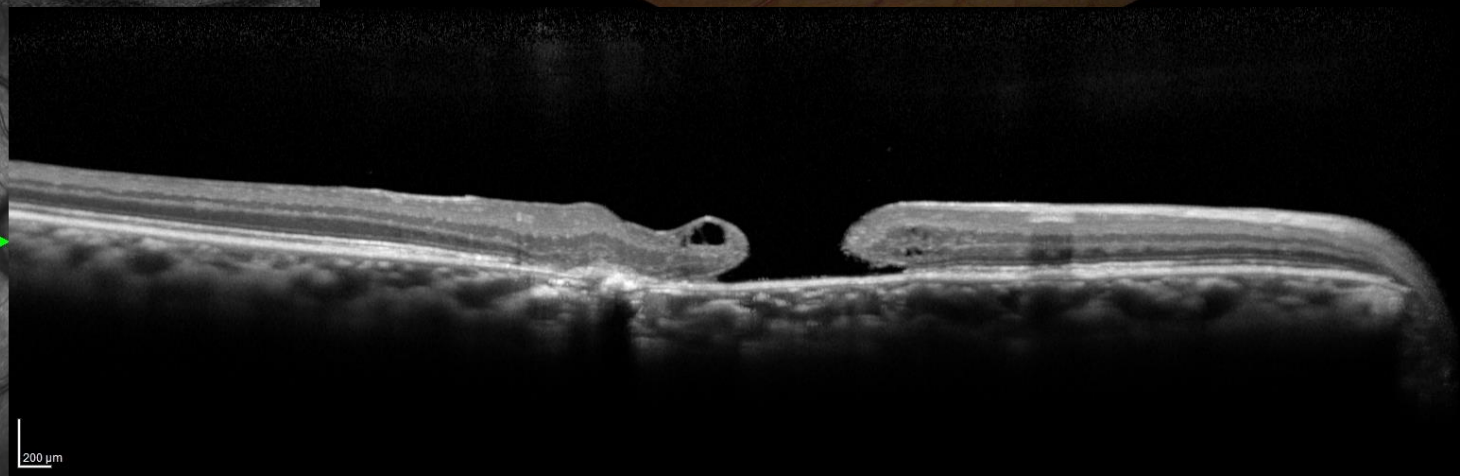
Non Invasive	Invasive
Color Fundus Imaging	Fluorescein Angiography
Optical Coherence Tomography	Indocyanine Green Angiography
Fundus Autofluorescent Imaging	Ophthalmic Ultrasound
Optical Coherence Tomography-Angiography	Intraoperative OCT or FA

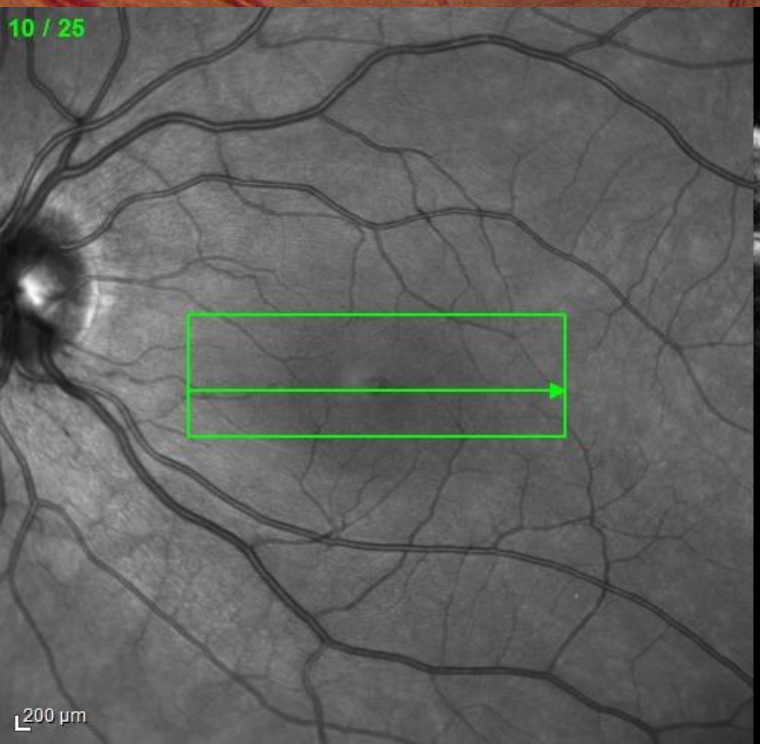
Retinal Imaging Modalities

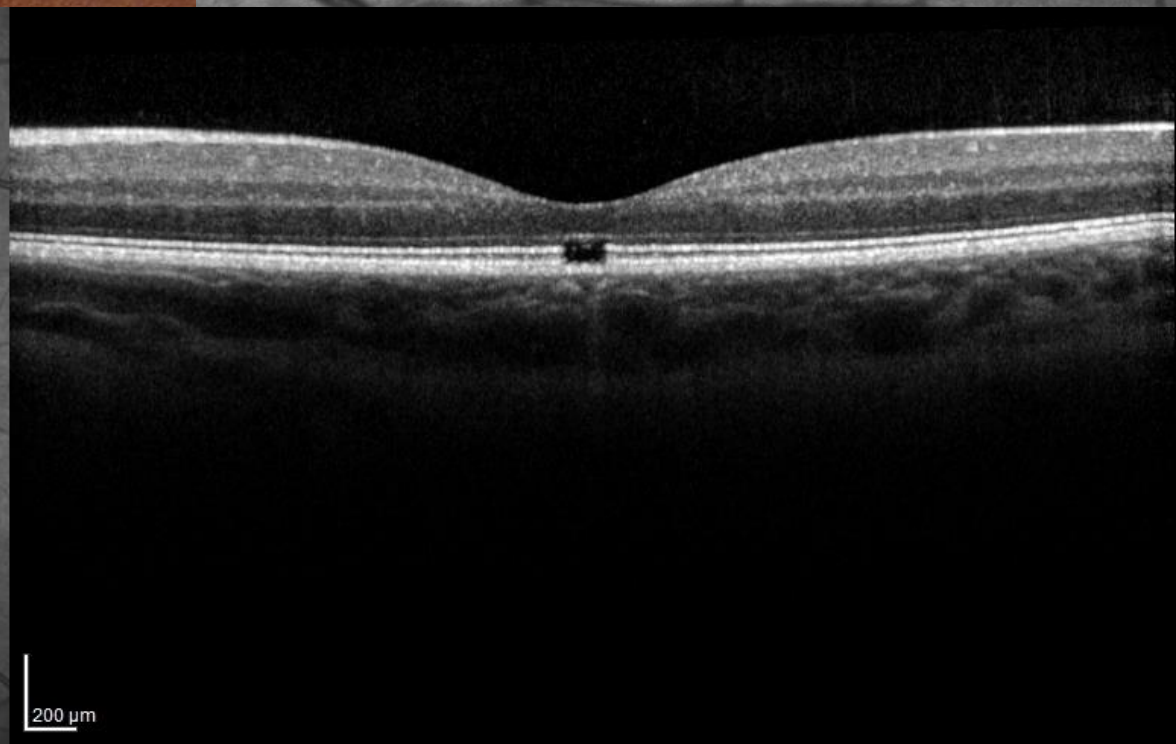
- Color Fundus Images (CFP)
- Wide Field or Ultrawide Field Color Images (WF/ UWF)
- Fundus Autofluoresence (FAF)
- Fluorescein Angiography (FA)
- Optical Coherence Tomography (OCT)
- Optical Coherence Tomography Angiography (OCT-A)
- Indocyanine Green Angiography (ICG)
- Ophthalmic Echography (US)


OU
11/04/2011
7

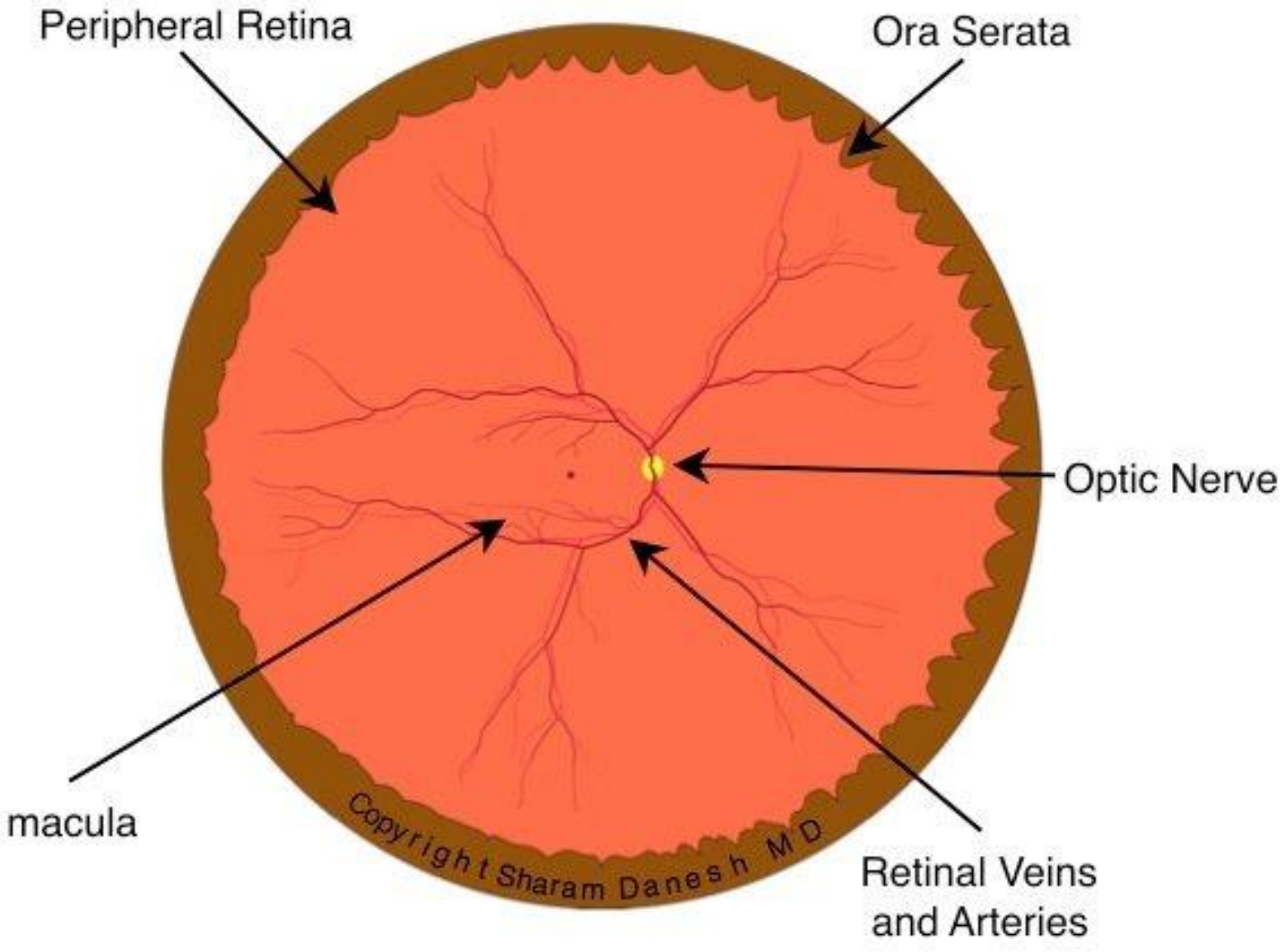

200 µm

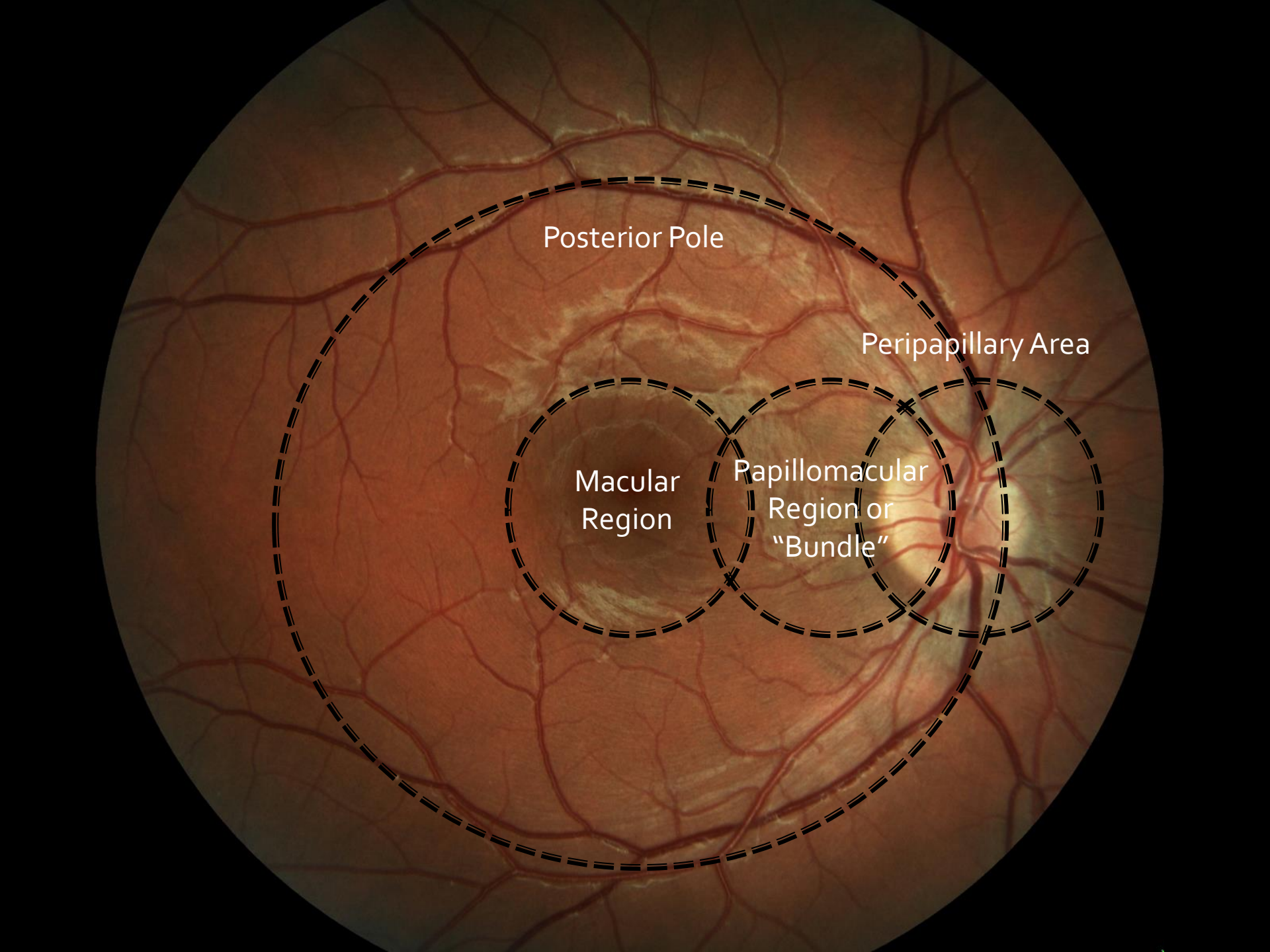

200 µm


10 / 25
200 µm

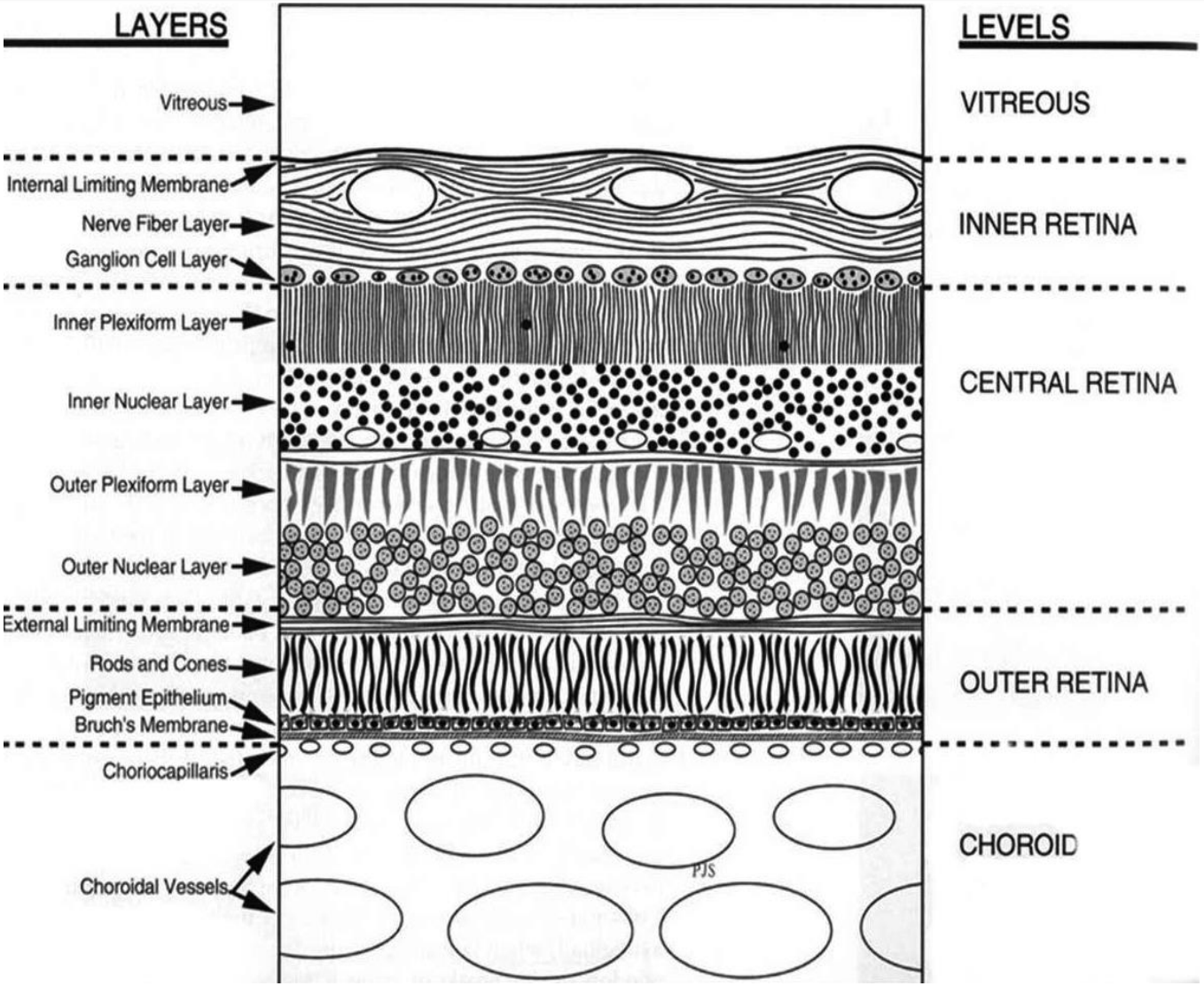

200 µm

Imaging: Geography



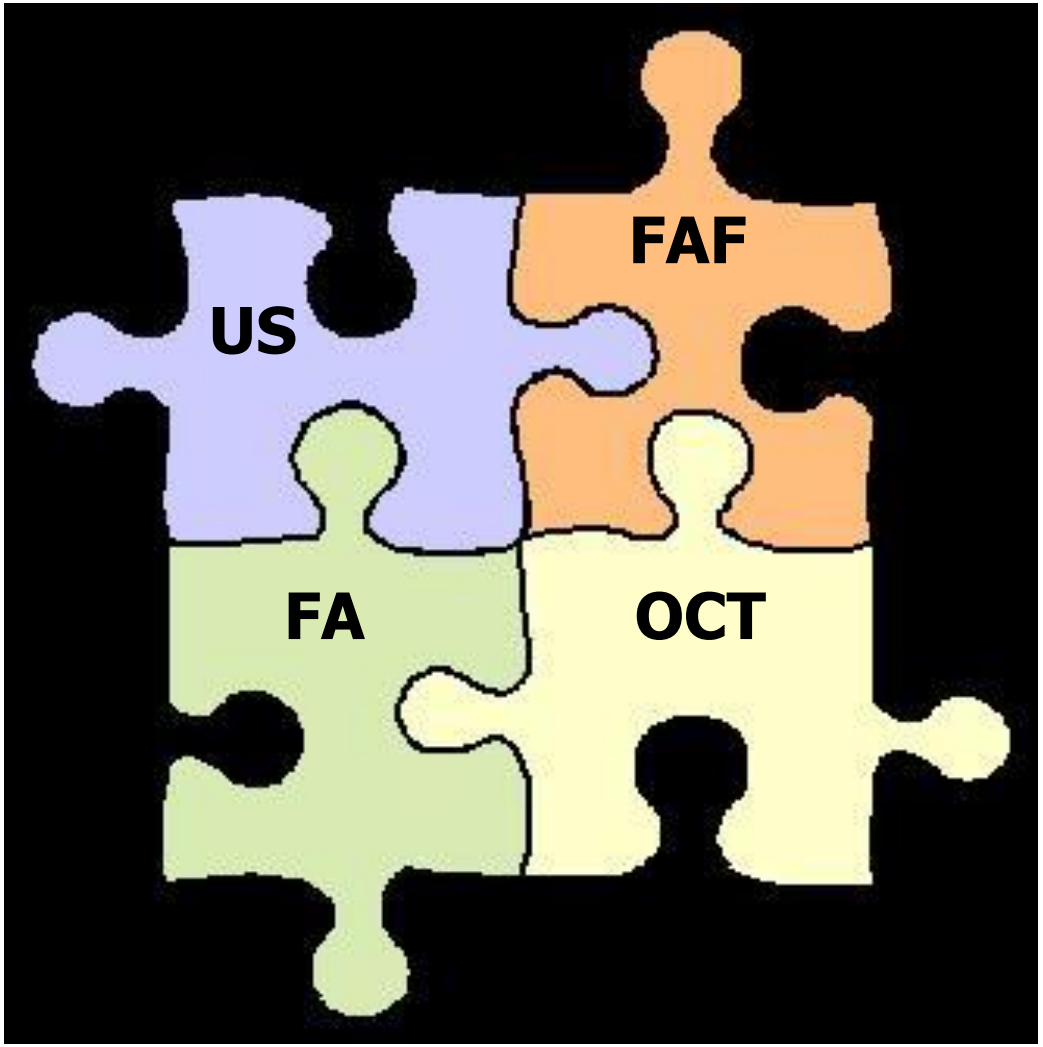

Posterior Pole
Peripapillary Area
Macular Region
Papillomacular Region or "Bundle"

Imaging: Location by Layer



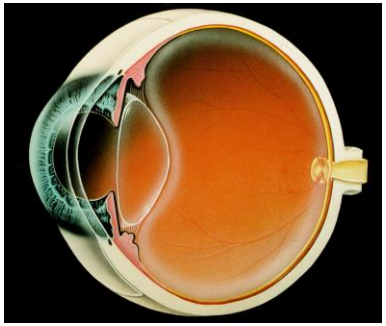
Assembling the Puzzle Pieces

- Different pieces of information help to draw conclusions that help the MD to decide how to most effectively treat a patient
- Often, one piece of the puzzle won't fit- thus the challenge



What is a Fundus?

- Latin for “Bottom”
- The large, hollow part of an organ, farthest from an opening; especially the top, hollow portion of the uterus
- **The back, interior part of the eye (retina)**
- The uppermost hollow of the stomach
- The deepest part of a sulcus, such as the sulci in the human cerebral cortex
- *We have lots of “fundi” in the human body….*



Classification of a Fundus Image

DIAGNOSTIC

- Lends new information
- Helps in differentiation of diagnosis
- Provides hemodynamic information

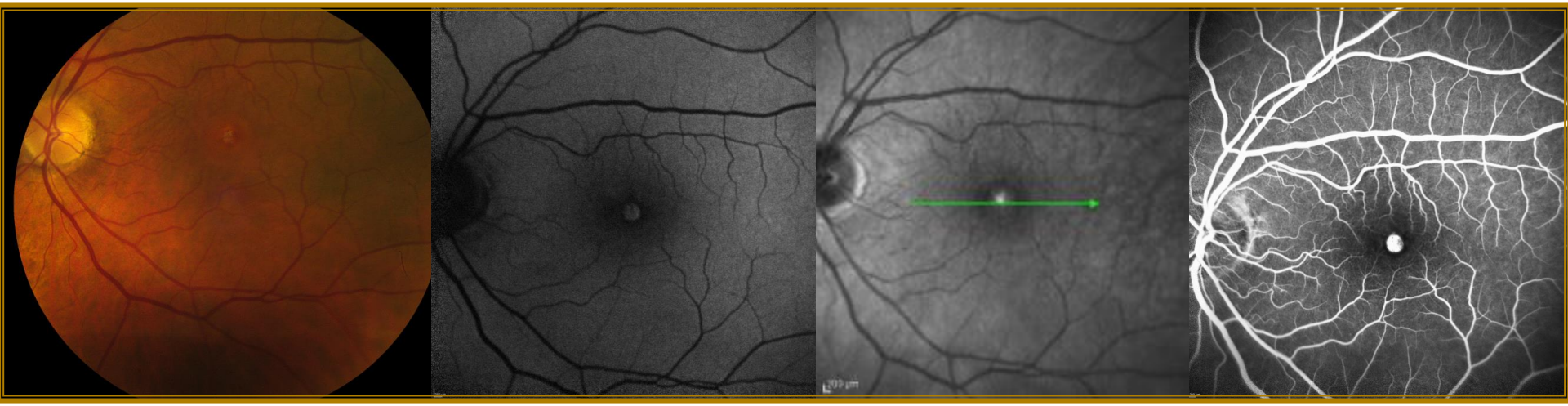
DOCUMENTARY

- Used for teaching
- Provides medicolegal "proof"
- Records change over time
- Just because it's cool…

Qualify the Wavelength…

- These are all “fundus” images
- Be specific by wavelength
- Not all devices offer all wavelengths

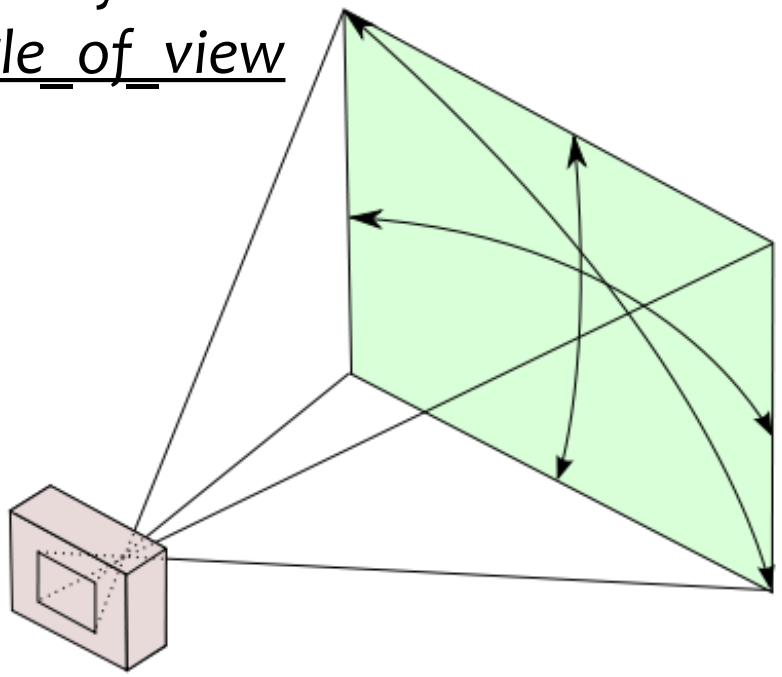
Color Autofluorescent Infrared Angio



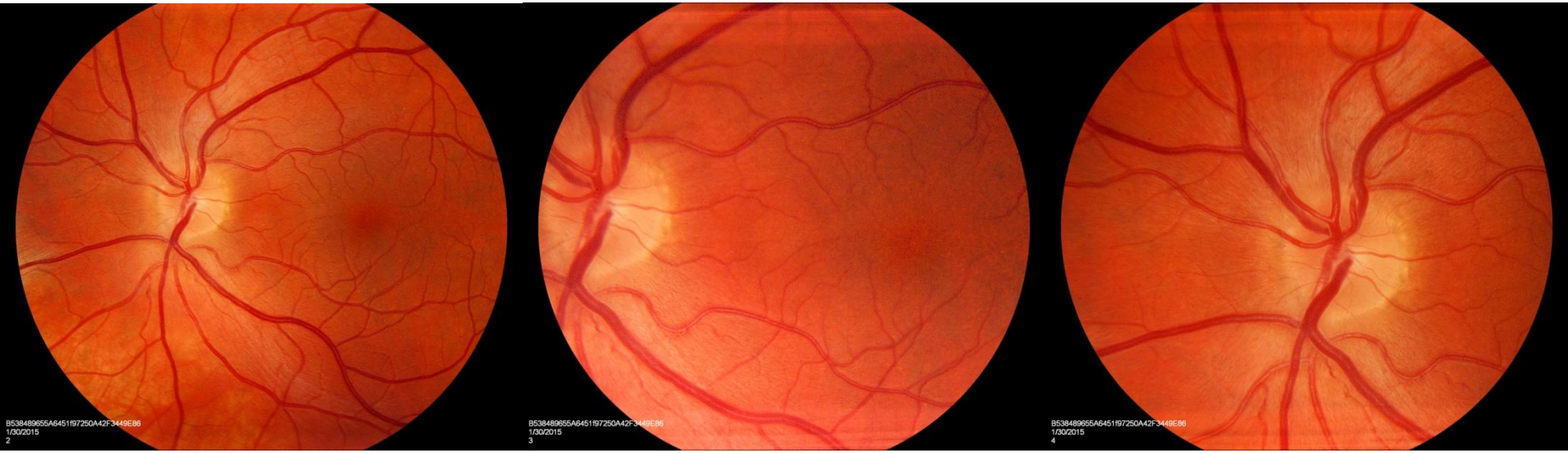
Field of View (FOV) = Angle of View

- "In photography, **angle of view** describes the angular extent of a given scene that is imaged by a camera. It is used interchangeably with the more general term **field of view**. A camera's **angle of view** can be measured horizontally, vertically, or diagonally". *http://en.wikipedia.org/wiki/Angle_of_view*

- *Depends on:*
  - *Size of Objective Lens*
  - *Size of Digital Sensor*
  - *Pt's K curvature, AXL*



Magnification / Angle of View



50 degrees

35 degrees

20 degrees

Not all instruments offer all angles of view

Definitions: OPS and ASCRS

- High magnification 20 degrees
- Normal 30-40 degrees
- Wide angle 50-50 degrees
- Ultrawide field >60 degrees, all modalities
- Some SLO's can provide max 200 degrees

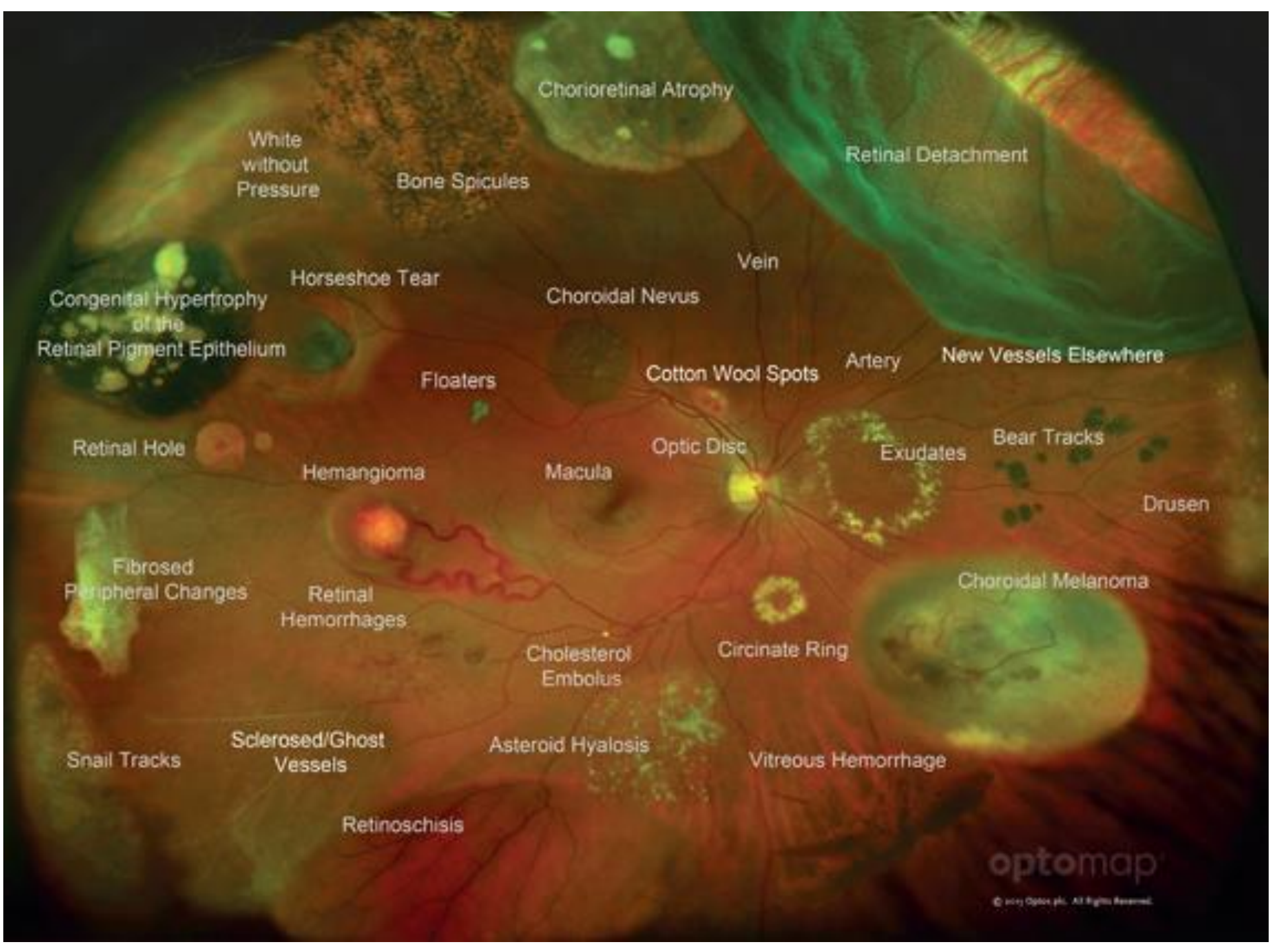
Definitions can vary between the experts and the design of fundus/SLO imaging devices

These are dimensions of optics of device and do not include montaged images…

Wide Field vs Ultra-wide Field

- Wide field and ultra-wide field imaging have been used interchangeably in literature without clear definitions.
- The Diabetic Retinopathy Clinical Research Network (DRCR.net) defined UWF images to have at least a 100° view of the fundus.[2] Recently, a consensus of experts in the field established that the term wide field should be used for images showing retinal features beyond the posterior pole but posterior to vortex vein ampulla in all four quadrants, while UWF should be restricted to images in a single capture showing retinal features anterior to vortex vein ampullae in all four quadrants.
- An image which can detail ora to ora in 360 degrees, is termed "Panretinal". Though montaging techniques with Optos can produce panretinal images, no device as of now can capture such an image in a single click.

Ultra-wide field retinal imaging
A wider clinical perspective
Kumar, Vinod; Surve, Abhidnya; Kumawat, Devesh[1]; Takkar, Brijesh[2]; Azad, Shorya; Chawla, Rohan; Shroff, Daraius[3]; Arora, Atul[4]; Singh, Ramandeep[4]; Venkatesh, Pradeep
Author InformationIndian Journal of Ophthalmology: April 2021 - Volume 69 - Issue 4 - p 824-835


Chorioretinal Atrophy
White without Pressure
Bone Spicules
Retinal Detachment
Vein
Horseshoe Tear
Choroidal Nevus
Congenital Hypertrophy of the Retinal Pigment Epithelium
Artery
New Vessels Elsewhere
Floaters
Cotton Wool Spots
Retinal Hole
Optic Disc
Exudates
Bear Tracks
Hemangioma
Macula
Drusen
Fibrosed Peripheral Changes
Choroidal Melanoma
Retinal Hemorrhages
Cholesterol Embolus
Circinate Ring
Sclerosed/Ghost Vessels
Snail Tracks
Asteroid Hyalosis
Vitreous Hemorrhage
Retinoschisis
optomap

The First Wide Field System

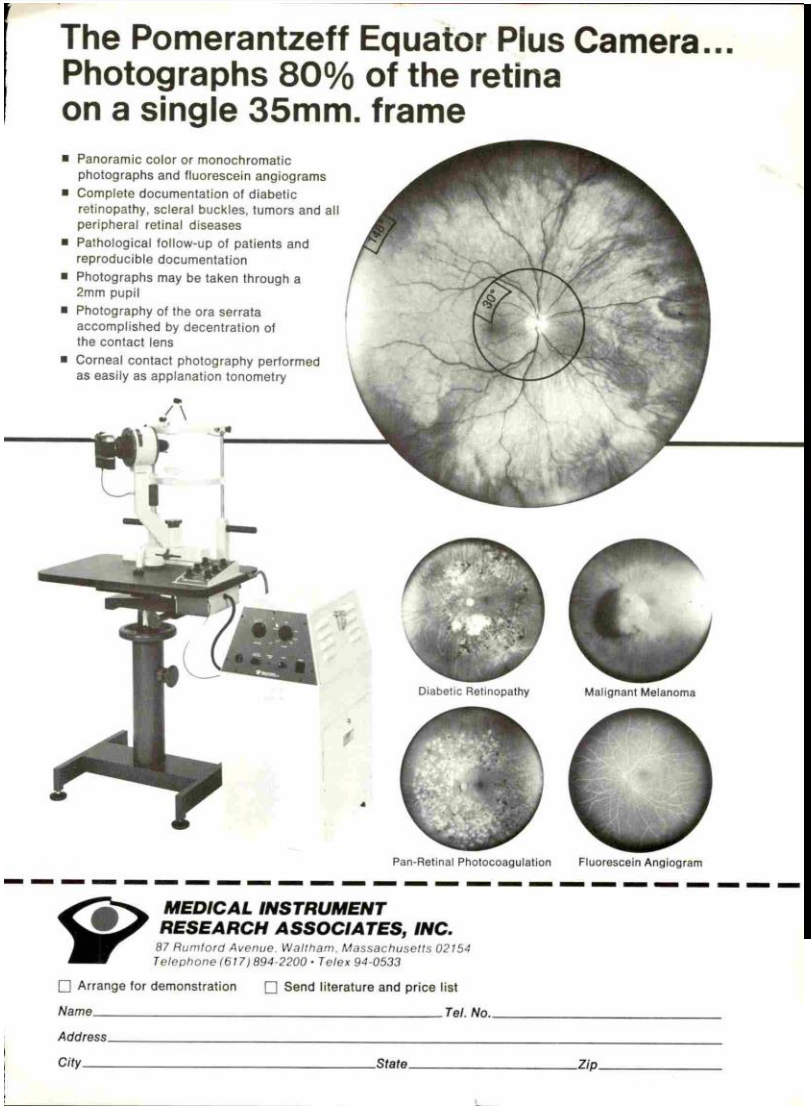


Photo: Denice Bartlett 1983

1979: 45 Degrees was Wide

My experiences with the Kowa RC-W wide-angle fundus camera

Sadao Kanagami
Tokyo, Japan

A wide angle fundus camera, the Kowa RC-W (Fig. 1), was first marketed in Japan in October, 1976. For one year prior to that time, I performed clinical tests on its prototype, and gave my suggestions for improvements and modifications to Kowa. I am now happy to have an opportunity to report my experiences with this camera.

The angle of view of the RC-W (Fig. 2A) is 45°, and its objective lens has a specially designed aspherical lens of high resolving power. Two close-up lenses are available, a 1.4×, yielding a 30° angle of view, and a 2× lens, giving a 21° angle of view (Fig. 2B and C). Insertion of these lenses on the camera body in front of the camera back converts the angle of view, and hence, magnification. Two controls are directly on top of the fundus camera body; one, a diopter compensation knob which can be changed in four stages, covering a total range of −33D to +36D. The second control is an Iris Diaphram Selector which is unique to the Kowa fundus camera. This selector is usually used at the closed position (small), but when the operator needs to have more light, he can pull it up to open the iris, so that the light quantity may be increased by 1 EV. Two filter wheels are situated on the left side of the camera, and will rotate filters into the illumination and photographic pathways for photography and angiography. Each filter wheel is marked with "W", P, F1 and F2 and inserts the respective filters as shown in Table 1. The shutter release button is located in the joy-stick; a horizontal pivot and tilting stand are available for optional use.

The camera backs are of two types, manual and motor driven, and produce a 22-mm round frame on 35-mm roll film. These camera backs are easily interchanged due to a specially designed bayonet mount system. The back cover may be replaced with a data recorder back, and data can be seen directly through the window on this back. The time counter records time digitally in four figures by 0.1 sec. A Polaroid back is available

Fig. 1: The Kowa RC-W fundus camera.

which produces a 60-mm round frame picture with erected image via pentaprism, as well as a 120 roll film back which produces an enlarged picture. Four marks on the 60-mm round frame indicate cutting lines for the Gepe 48 × 48 super slide mount.

Three power supply units are available.

1) PU-300: This standard power unit (Fig. 1) is specially for 35-mm color photography and has six stages of flash intensity, 25, 50, 75, 100, 200 and 300 watt/seconds. This unit is capable of Polaroid photography.
2) PU-300F: This power pack is used for fluorescein angiography. Charging can be done under 1 sec. There are three

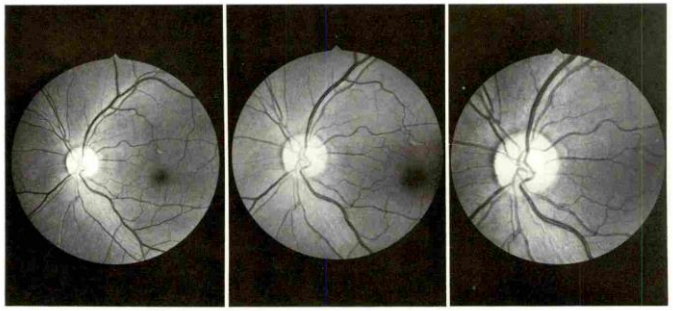
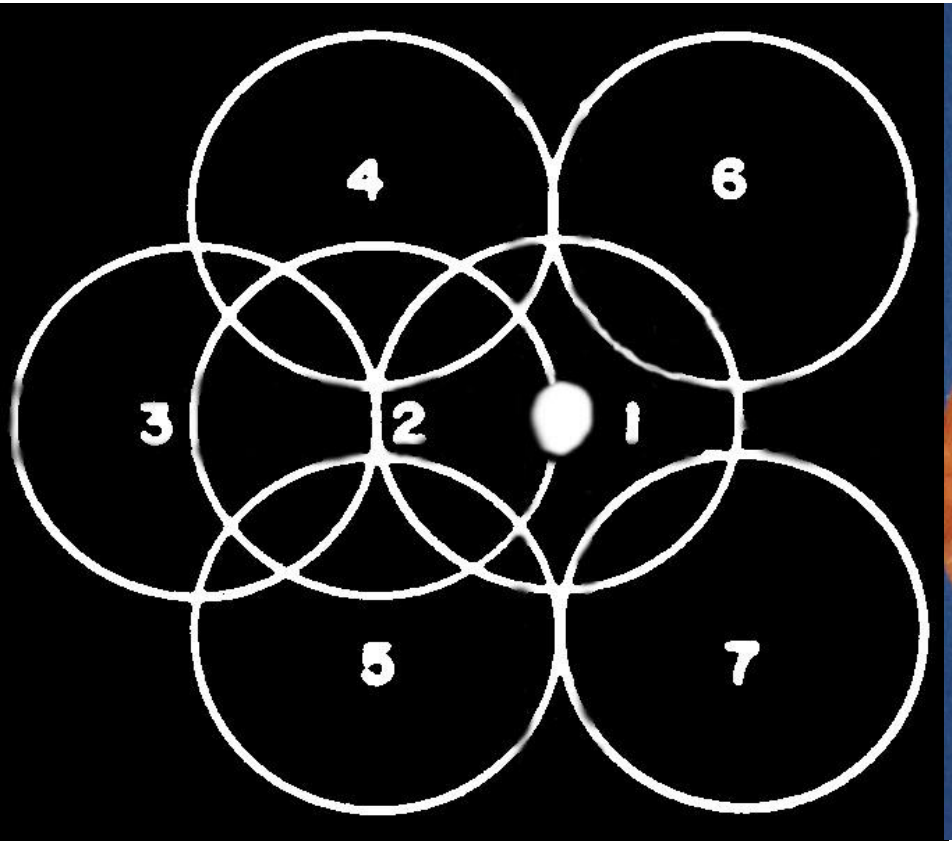


Fig. 2A: A fundus photograph with 45° angle of view.

Fig. 2B: A fundus photograph utilizing a 1.4× magnifying lens yielding a 30° angle of view.

Fig. 2C: A fundus photograph with a 2× magnifying lens yielding a 21° angle of view.

Old School: The "7 Standard Fields"

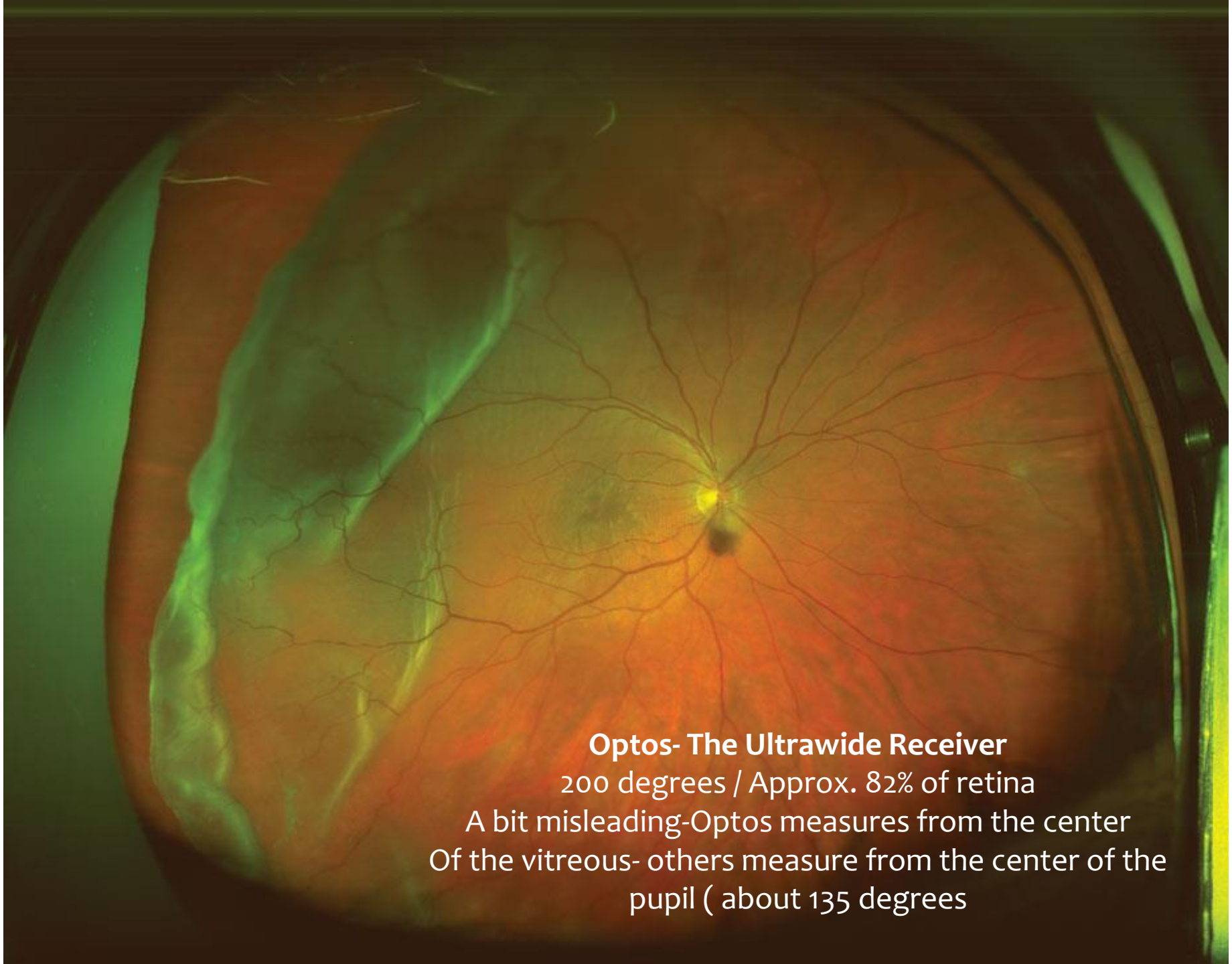


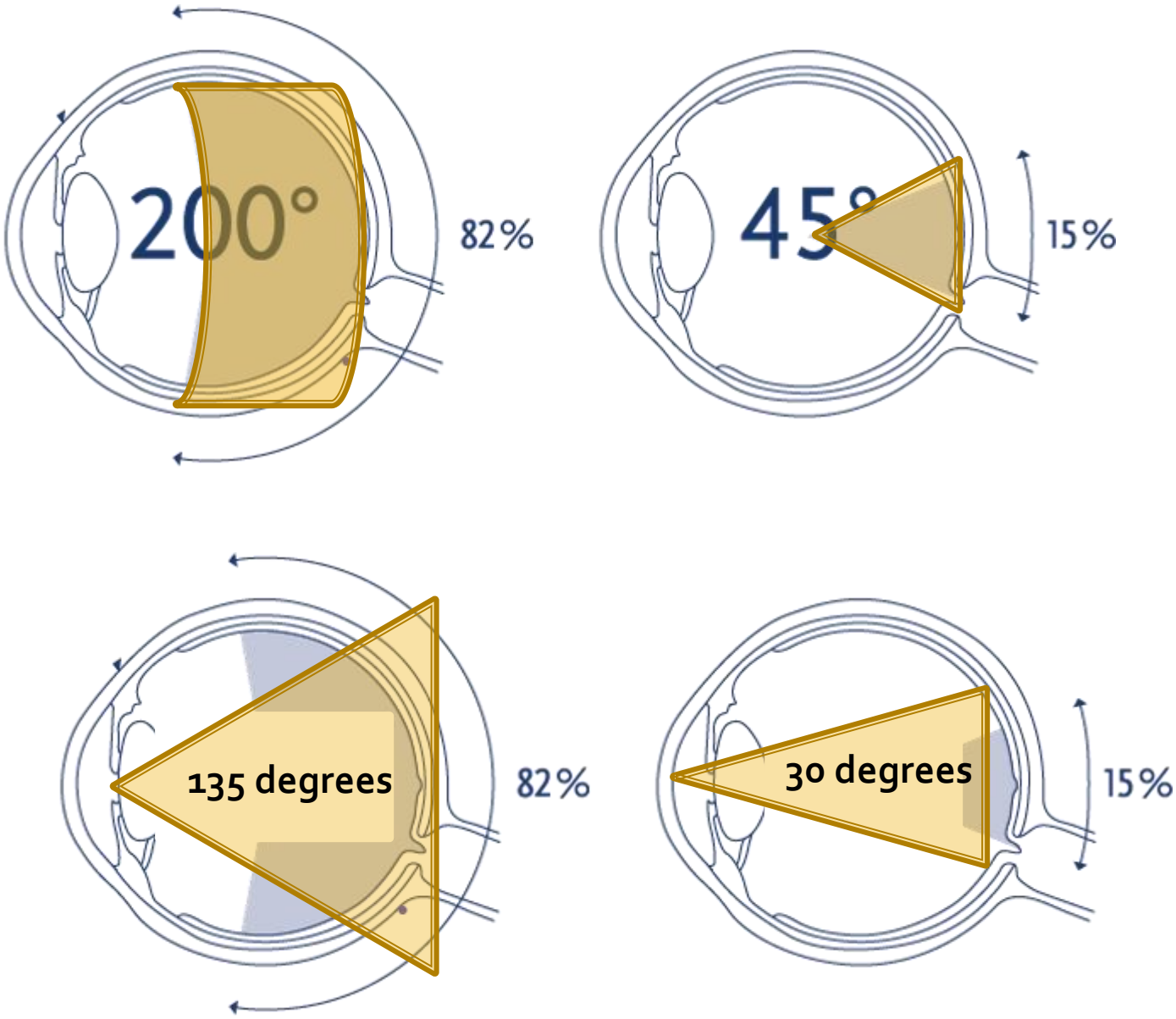
30 degree Field of View x 7 slides
Approximately 75 degrees of retina

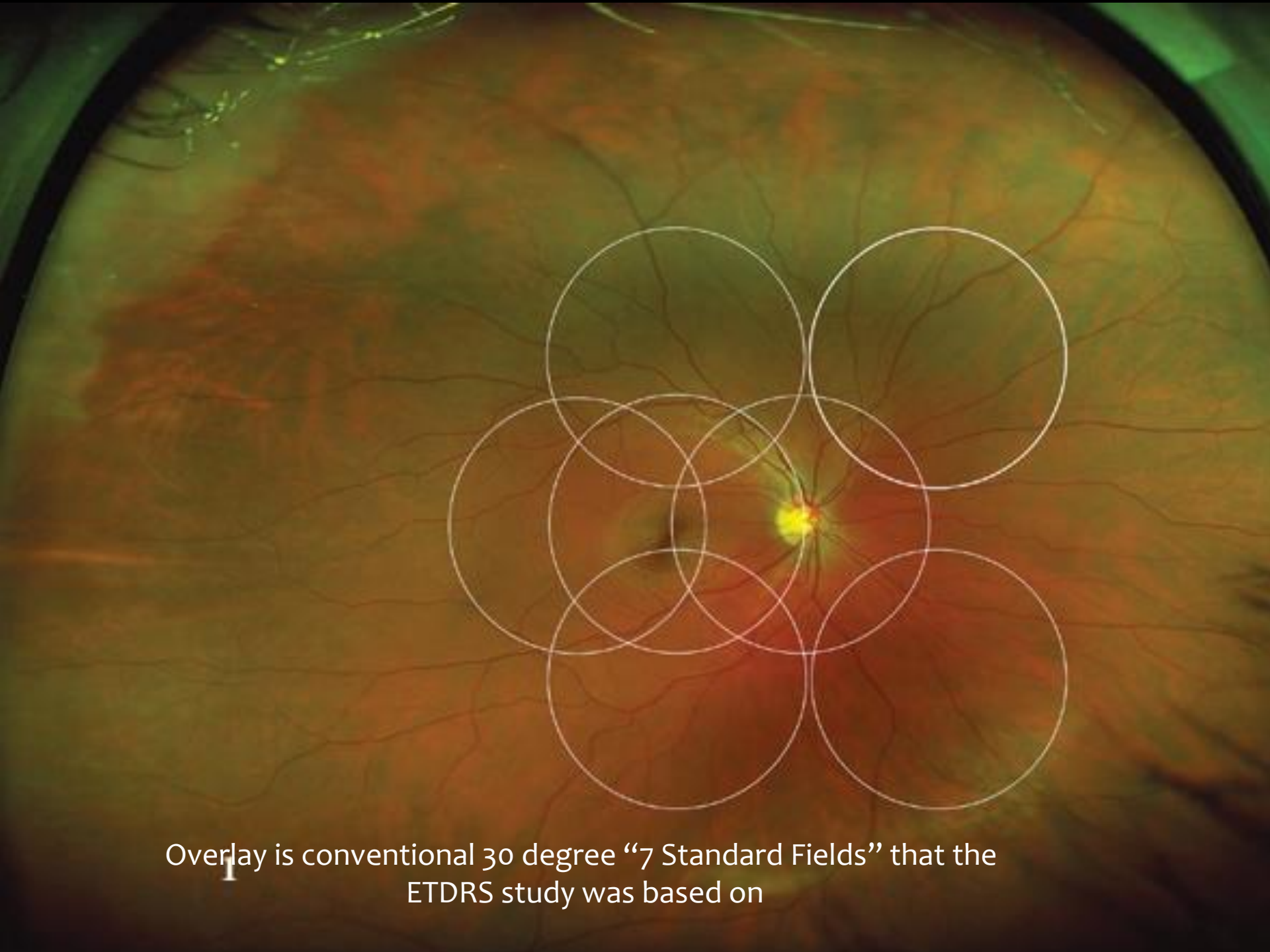
Photoshop montage of retina.

“Panretinal”

Photo courtesy of West Coast Retina


Optos- The Ultrawide Receiver
200 degrees / Approx. 82% of retina
A bit misleading-Optos measures from the center
Of the vitreous- others measure from the center of the
pupil (about 135 degrees


200°
82%
45°
15%
135 degrees
82%
30 degrees
15%

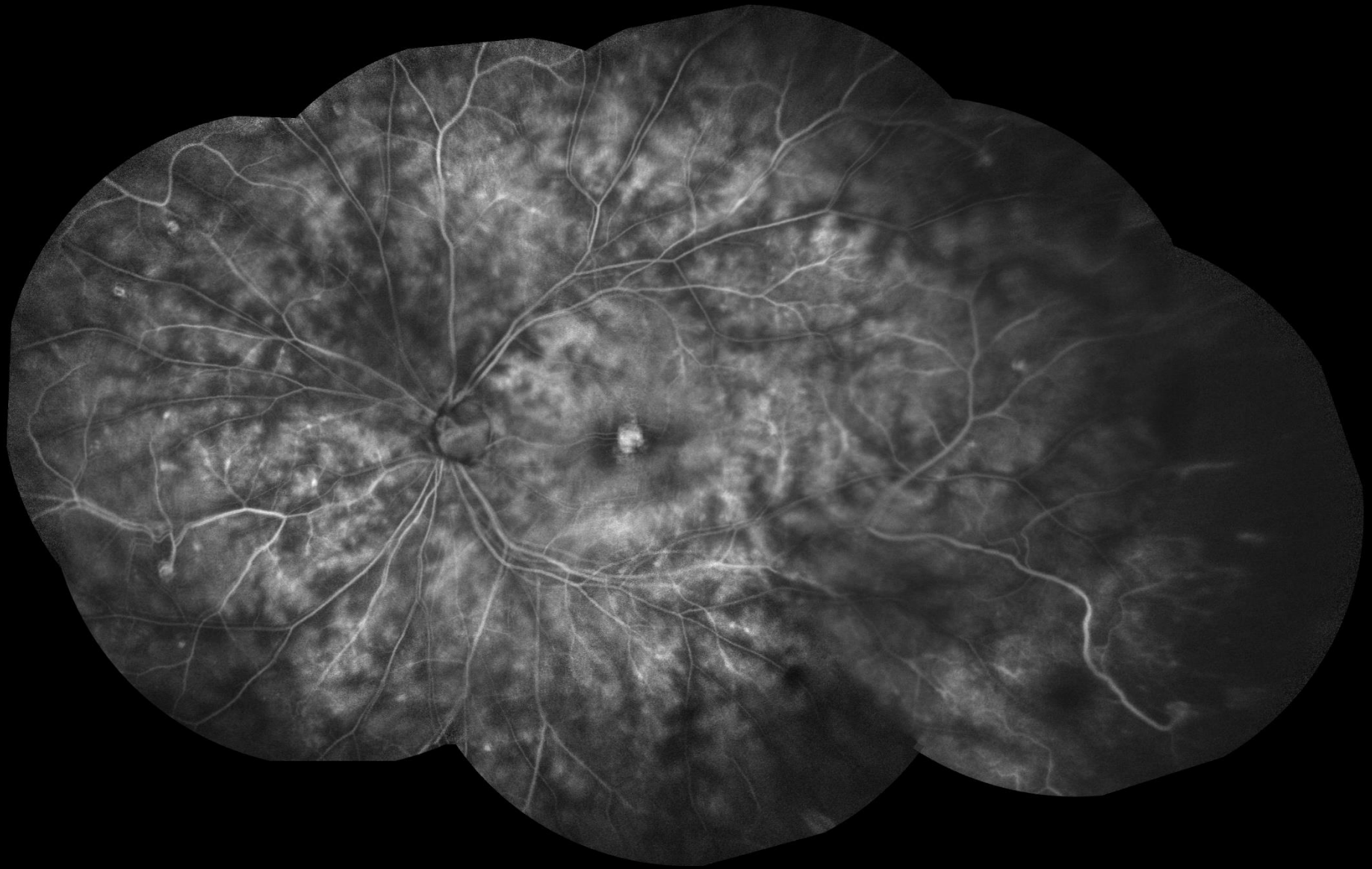


Overlay is conventional 30 degree “7 Standard Fields” that the ETDRS study was based on

4 50 degree images
“Stitched” using OIS software
Topcon 50ex fundus camera
CPPAEYE1
02/01/2016
1
Not original data

Fundus vs cSLO

Zeiss Clarus 700 133 degrees



Handheld Retinal Imaging

Intraoperative or EUA

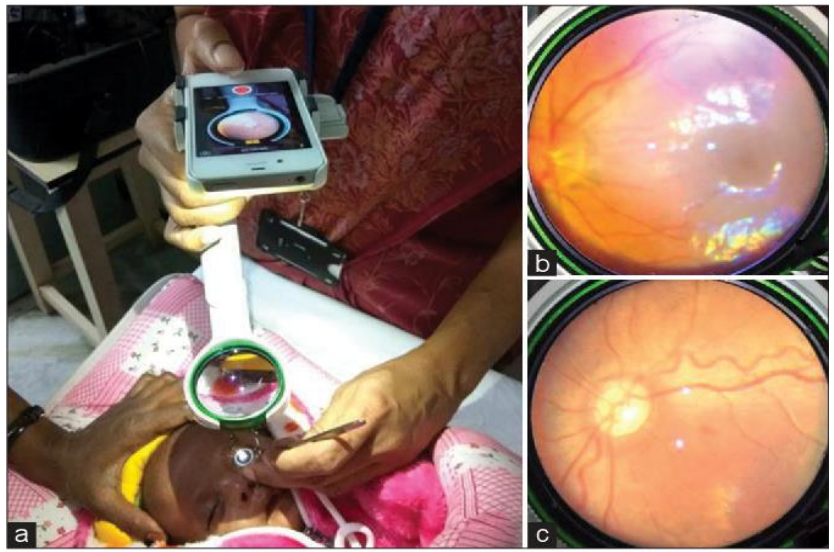
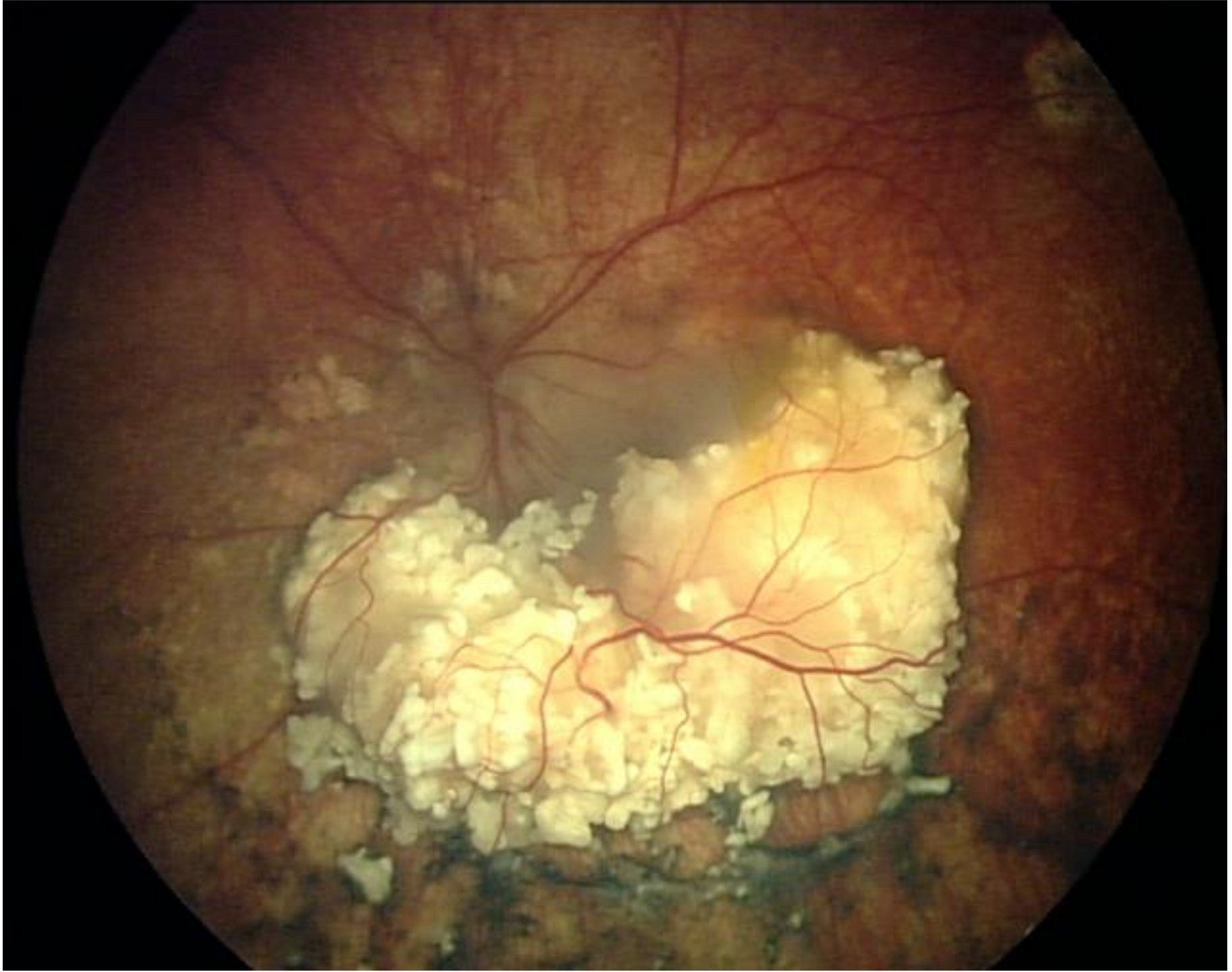
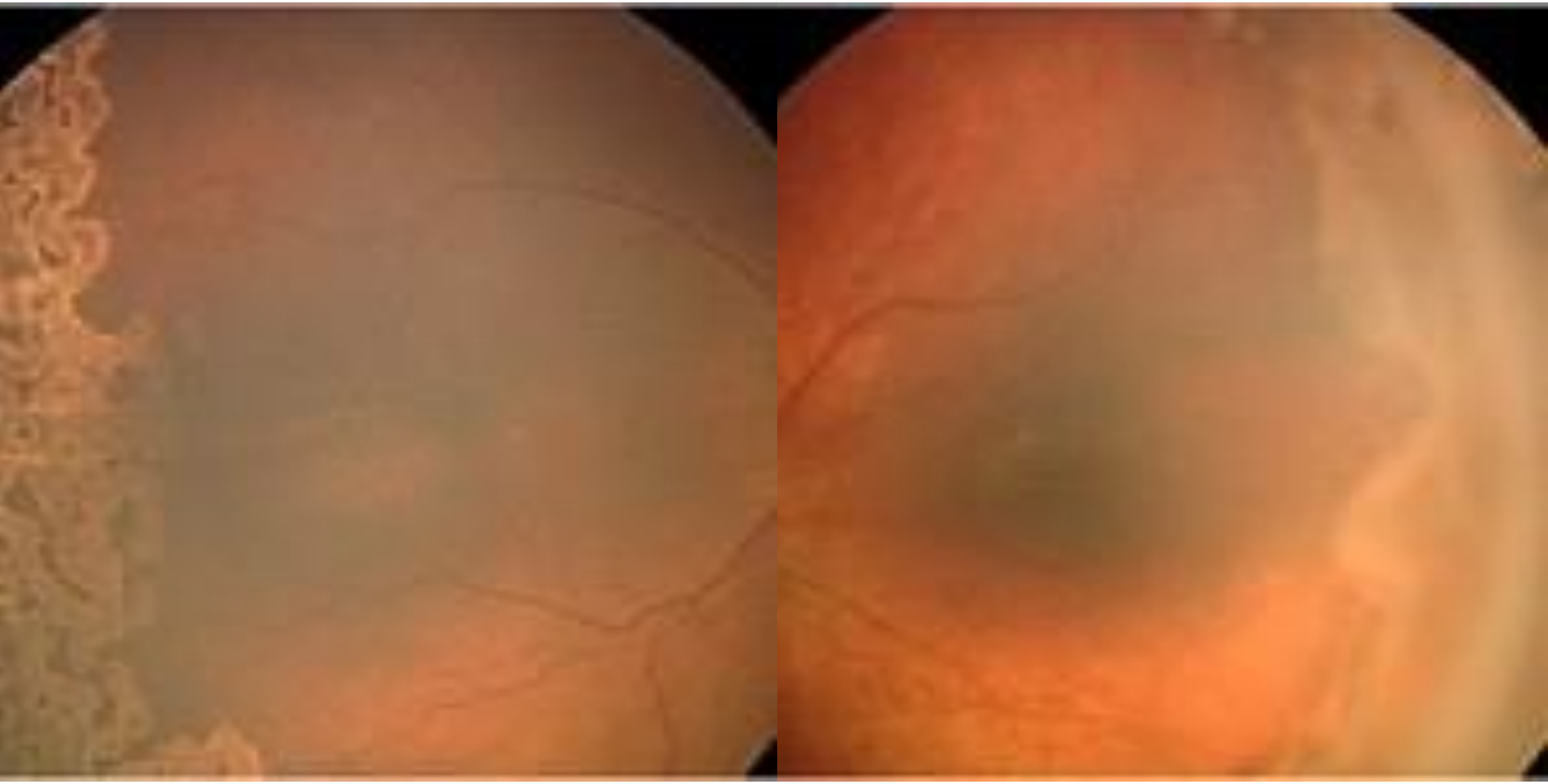
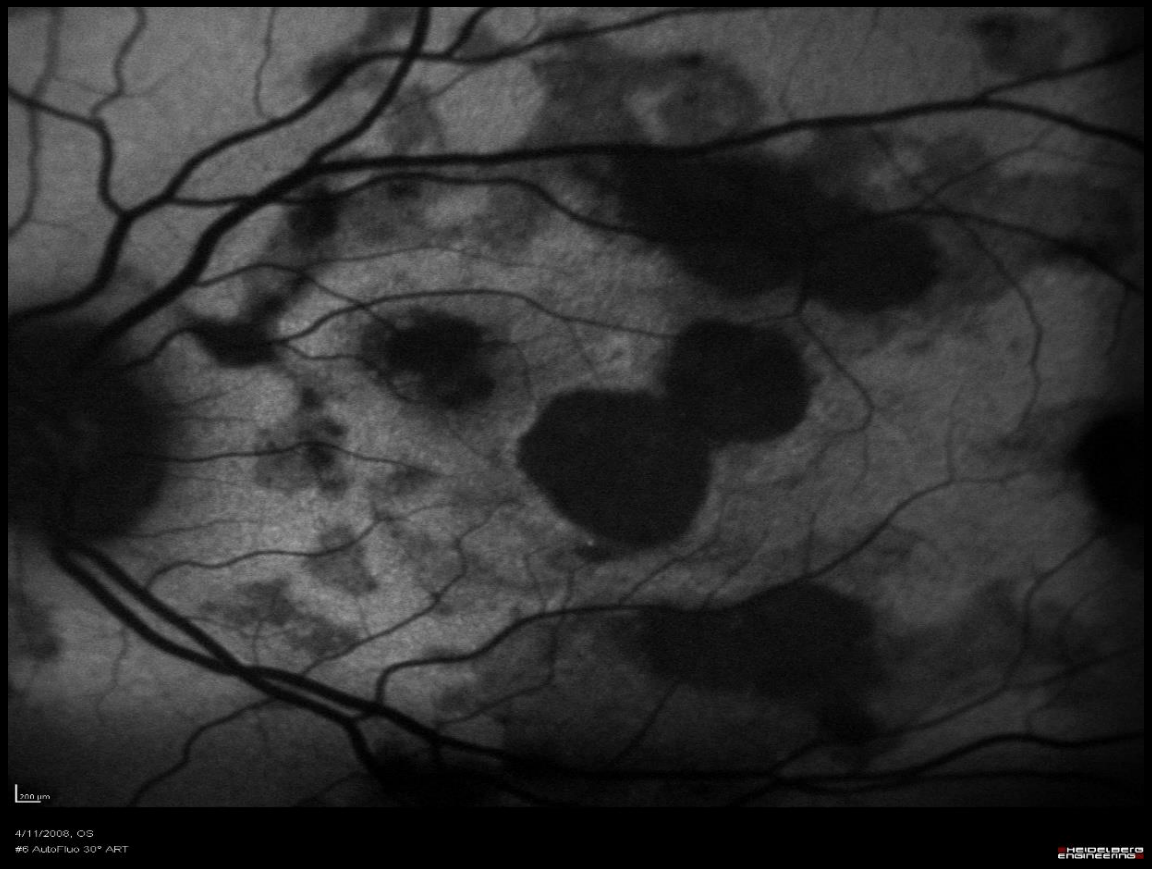


Figure 1: Showing (a) the technique of MI RetCam assisted fundus





Smart Phone based Systems



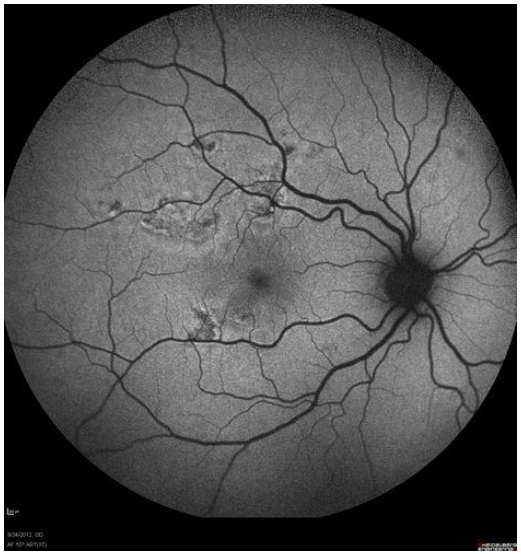
Fundus Autofluorescence

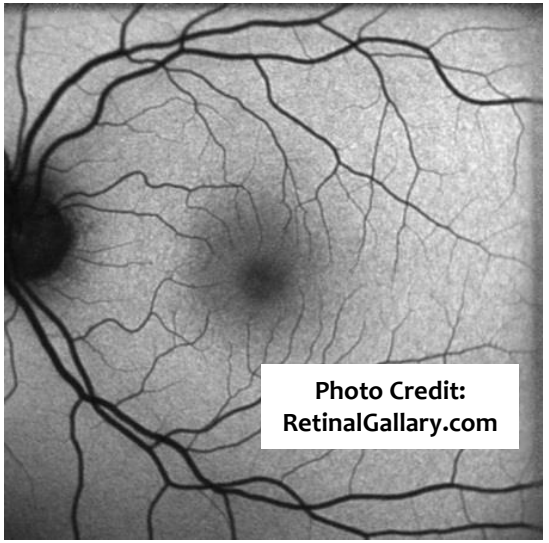
What is FAF Imaging?

- **Fundus auto fluorescence** (FAF) is a non-invasive retinal imaging modality used in clinical practice to provide a density map of lipofuscin, the predominant ocular fluorophore, in the retinal pigment epithelium.
- This fluorescence makes it possible to visualize lipofuscin *non invasively* with fundus imaging devices
- Can be done with a standard fundus camera with special filters, or increasingly, using a scanning laser ophthalmoscope (slo, cSLO)
- FAF is increasingly used and has been proven to be a useful tool in the diagnosis of many retinal disorders, and determination of appropriate treatment options and therapies

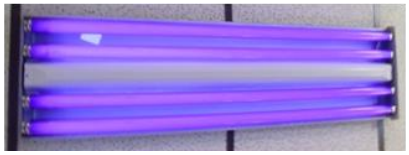
Origins of FAF Imaging

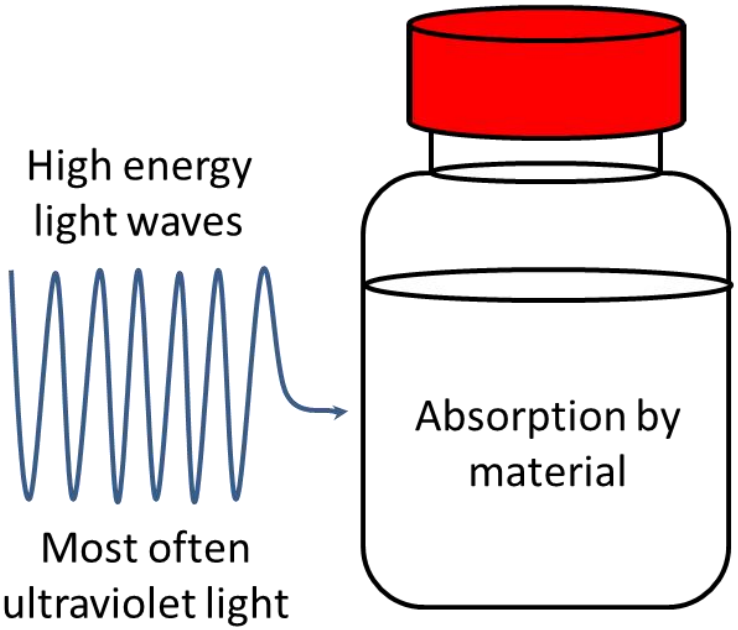
- Fundus autofluorescence (FAF) imaging using a confocal scanning laser ophthalmoscope (cSLO) was initially described by von Rückmann et al. in 1995.
- Pioneering work on the spectral analysis of the origin of the autofluorescence signal was performed by Delori and coworkers in parallel.
- In 2003, Spaide introduced a modified fundus camera (using longer wavelengths as originally applied) for FAF imaging. Further, near-infrared autofluorescence imaging has been described
- Improvement in imaging devices and filters continues to play an important role in the use of FAF as a diagnostic modality





Fluorescence





Fluorescence happens when something absorbs light of a certain energy and then emits light with (typically) less energy.

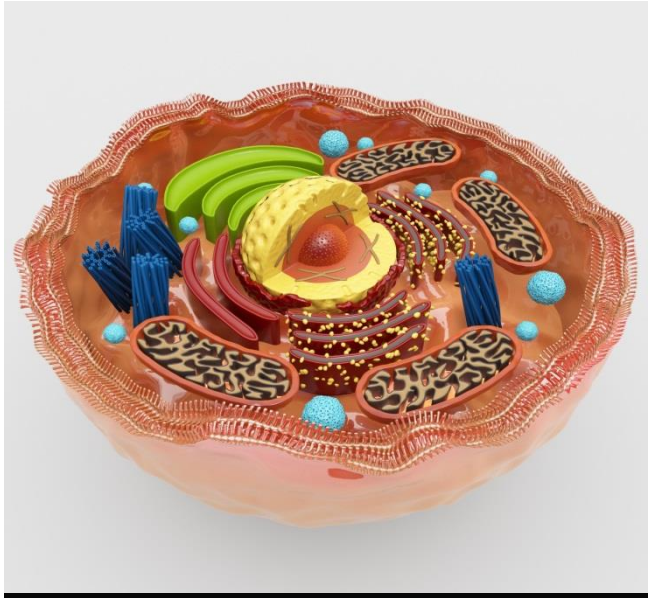
What is Lipofuscin?

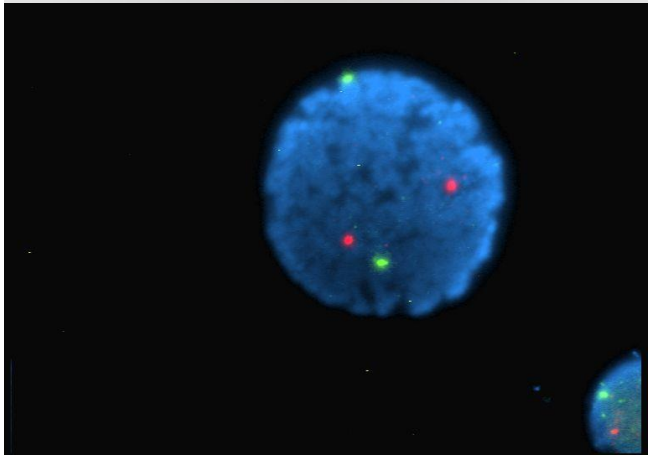
- Lipofuscin is a lipopigment, which is material in the lysosomal (the part of the cell that contains enzymes which break down wastes) compartment of non-dividing calls that cannot be degraded, and thus, it accumulates… **Molecular poop!**
- Lipofuscin contains fluoreophores that glow
- Lipofuscin is found in the **RPE**, the liver, kidneys, heart muscle, adrenals and **nerve and ganglion cells**

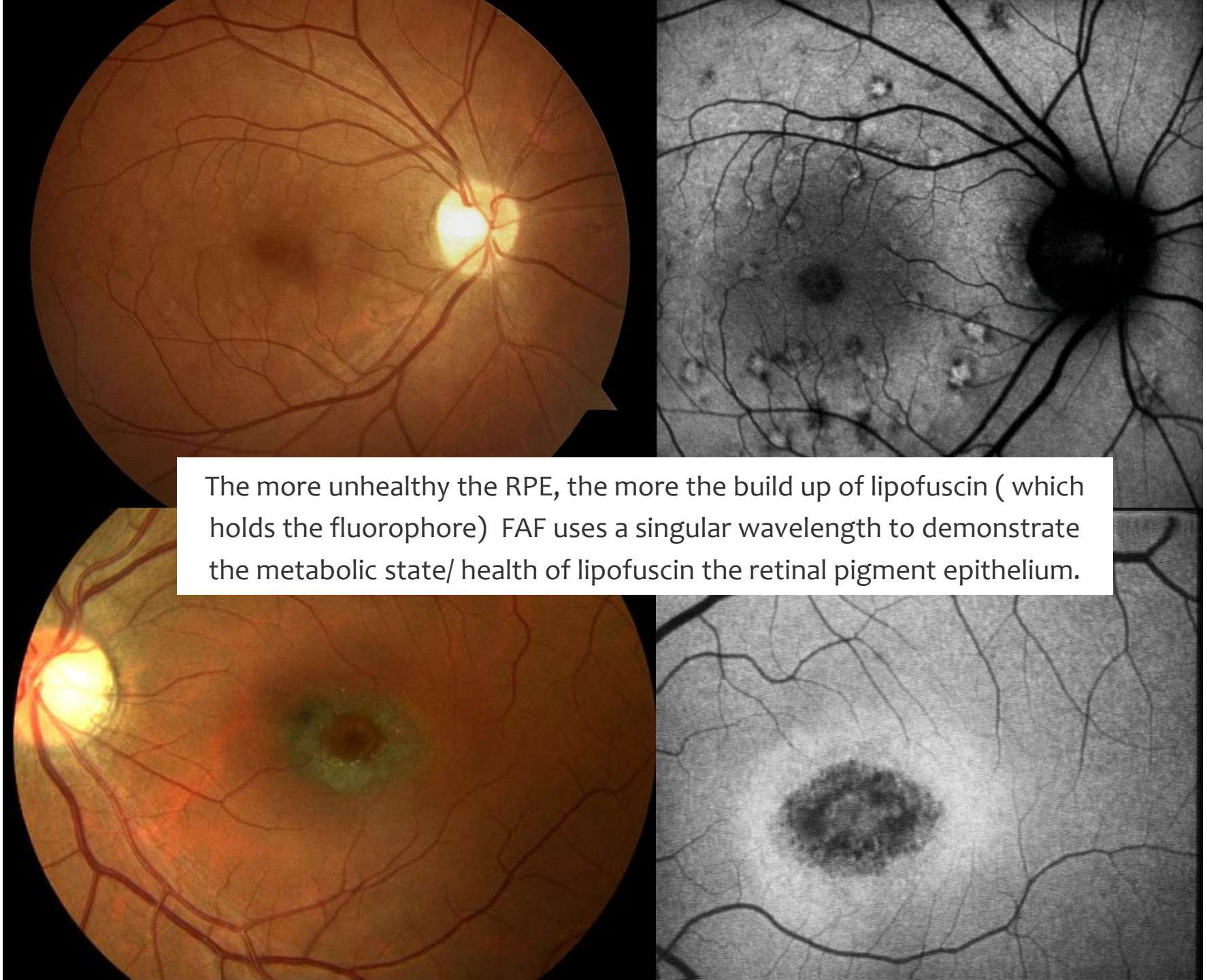
Liver or age spots Are Lipofuscin

What is a Fluorophore?

- A part of a molecule which causes that molecule to fluoresce
- This part of the molecule absorbs energy of a specific wavelength and re-emits energy at a different wavelength
- **The eye has over 10 naturally occurring fluorophores**
- **A2-E lipofuscin** granules within the cell cytoplasm are a dominant fluorophore

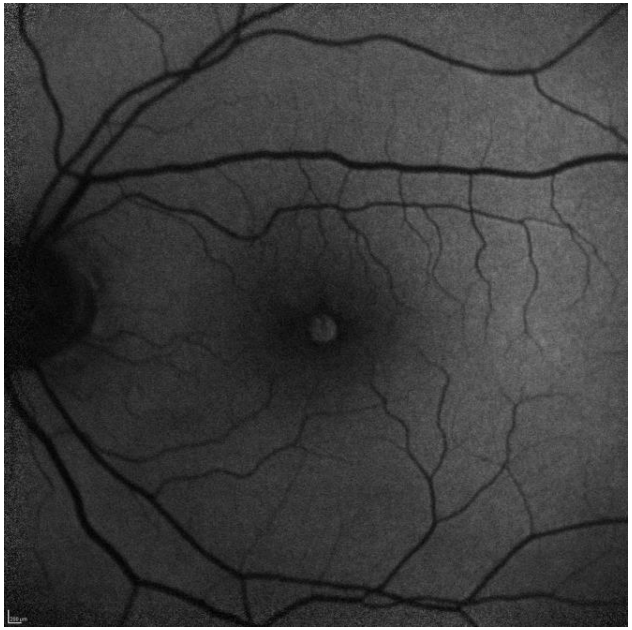


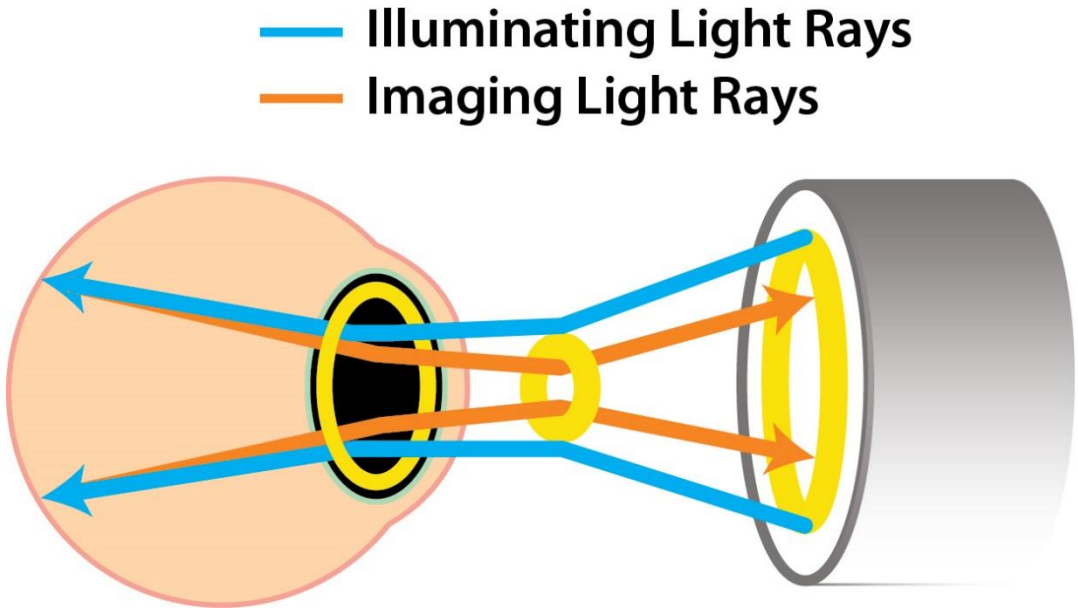




The more unhealthy the RPE, the more the build up of lipofuscin (which holds the fluorophore) FAF uses a singular wavelength to demonstrate the metabolic state/ health of lipofuscin the retinal pigment epithelium.

How FAF Imaging Works

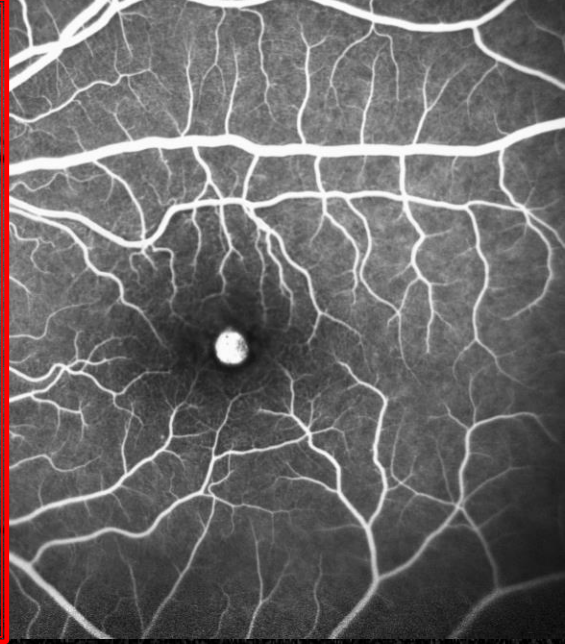


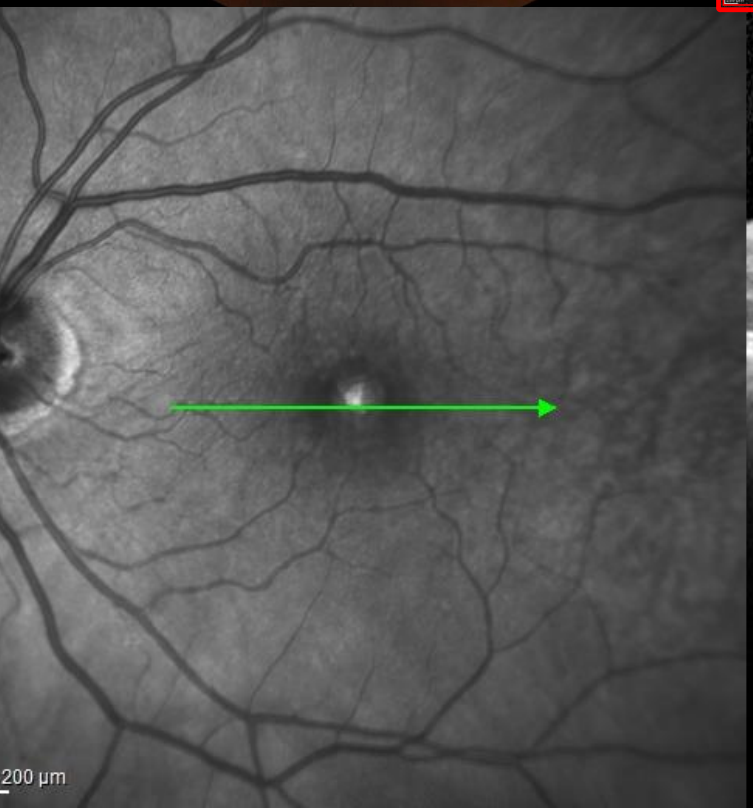


Camera light/ filters excites a molecule

Something in the RPE fluoresces (at about 400-450 nm)

Emission of fluorescence is sent back to create image




200 µm

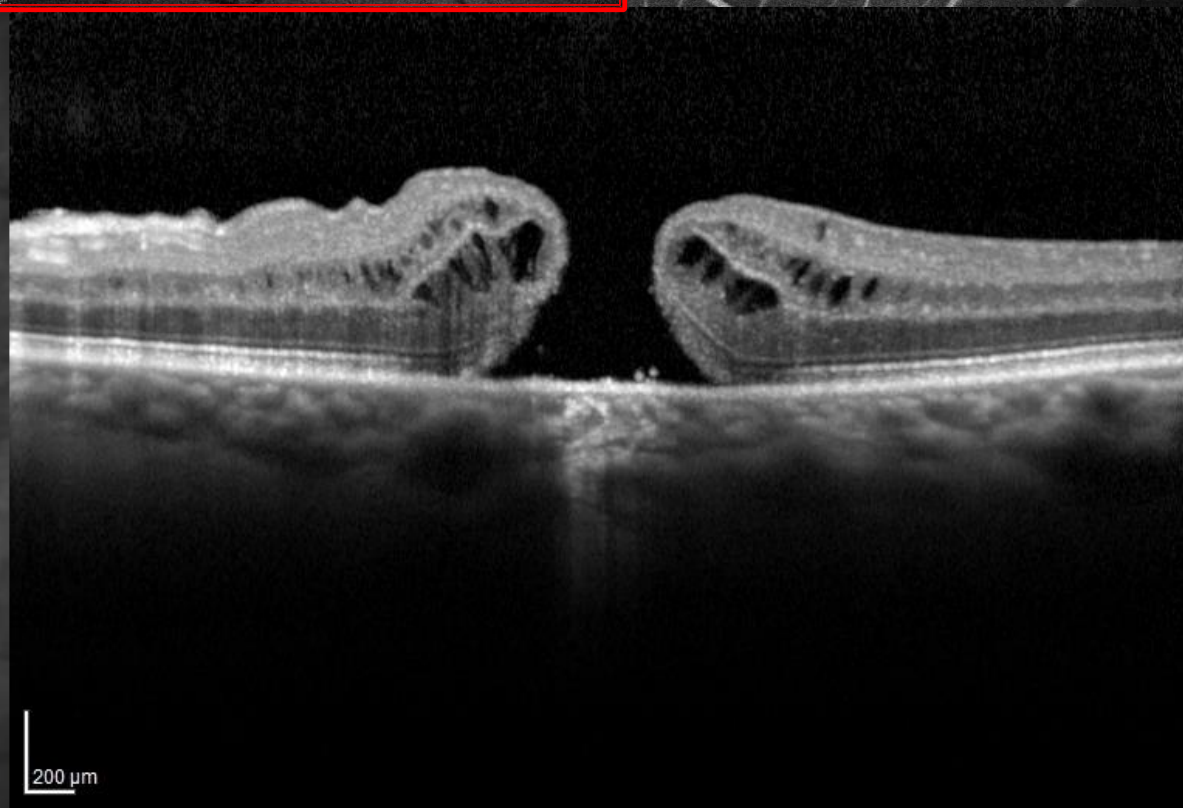

200 µm

Photo: AIOP Photo Gallery
Retinitis Pigmentosa

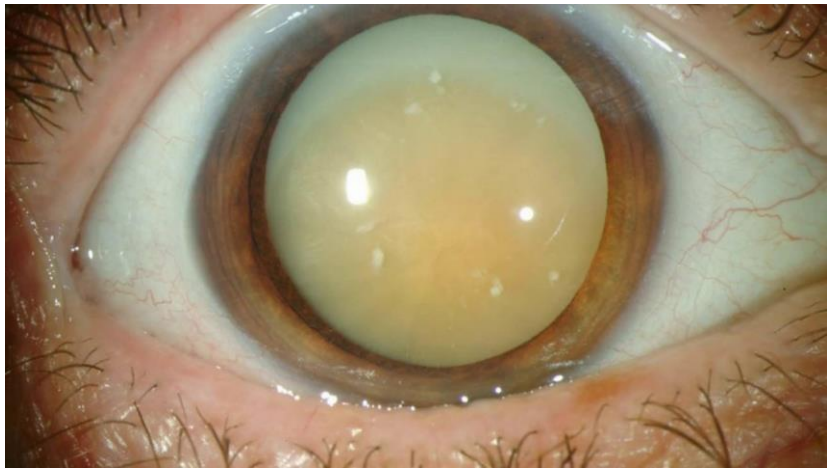
Barriers to Good FA Imaging

- In principle, the intensity of the autofluorescence signals depends on the *concentration of* lipofuscin in a structure

However, the signal intensity can be affected by a complex interaction between the distribution of fluorophores, structure of the retina, and pathologic changes

The main barrier is the crystalline lens which has highly fluorescent characteristics in the short wavelength range (excitation at 400-600 results in peak, or best emission at about 520 nm)

The yellowing of the lens is in direct conflict with the FAF filters



Autofluorescence

Visualizes lipofuscin at the level of RPE

Increased

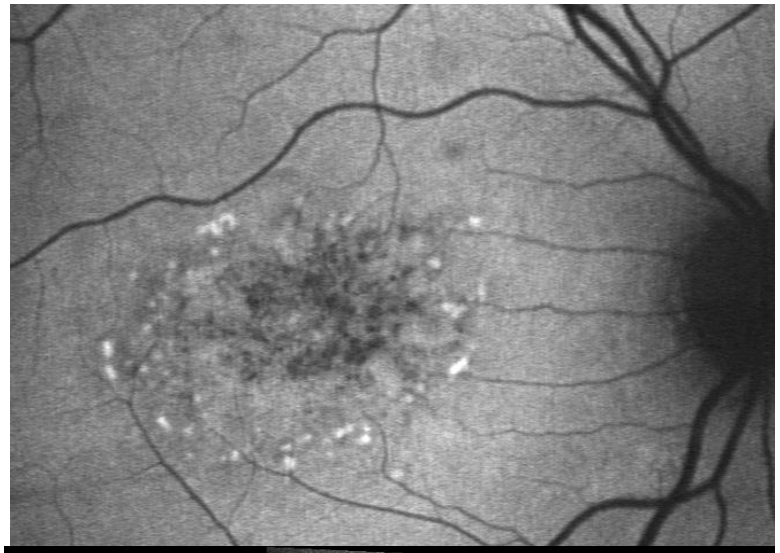
- High turnover of photoreceptor segments
- Inability to recycle metabolites

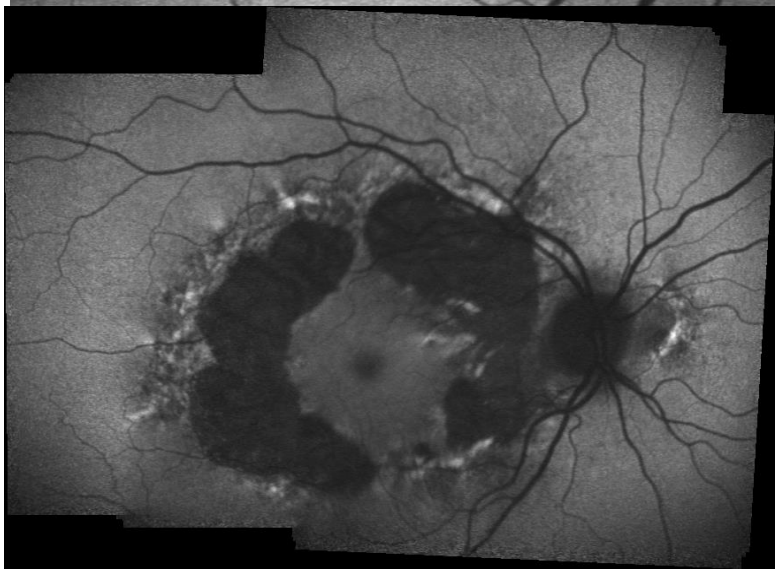
Decreased

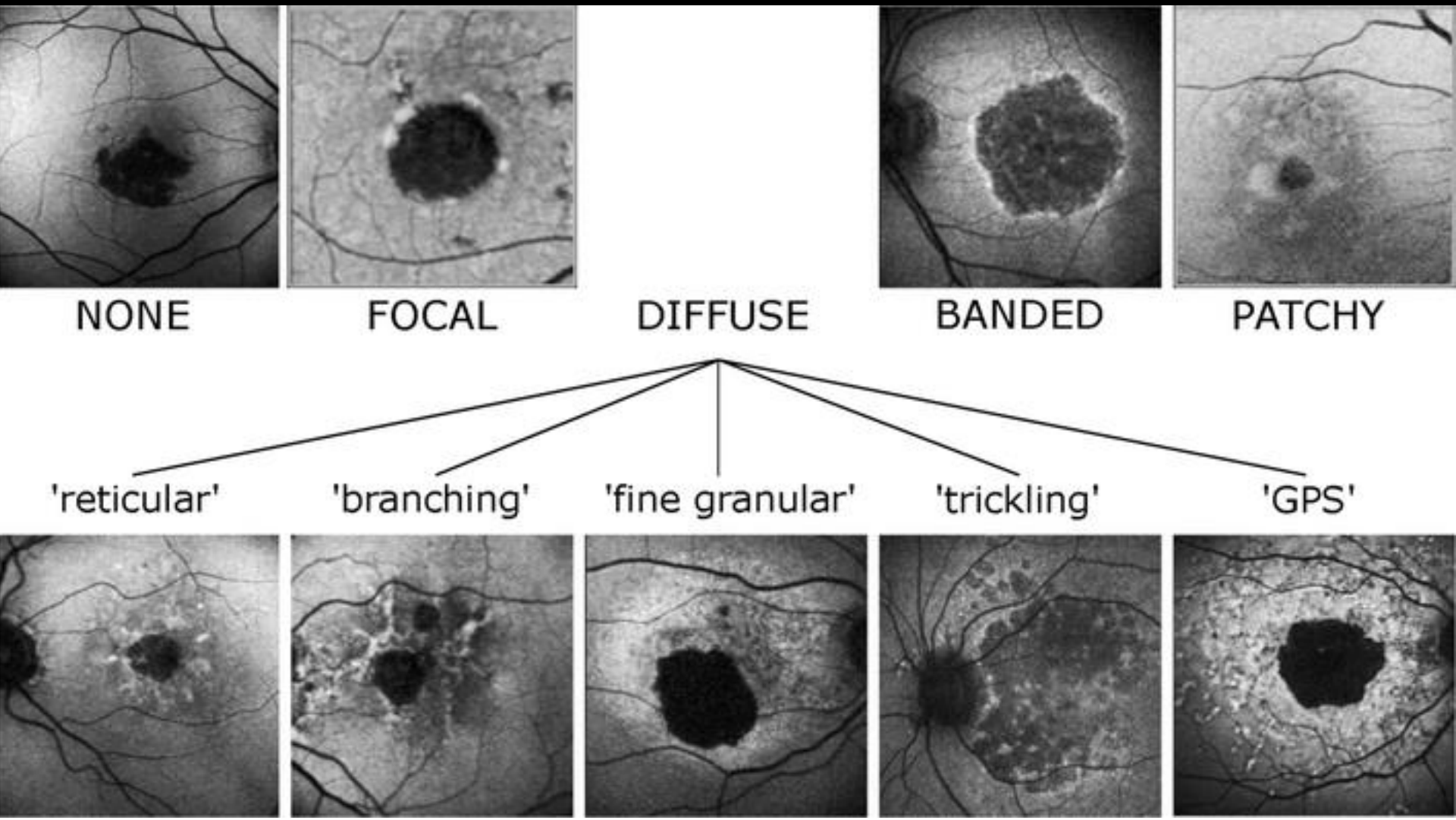
- Reduced metabolic demand

Absent

- Atrophy or death







Visualizes lipofuscin at the level of RPE

Increased

- High turnover of photoreceptor segments
- Inability to recycle metabolites

Decreased

- Reduced metabolic demand

Absent

- Atrophy or death

Simultaneous FAF and OCT

Simultaneous FAF and OCT imaging provides a new perspective on structure and metabolic activity within the retina.

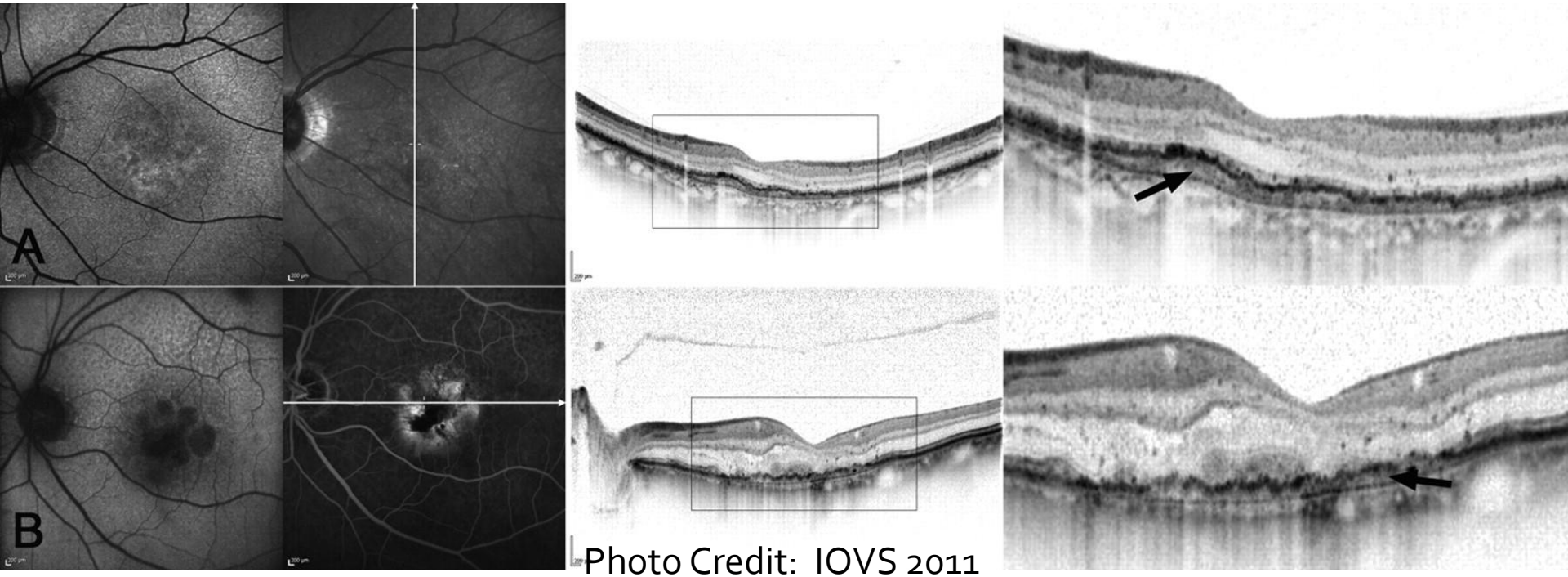
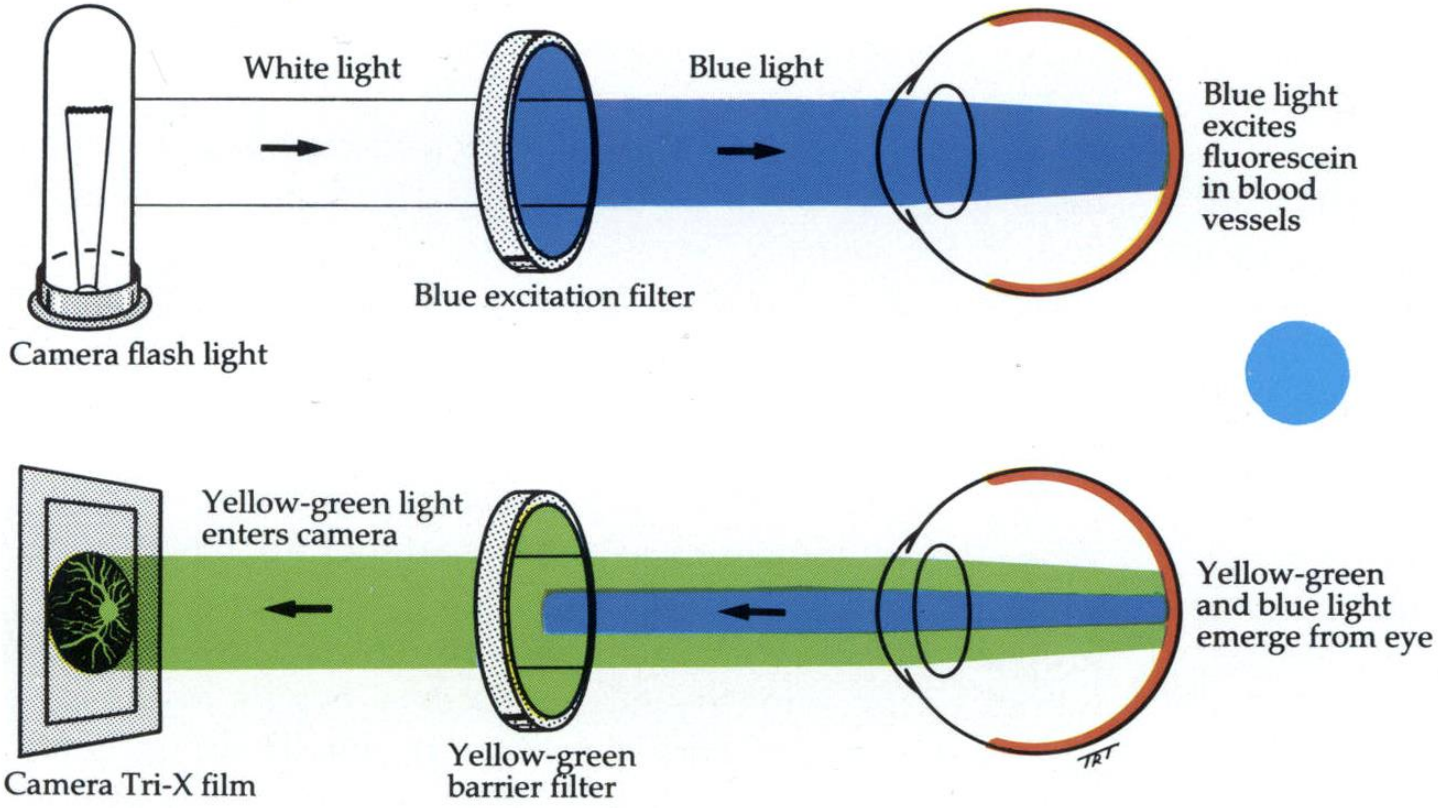


Photo Credit: IOVS 2011

Fluorescein Angiography

Fluorescein Filters

When illuminated using a retinal camera with a blue filter, the dye fluoresces and emits light in the yellow-green spectrum.



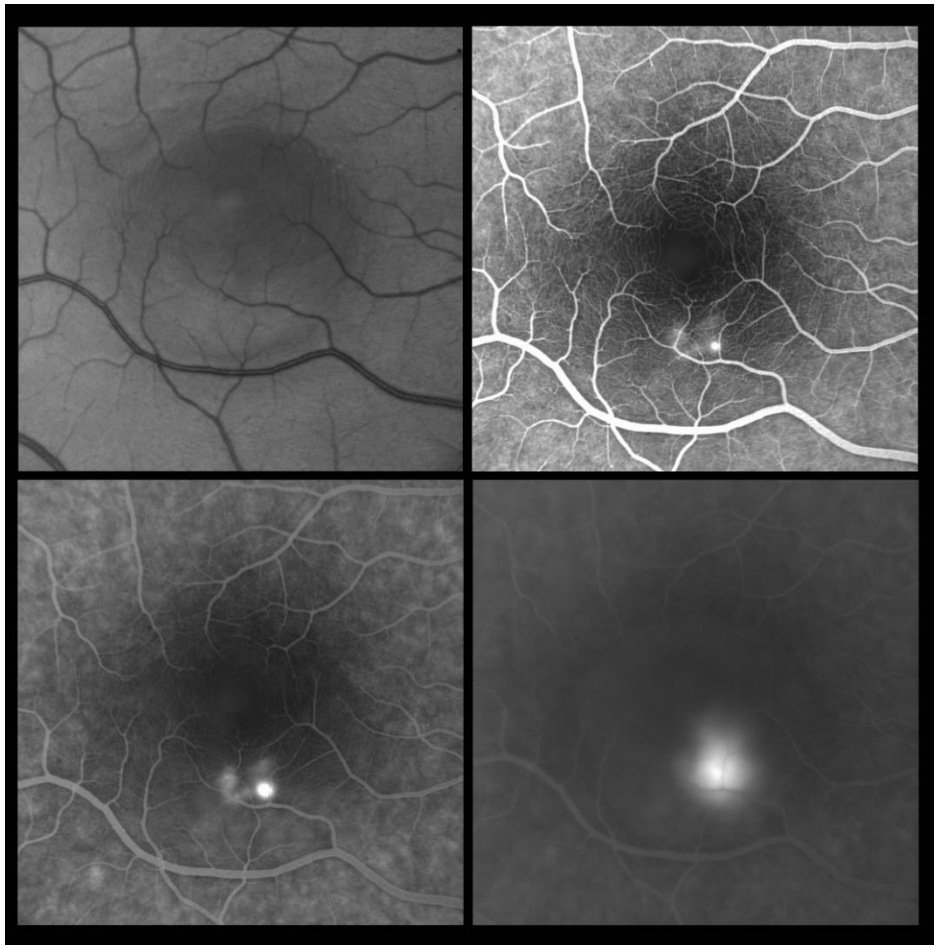
Fluorescein Angiography

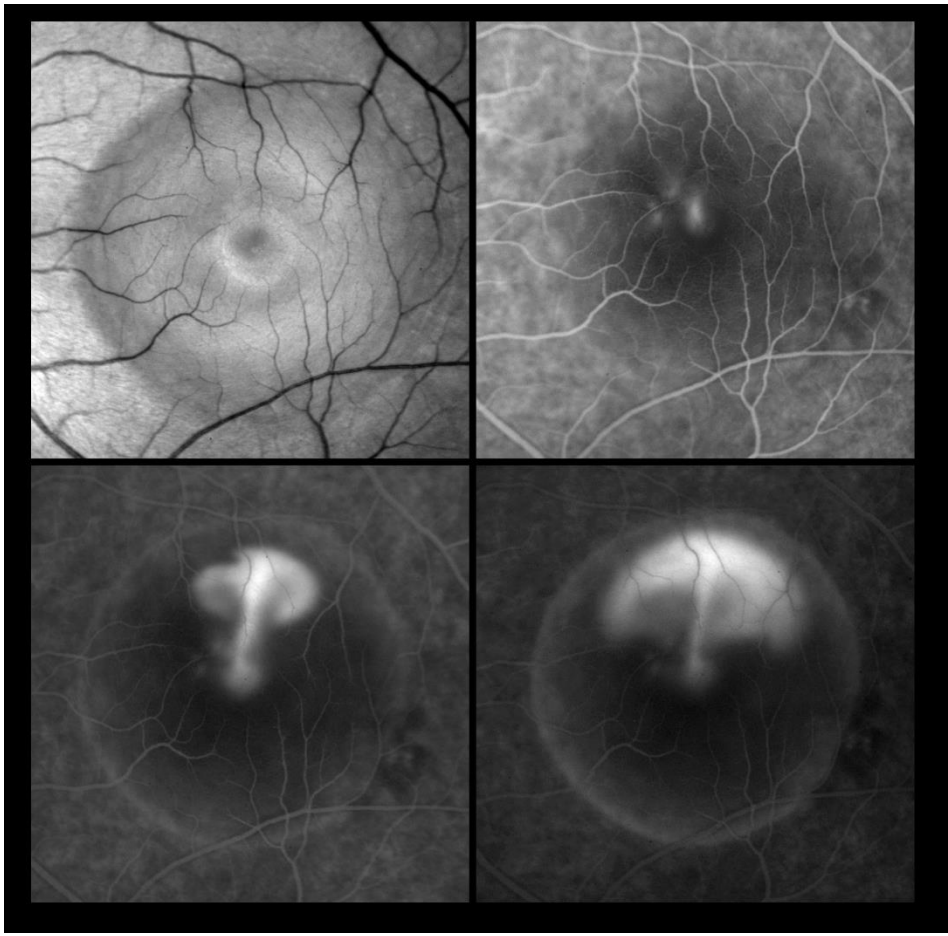
Through the use of either IV or oral sodium fluorescein dye, fluorescein, angiography allows for the visualization and documentation of ***flow*** through the retinal vasculature over time

Color vs Angiography

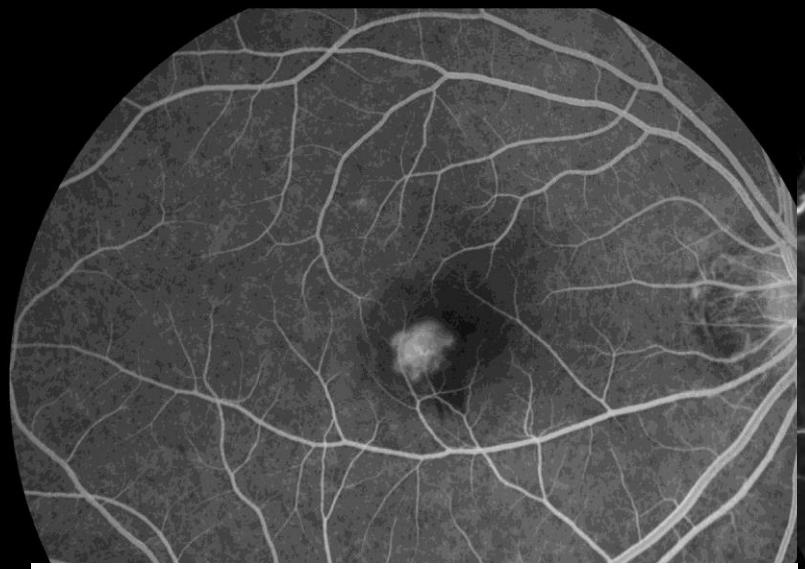
These color images record the appearance of the retina as the ophthalmologist has seen it during the exam, providing a reference image to compare with various frames of the angiogram

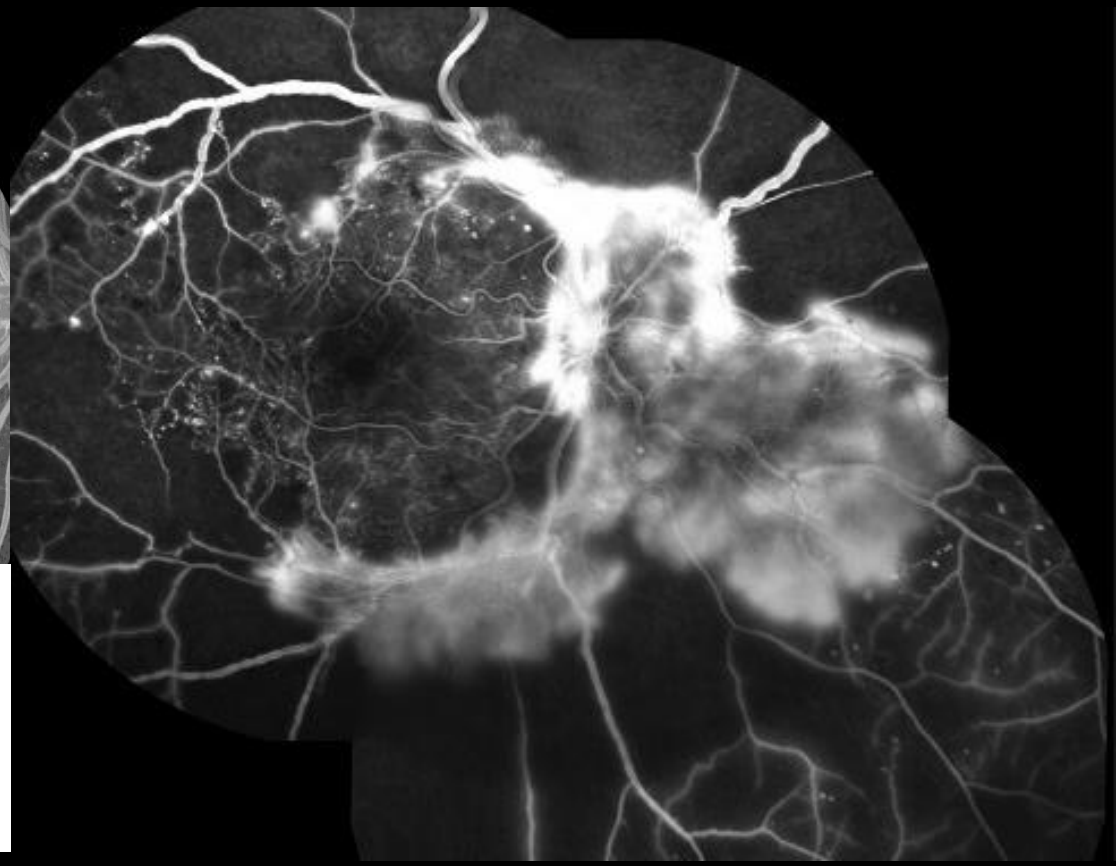
50 years of Fluorescein Angiography





Prime Contenders for FA



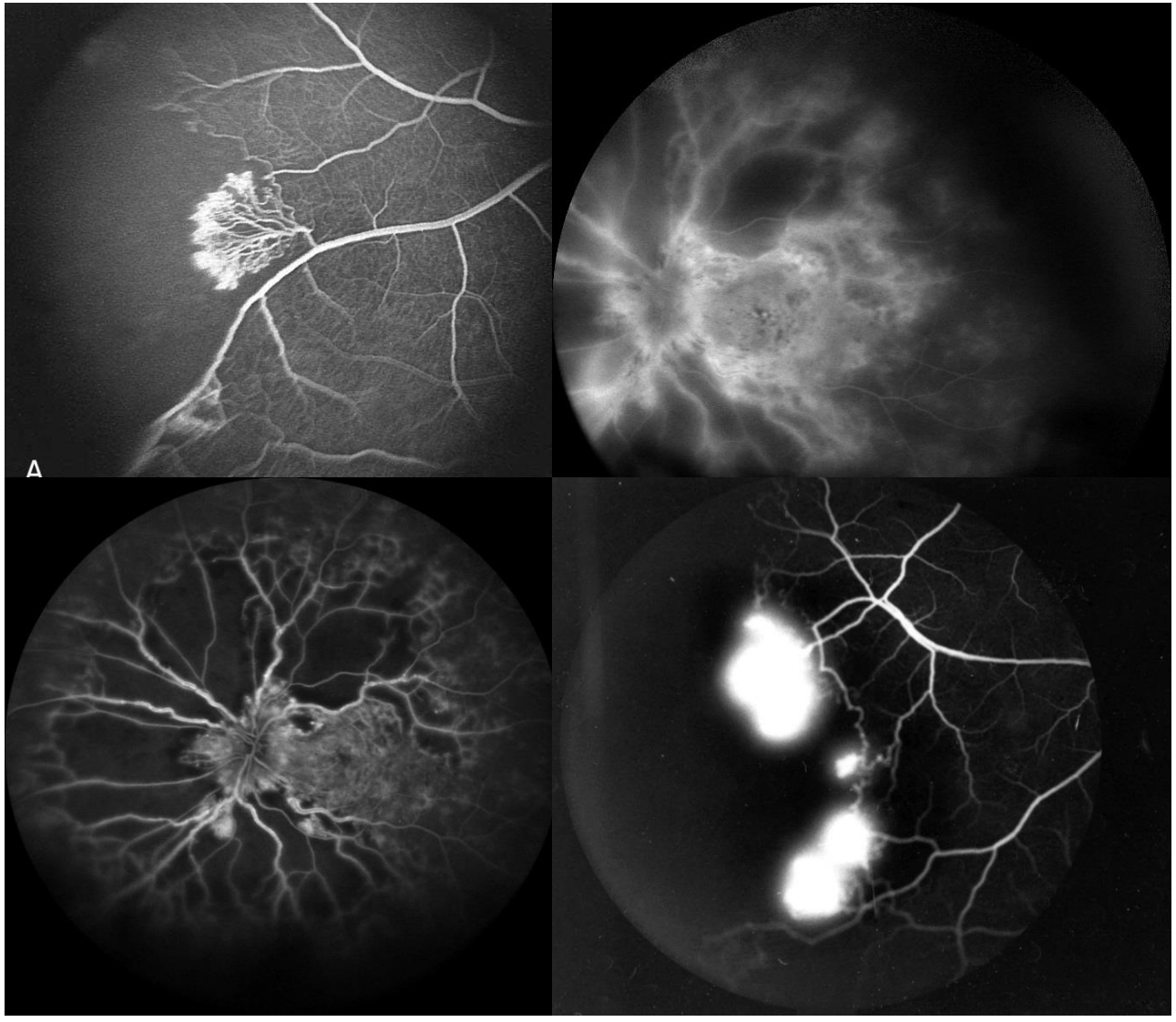


- Things that leak, plug up or are inflamed
- Retinal / choroidal vascular diseases
- Used to determine the extent of damage, to develop a treatment plan, or monitor results of a treatment / Still used in many Clinical Trials

Current SD-OCT systems do not have the resolution to see abnormal vessels in CNVM. In Diabetes, the leakage, blockage and new vessel growth demands a good hemodynamic study

Is Angiography Dead?

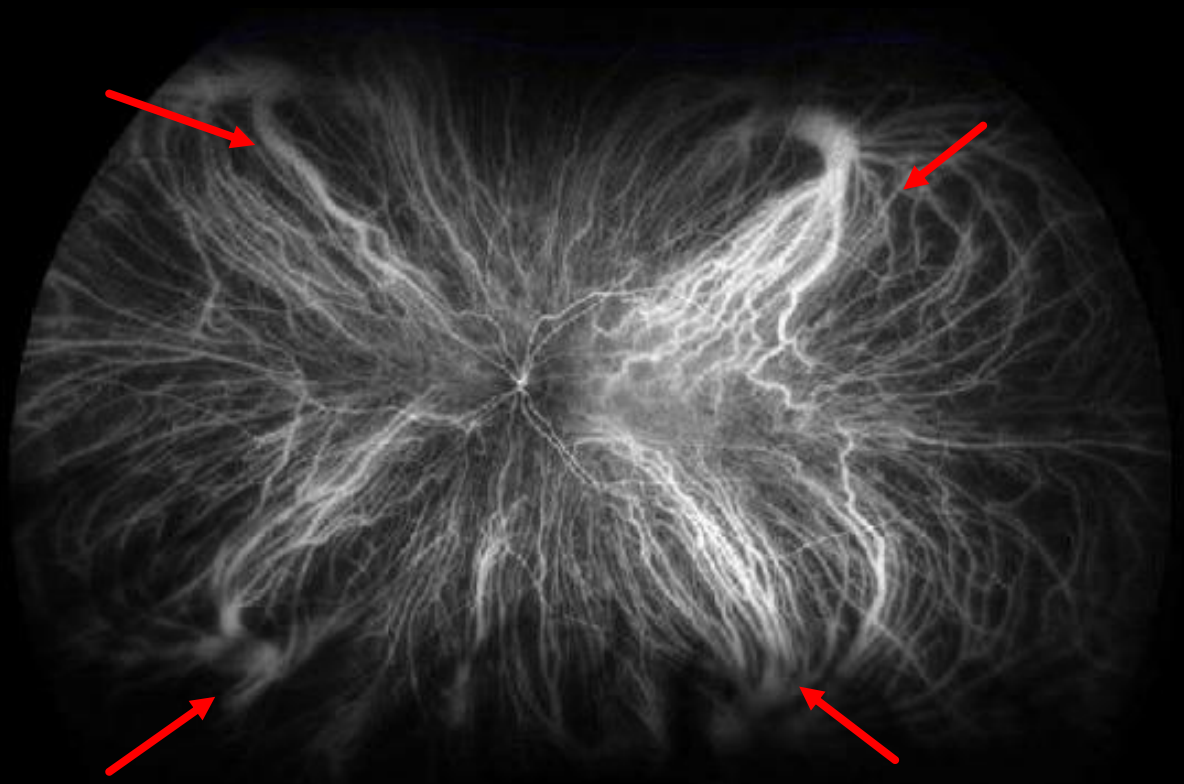
Why We Still Need IV FA



Sodium Fluorescein demonstrates ***LEAKAGE…***

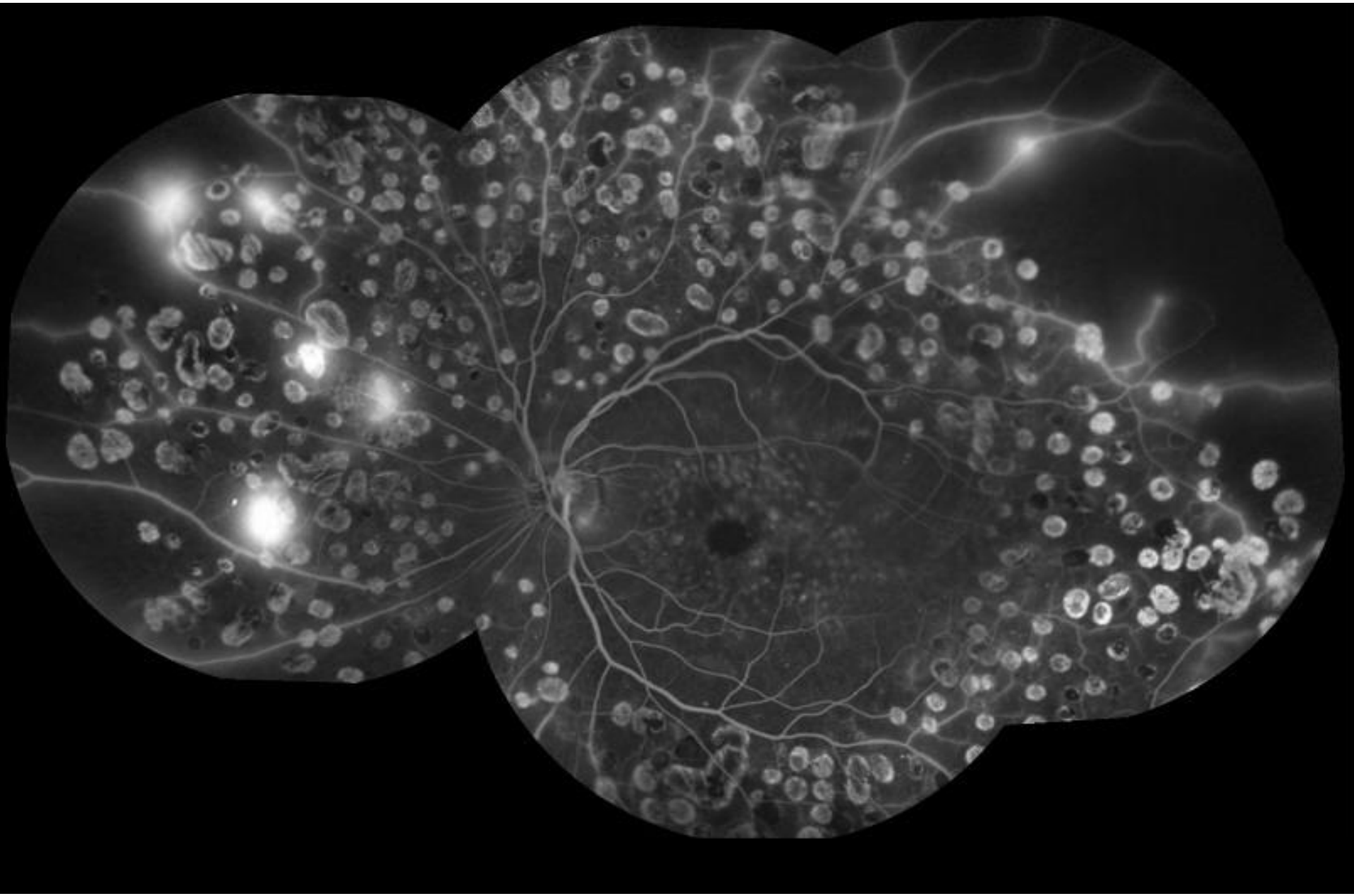
Are Wide field Color images enough to treat/ Laser?

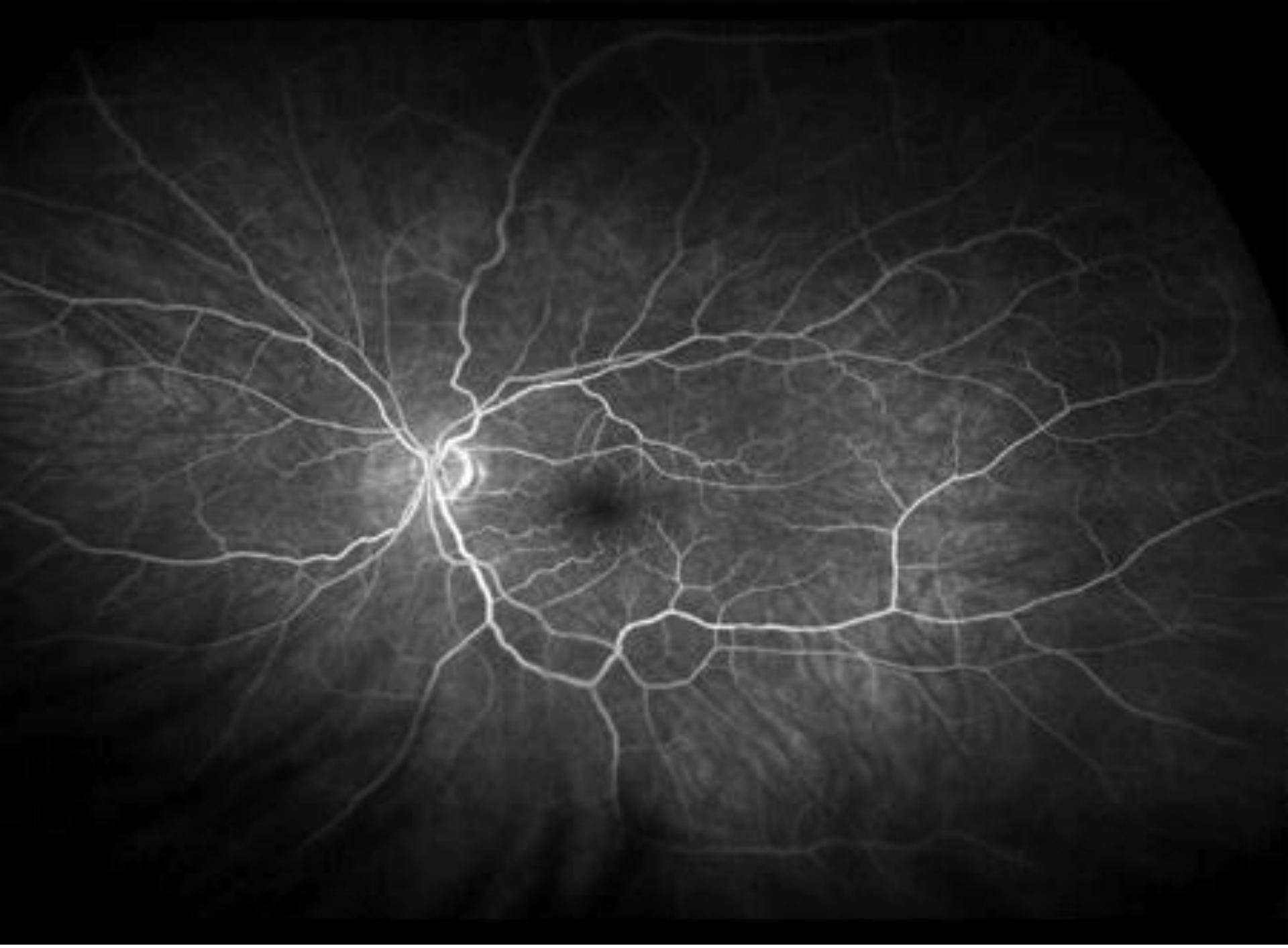
Do we yet have enough confidence to trade conventional FA for OCTA?

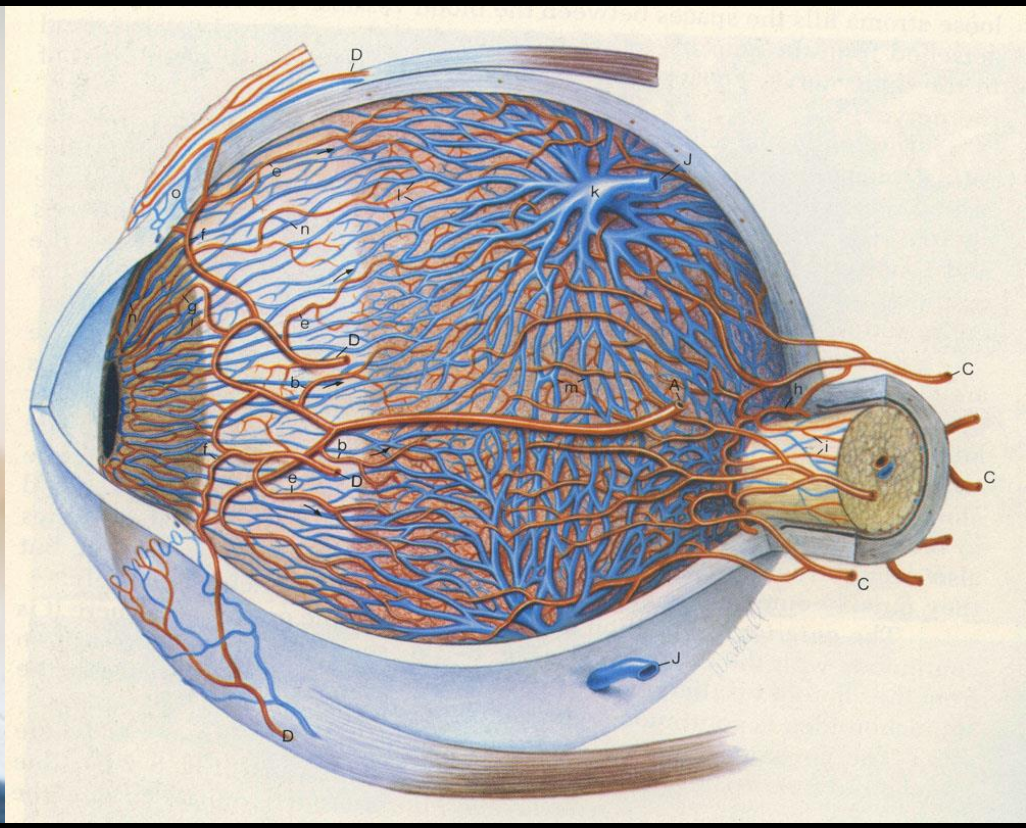


Wide Field Angiography

The edge of the fundus: clinically one of the easiest ways to locate equator of the fundus is to find the vortex veins.







Choroidal Angiography (ICG)

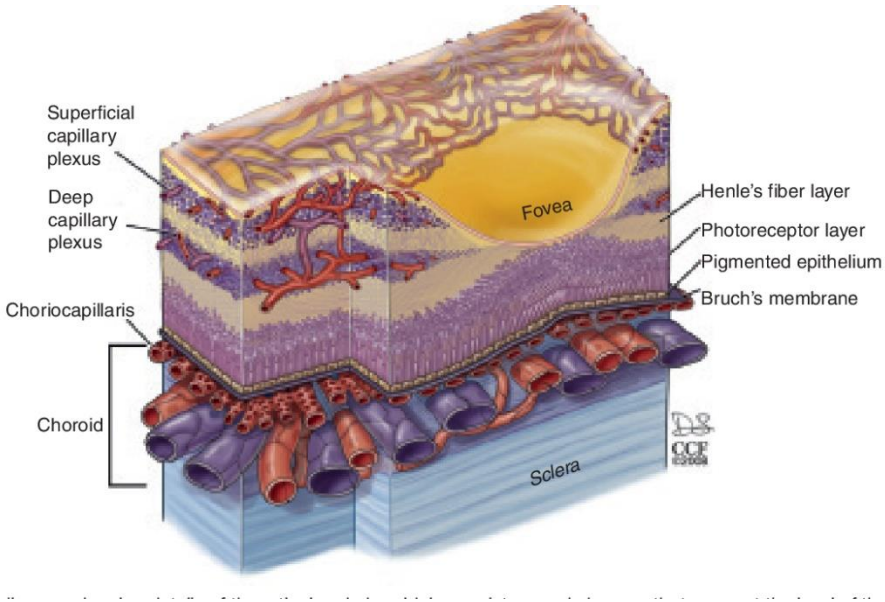
When you want to “Look Under the Rug”, the Retinal Rug that Is…

History of ICG

- Mid-50's with help of a grateful patient at Eastman Kodak
- Dyes used in color films and filters-ICG
- Kodak and Mayo clinic developed modern ICG dye
- First used in 1956 in field of cardiology looking for cardiac output defects
- First sterile, medicinal version "Cardio Green"
- Use in renal blood flow in 1964
- By 1970's widely used for cardiac output, hepatic function and liver blood flow
- **1980 first commercial ICG fundus camera system (TRC-W) but not marketed until 1987 (TRC-IA)**
- First Workshop n Clinical Choroidal Angiography in October 1984 (Spain) and International Symposium of Fluorescein Angiograph (1985) in Germany
- **First International Symposium on ICG Angiography in New York January 1993 (Yannuzzi)**

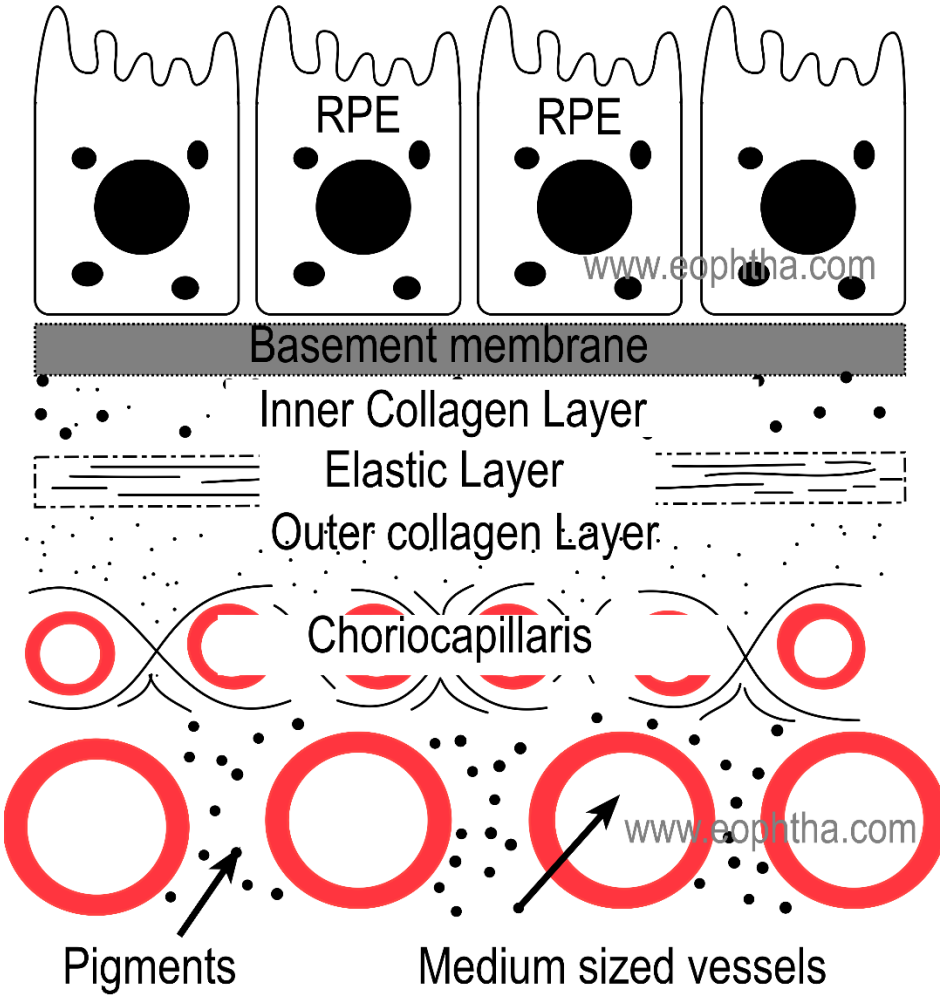
Indocyanine Green Angiography?

- Uses a Tricarbocyanine dye which is highly protein bound(Around 98%) ICG dye has a larger molecular structure too large to leak through natural fenestrations of the choriocapillaris.
- Like FA, ICG dye is injected intravenously, flows through the retinal and choroidal circulation- however, ICG Dye is retained in choriocapillaris- unlike FA which extravasates rapidly from the CC and fluoresces in the extracellular space

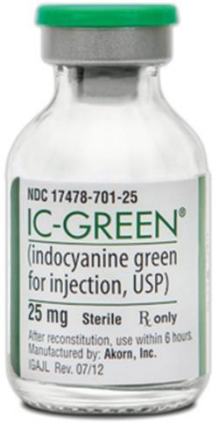


The remaining 20% or so of unbound molecules "glow" or "excite" when exposed to light of 520-530 nm. This is different than FA and FAF which allows ICG to see past the retina , by rendering the RPE "invisible" and allows for visualization of the underlying choriocapillaris

Sodium Fluorescein vs. Indocyanine Green

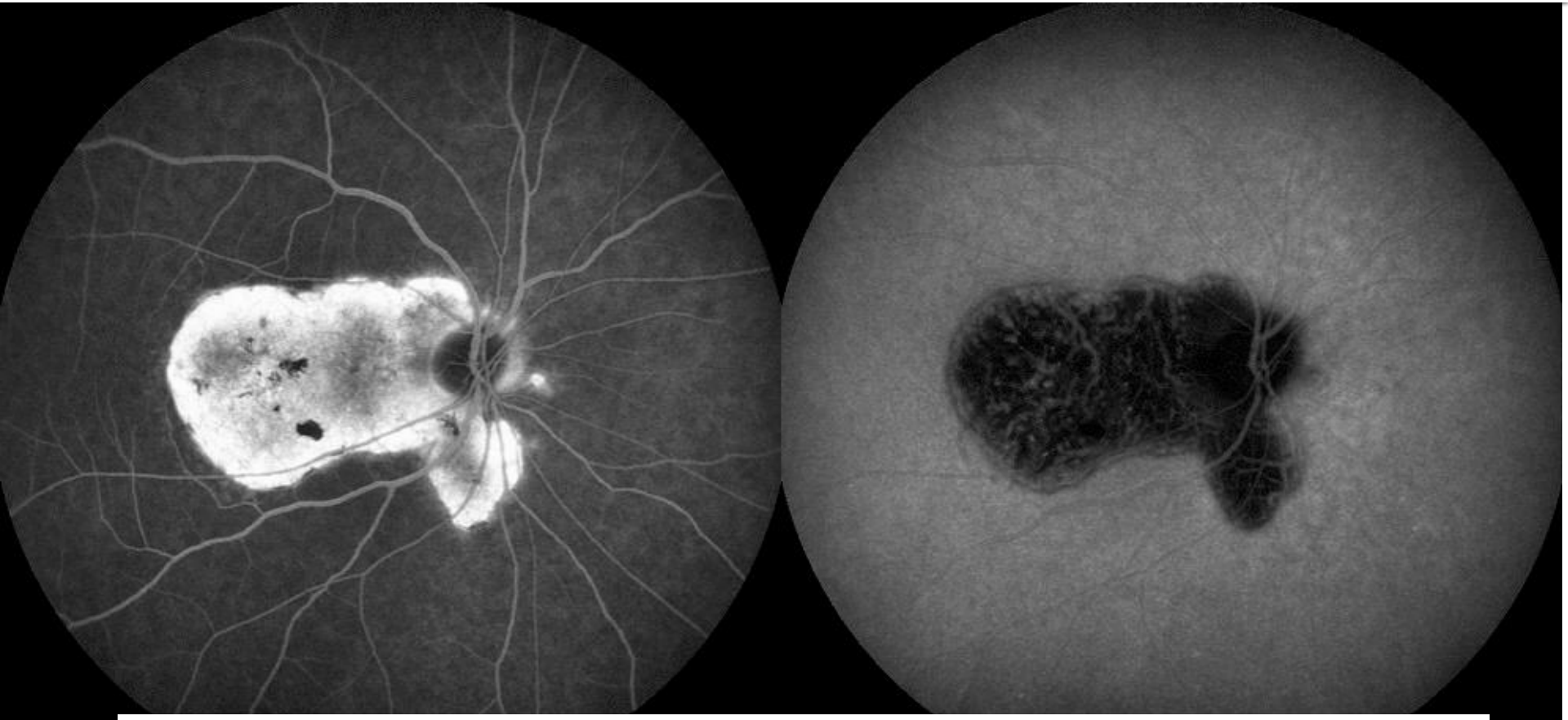


376.7 kilodaltons

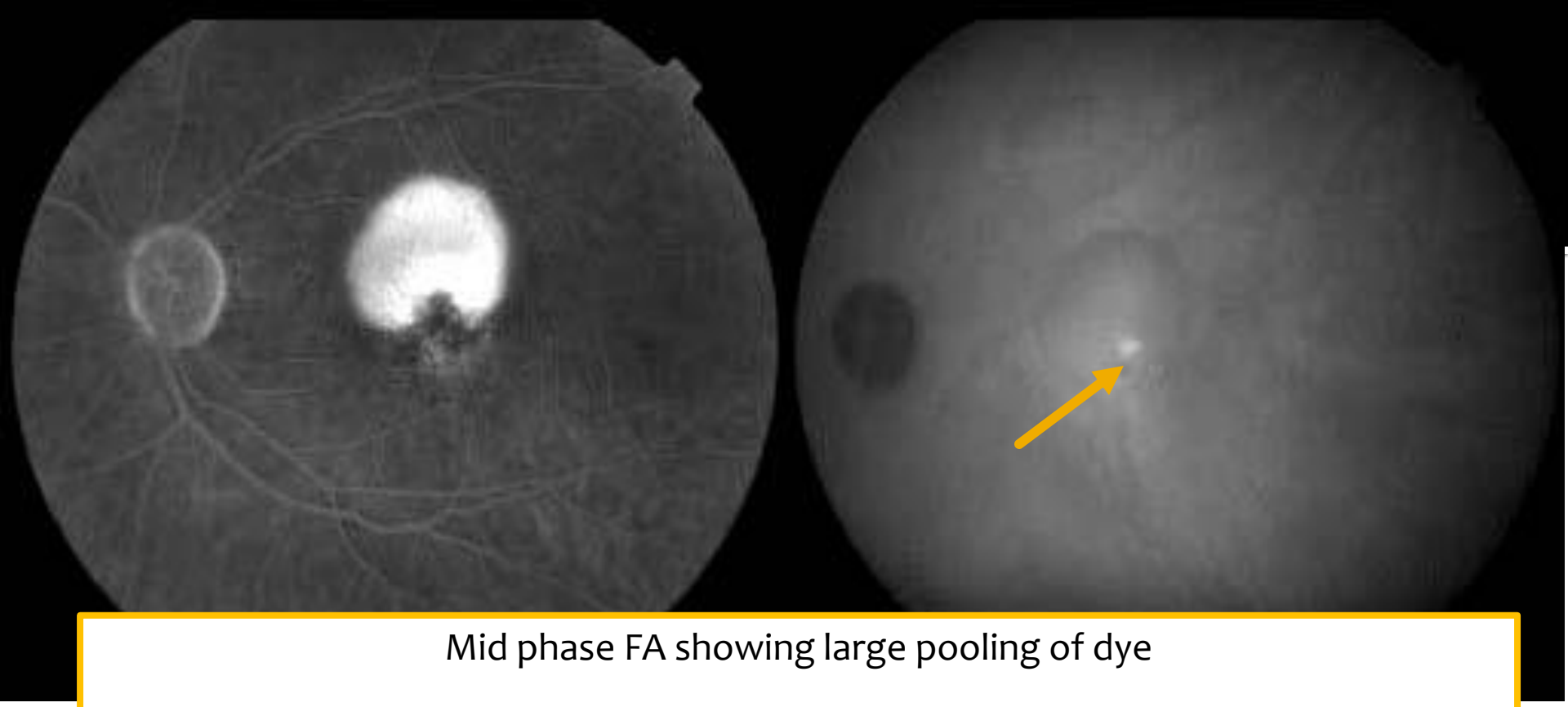


776 kilodaltons

FA and ICG



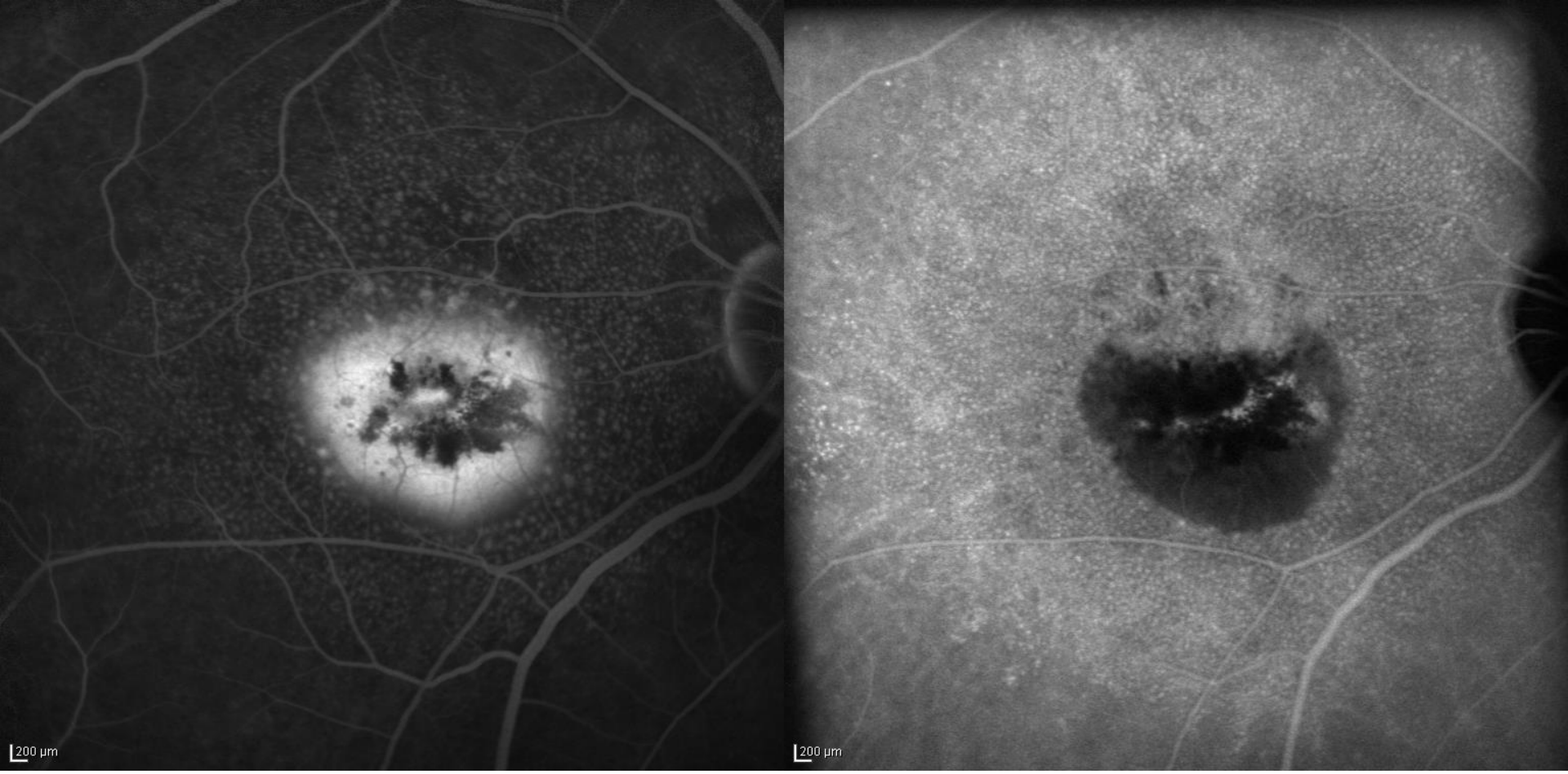
Some imaging systems allow for simultaneous FA/ICG studies with the proper filters and protocols for a combined sodium fluorescein/ tricarbocyanine green intravenous injection



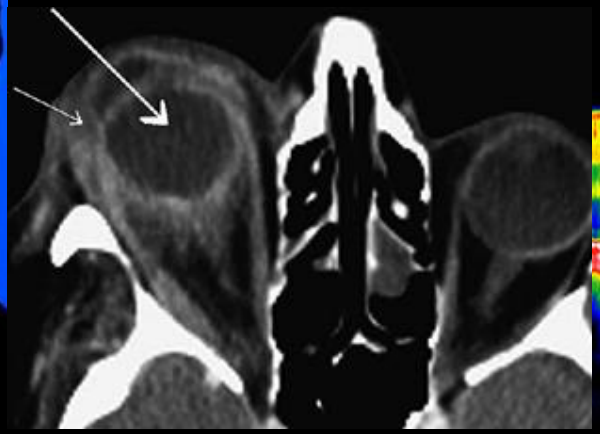
Mid phase FA showing large pooling of dye

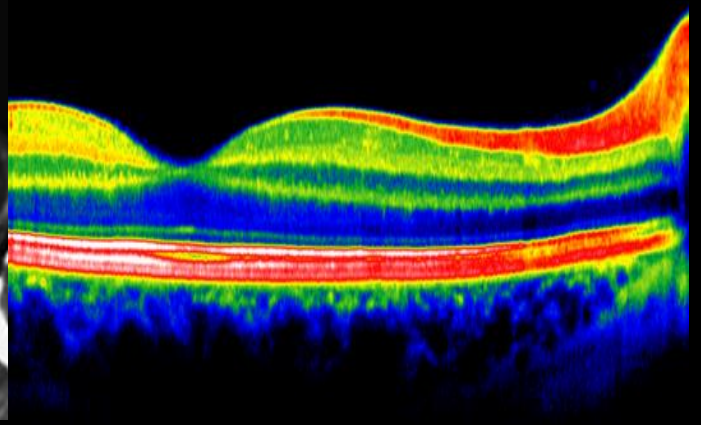
Late phase ICG highlights discrete area of leakage

Most useful for evaluation of diseases such as polypoidal **choroidal** vasculopathy , **choroidal** neovascularization, intraocular tumors (which originate in the **choroid**) retinal angiomatous proliferation (where retina and **choroid** abnormally communicate or RAP) and central serous **chorio**retinopathy.



- Retinal vessels appear as darker structures
- Rapid decrease in retinal vessel fluorescence reflect the fast kidney extraction of dye from the blood
- Plasma gaps in choroidal capillaries must be sufficiently large to become the main source of late choroidal fluorescence





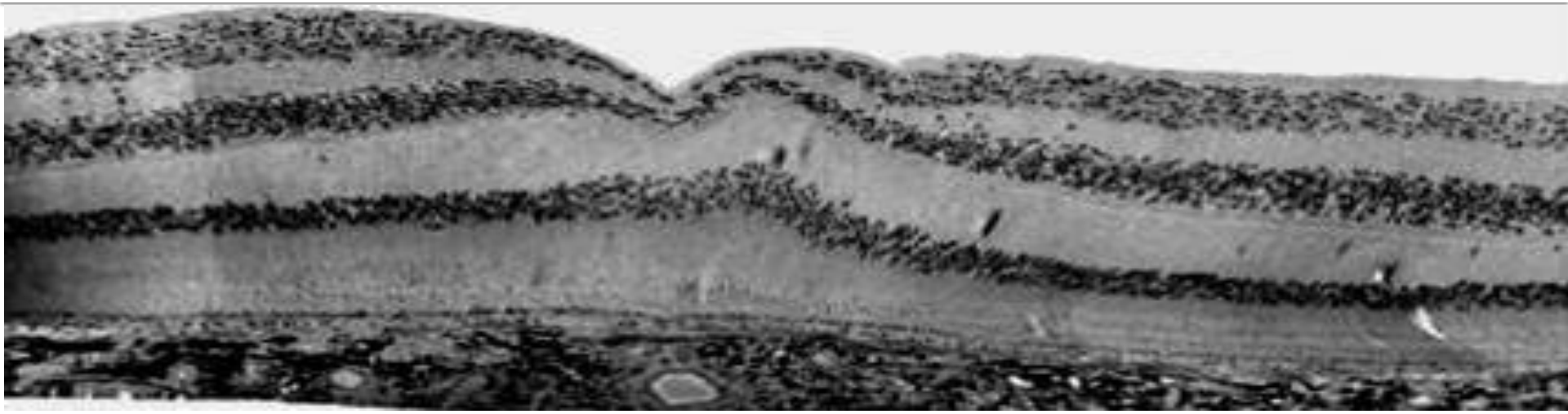
Optical Coherence Tomography

Tomography:
Tomos (To Cut or Section)
Graphein (To Write)

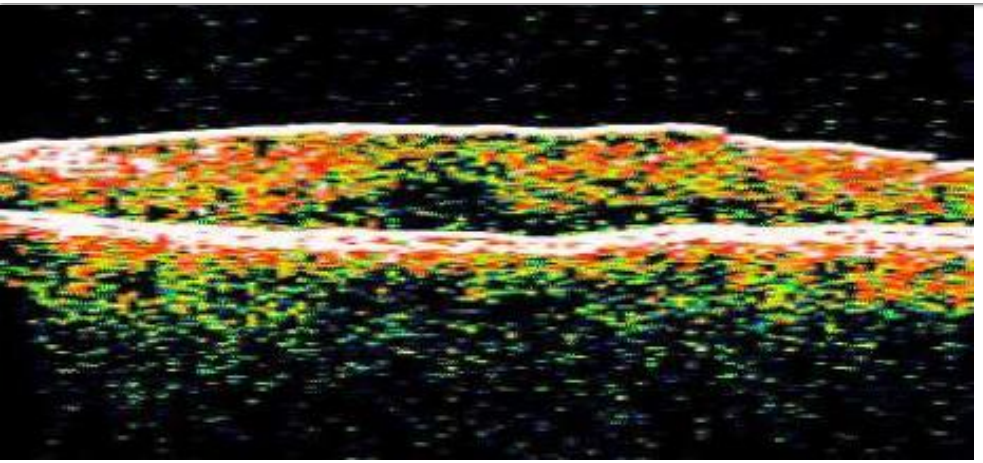
Is there any one out there NOT performing OCT??? Raise Your Hand…

OCT revolutionized Ophthalmology in the late 90's, just as Angiography did in the late 70's. Consider it a vital sign in ophthalmology…

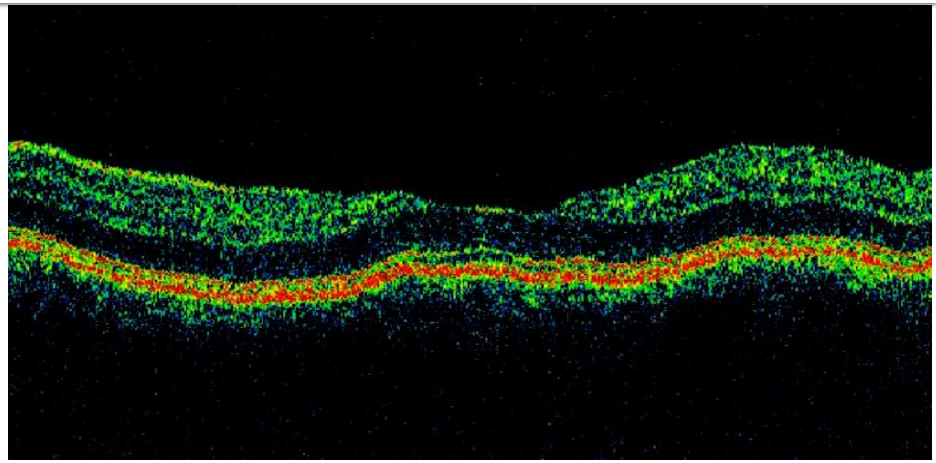
True Histology vs OCT "Histology"



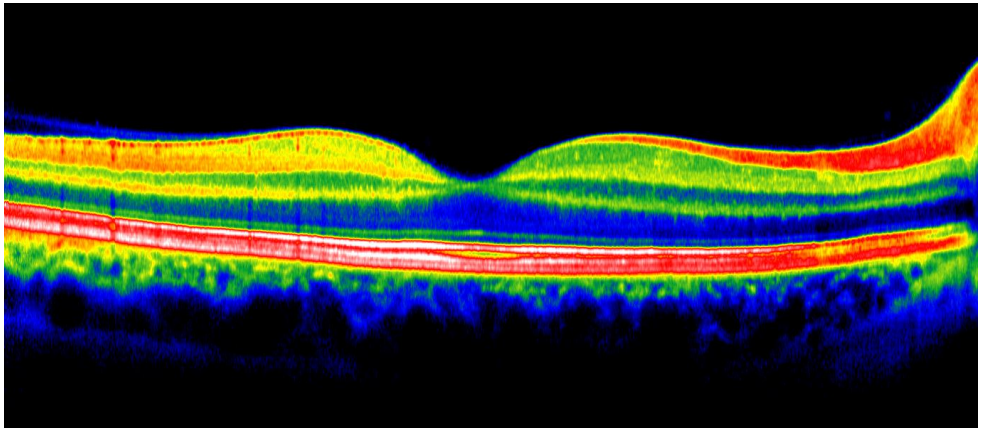
Evolution of Optical Coherence Tomography



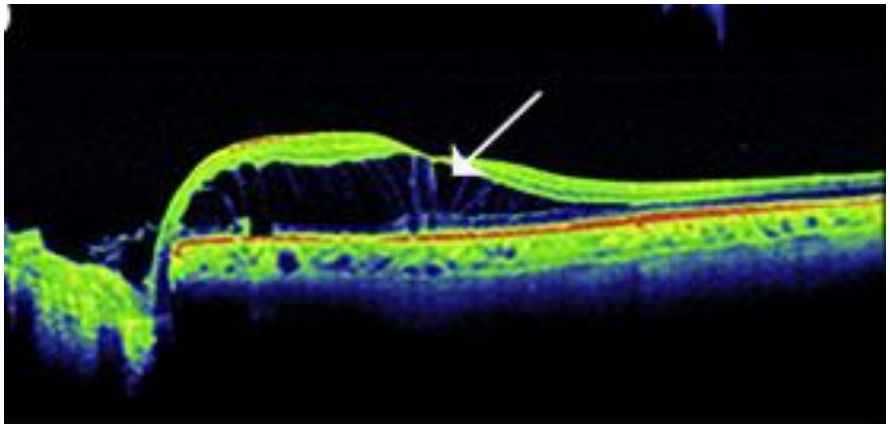
Zeiss OCT 1 TD-OCT introduced 1996
(approx. 20 micron resolution)



Zeiss Stratus TD- OCT 3 2000-2006 (Patent expired) approx. 10 micron resolution



SD-OCT 2007
(Approx. 3-4 micron resolution)



SSD-OCT about 2016
(Approx. 3-4 micron resolution)

Current OCT Technology

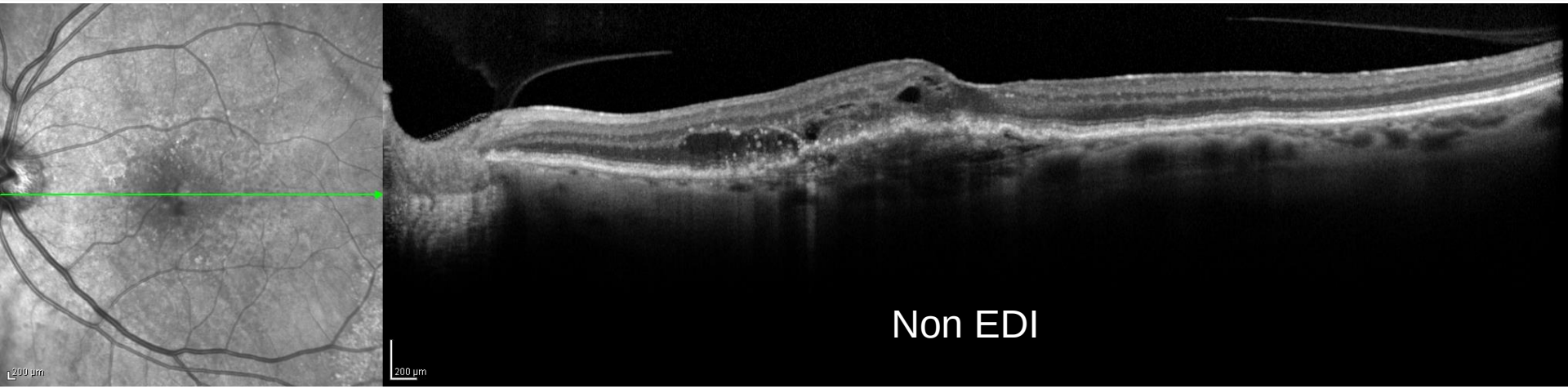
SD-OCT : Spectral Domain OCT

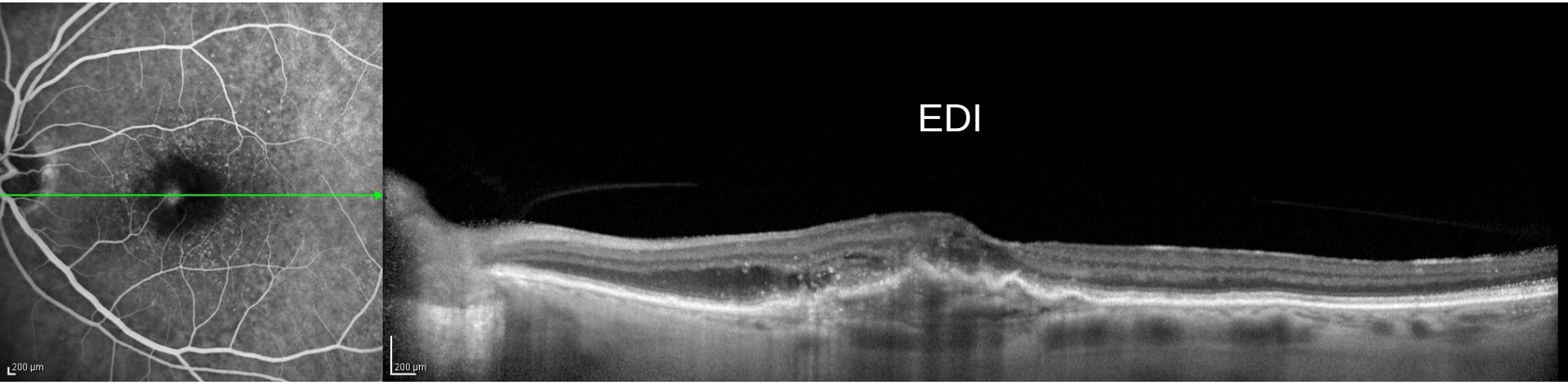
- Utilizes an detector known as a Spectrometer
- Near infrared wavelength (approx. 840 nm) and low interference interferometry

SS-OCT: Swept Source OCT

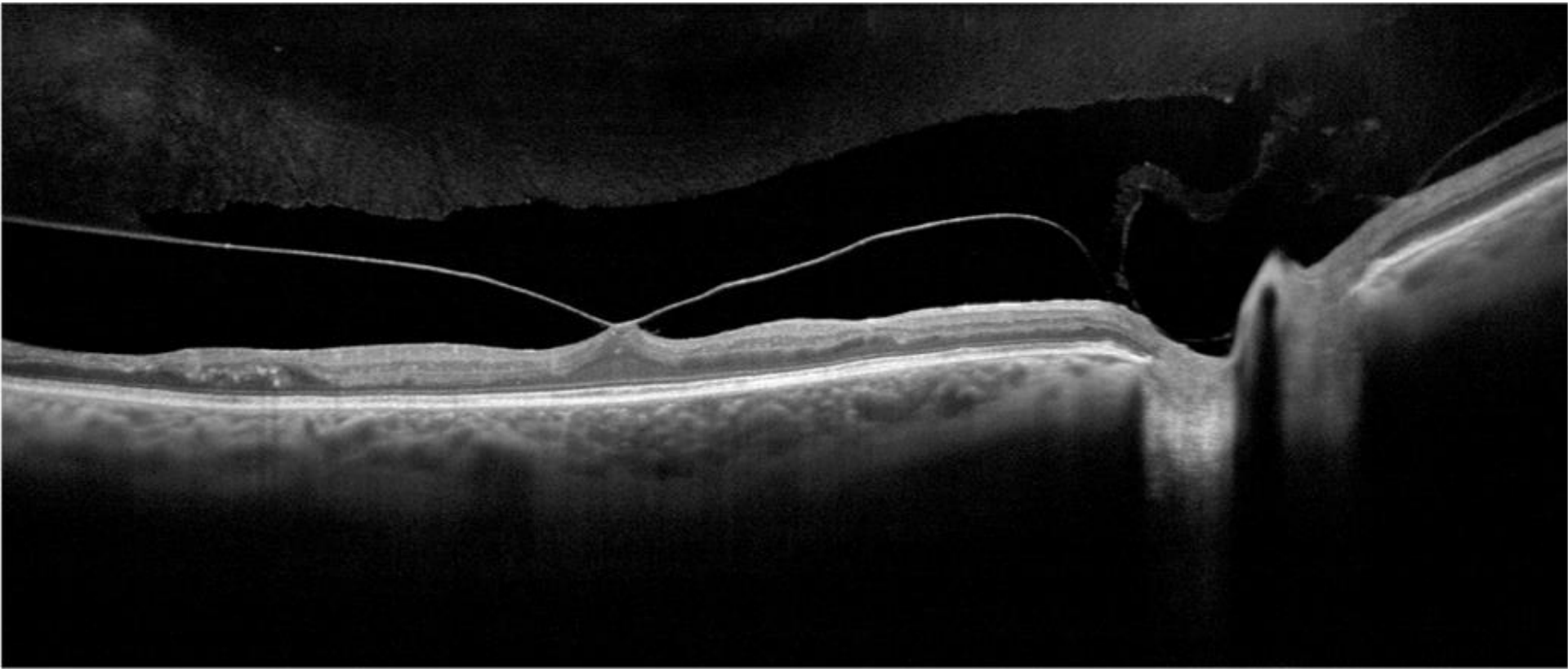
- Utilizes a laser that sweeps over a broad range of longer near infrared wavelengths (a median of 1050 nm) allowing for faster speed and greater penetration through the retina for evaluation of the choroid

OCT Enhanced Depth Imaging (EDI)





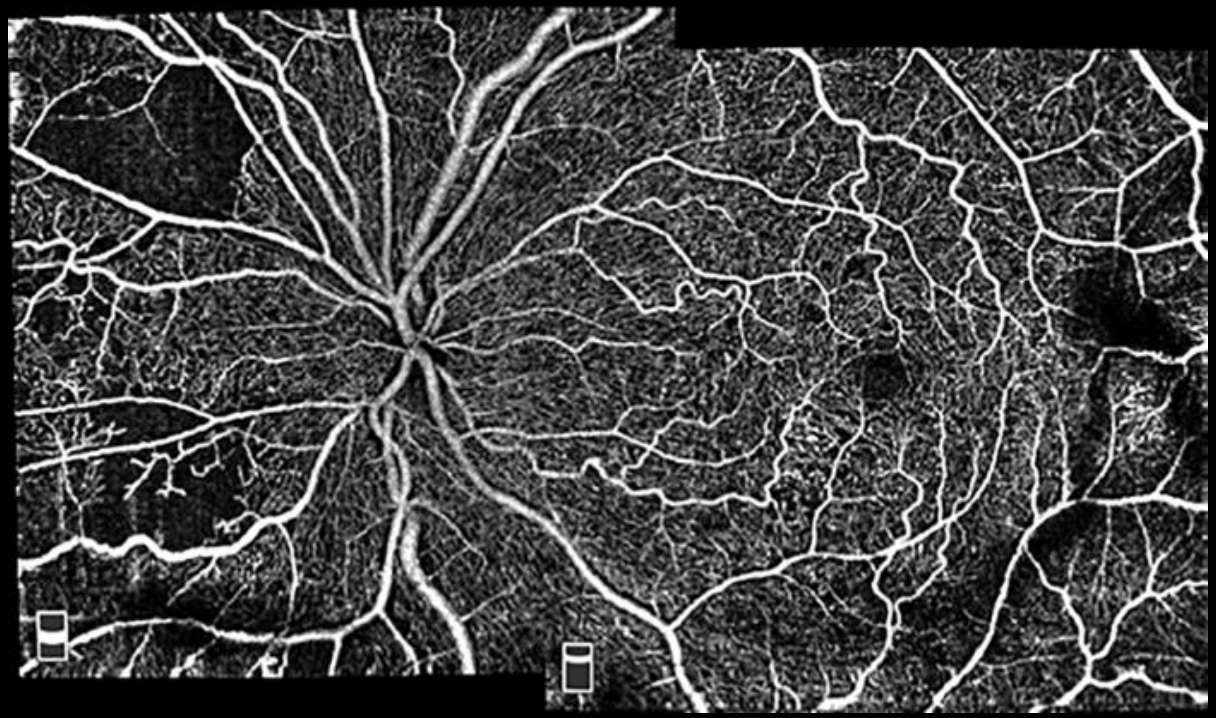
Wide View OCT



Heidelberg Composite

Intraoperative OCT

Angiography & OCT
The Perfect Marriage?



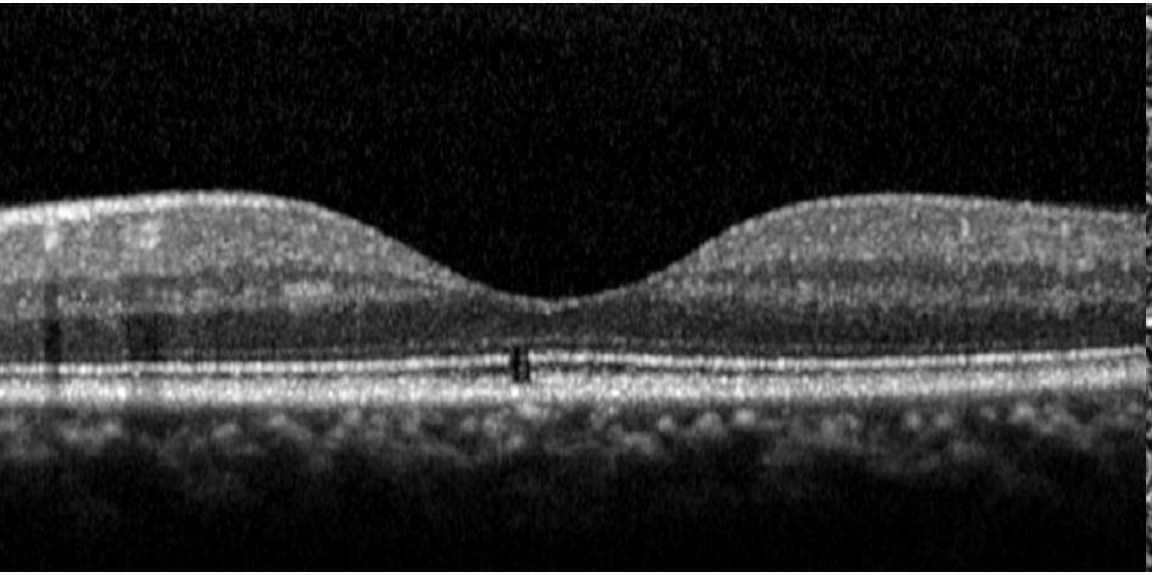
Optical Coherence Tomography-Angiography (OCTA)

What is OCTA?

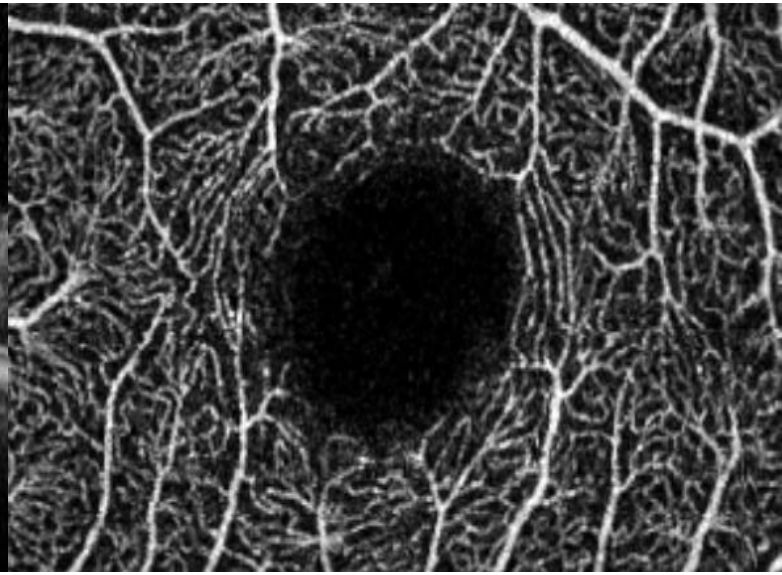
- Built on the platform of OCT technology (1993-present) and *launched at the 2015 AAO in Las Vegas)*
- provides 3-dimentional, depth encoded images that can reveal the vessels in which there is flow in the different vascular layers (called "Slabs") of the retina and choroid.
- OCTA is an extension of traditional OCT that takes multiple BScNs though each point along the volume of the retina, the plotting of the signal difference from the moving erythrocytes between B scans taken in the same location allows the computer to recreate a map of blood flow through the retina
- Has become a critical tool for evaluation and management of retinal disease in a non-invasive and real-time image acquisition manner by looking for perfusion defects, abnormal vessel distribution and pathologic vascular layer features.
- ***Detects FLOW or absence of flow, not LEAKAGE of the retinal and choroidal microvasculature.***
- At this time is limited by the field of capture- limited peripheral and widefield options.
- Unlike FA, OCTA doesn't require either the use of IV or oral fluorescein.

OCT vs. OCTA

OCT: Illustrates ANATOMICAL Structure (Morphology)



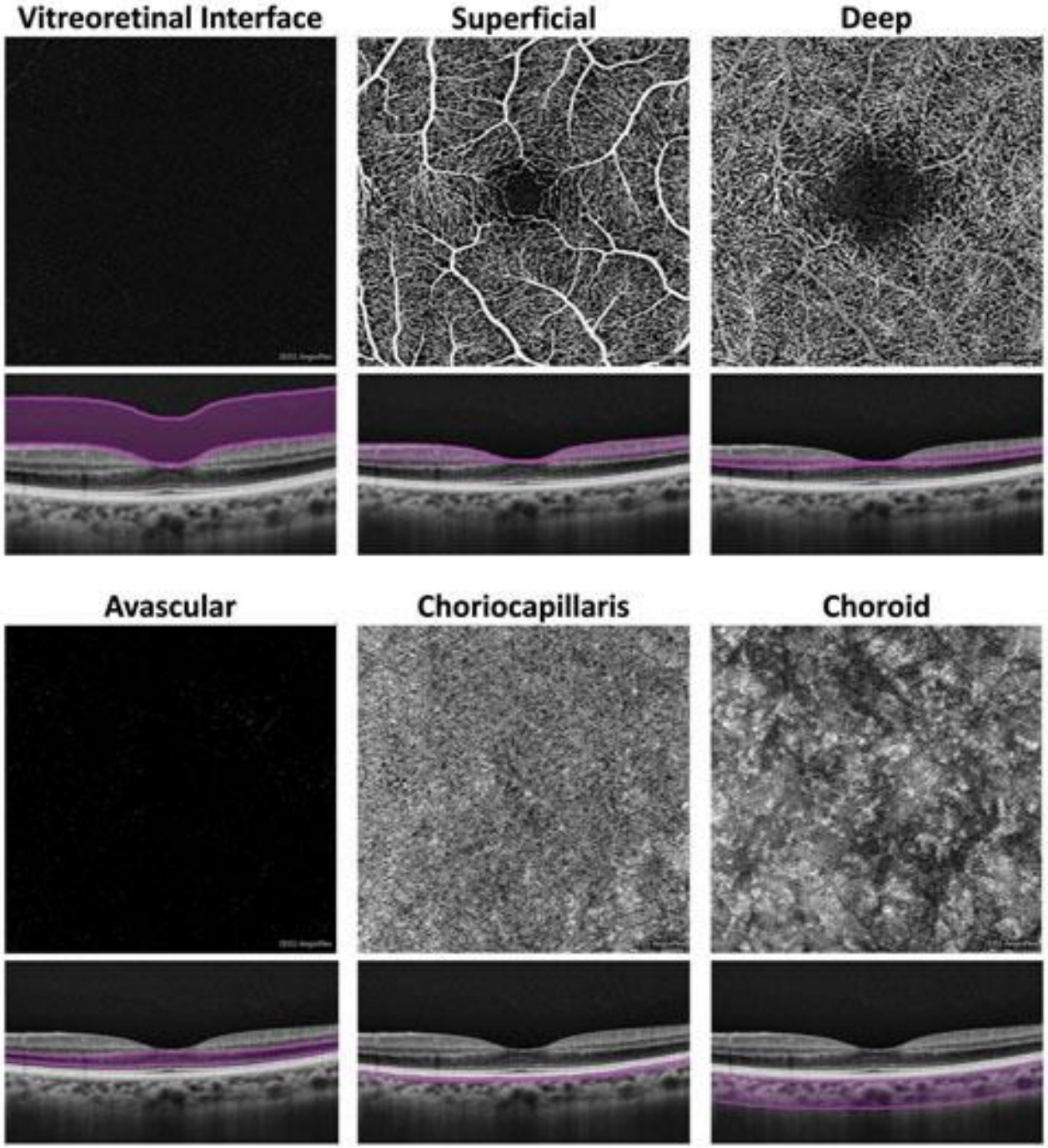
OCTA: Illustrates VASCULAR Structure (Flow)



En Face
(French for “to face forward”)
flow by “slab” or layers
allows for both qualitative and quantitative evaluation of depth-encoded retinal vasculature.

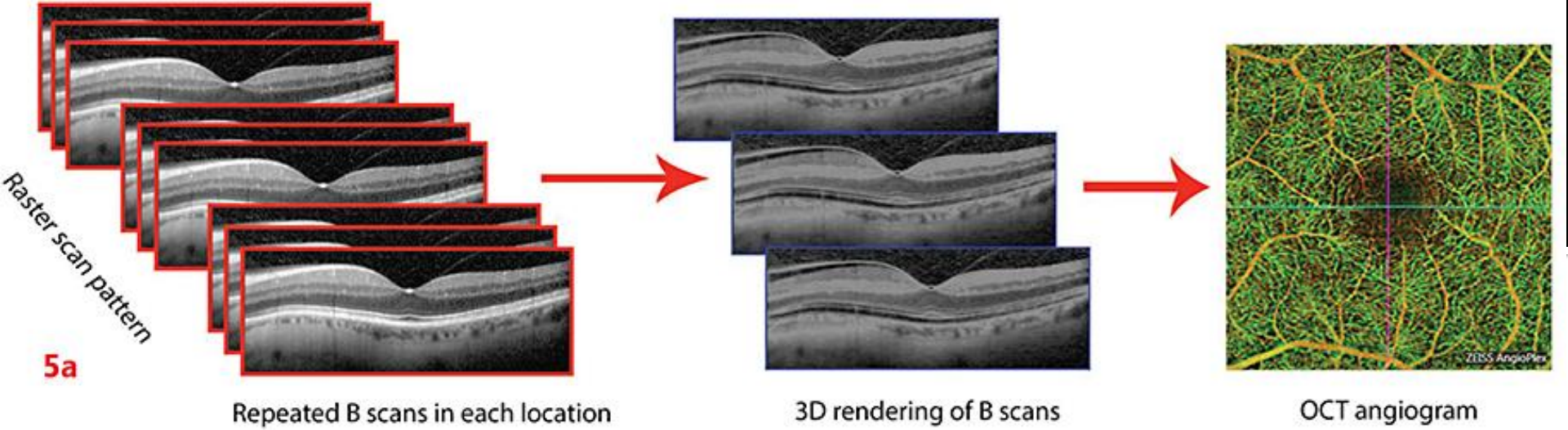
OCTA can quantify and compare vessel density

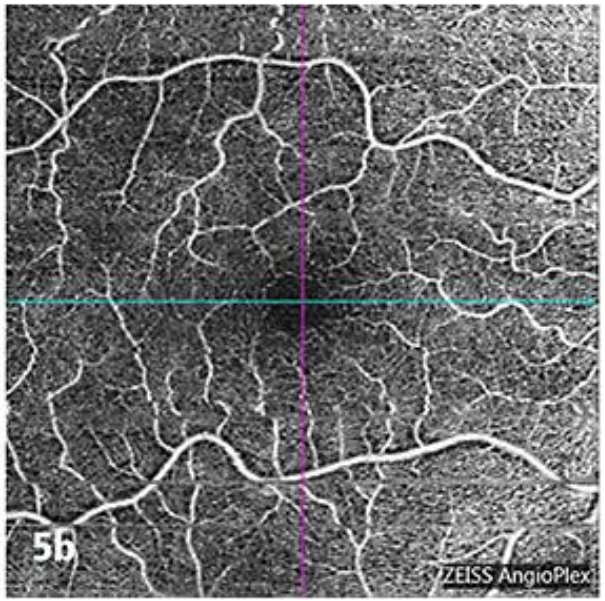
Excels at showing the FAZ in a way that dye based FA cannot

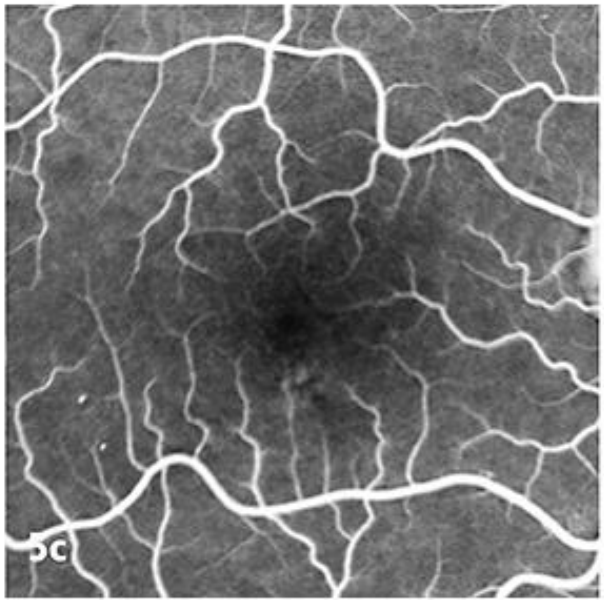


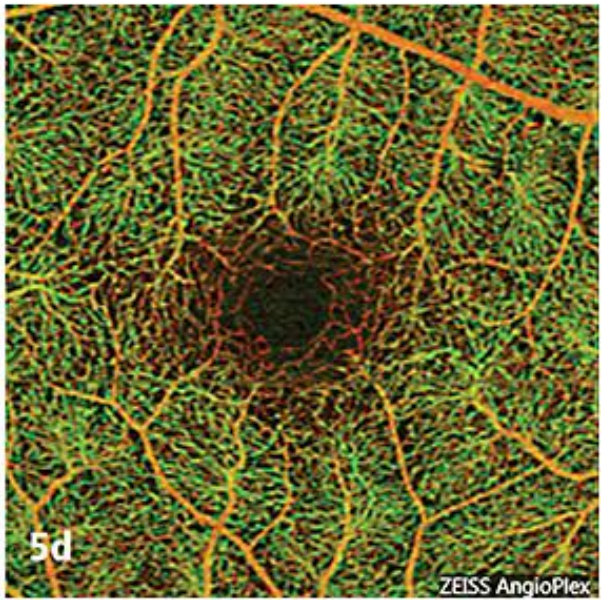
A network of vessels in the body
An intricate network or web like formation

Plexus





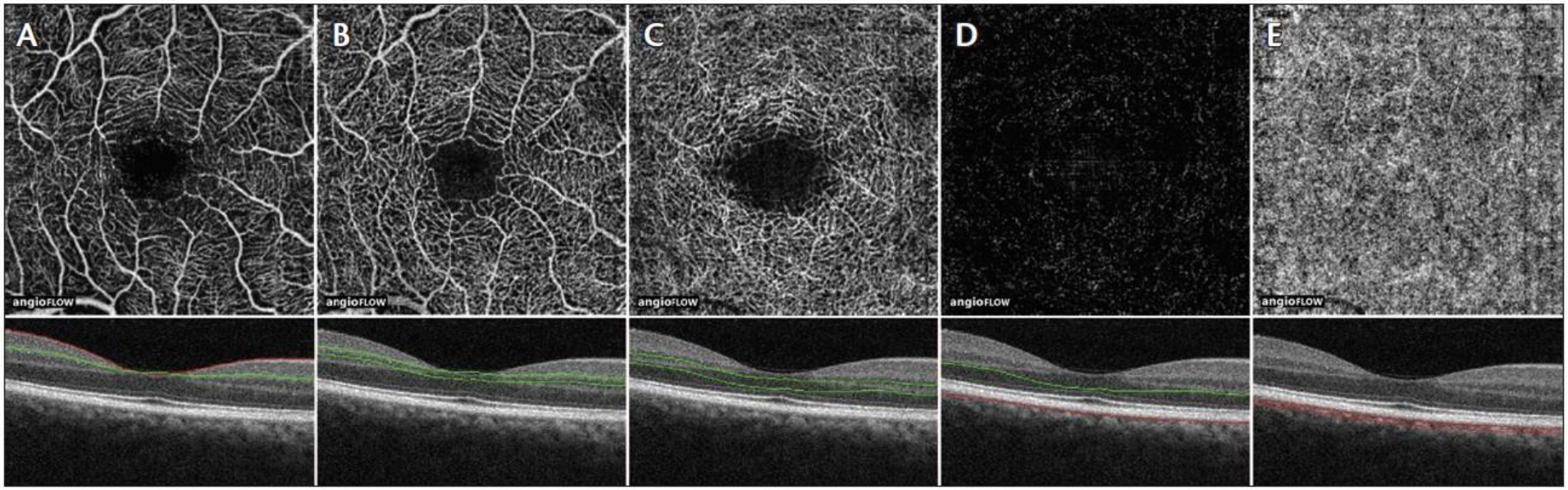


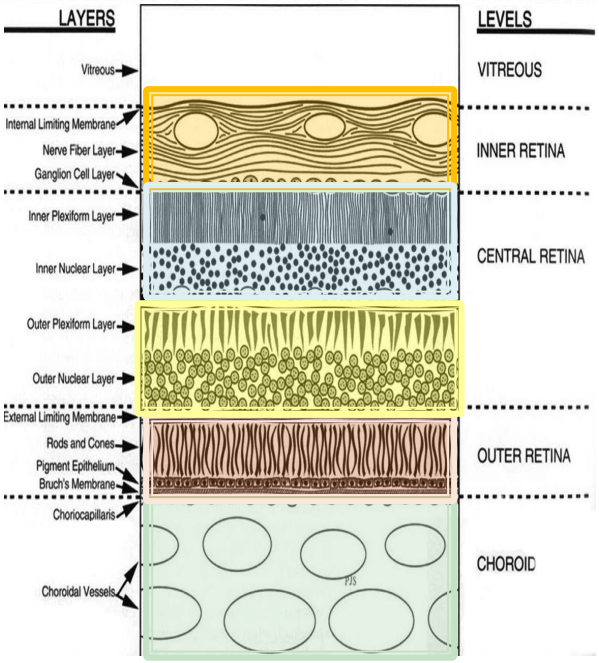


At each location in a volume scan multiple B-scans are obtained and compared. Regional variation in images is inferred to represent motion.

As OCTA is based on motion detection, the acquisition time is limited by how long the patient can keep his or her eye open without blinking.

Dissected Layers of the Retina





A. Superficial Plexus (ILM, RNFL, GCC)

B. Intermediate Plexus (border of the inner plexiform layer (IPL) and inner nuclear layer (INL)

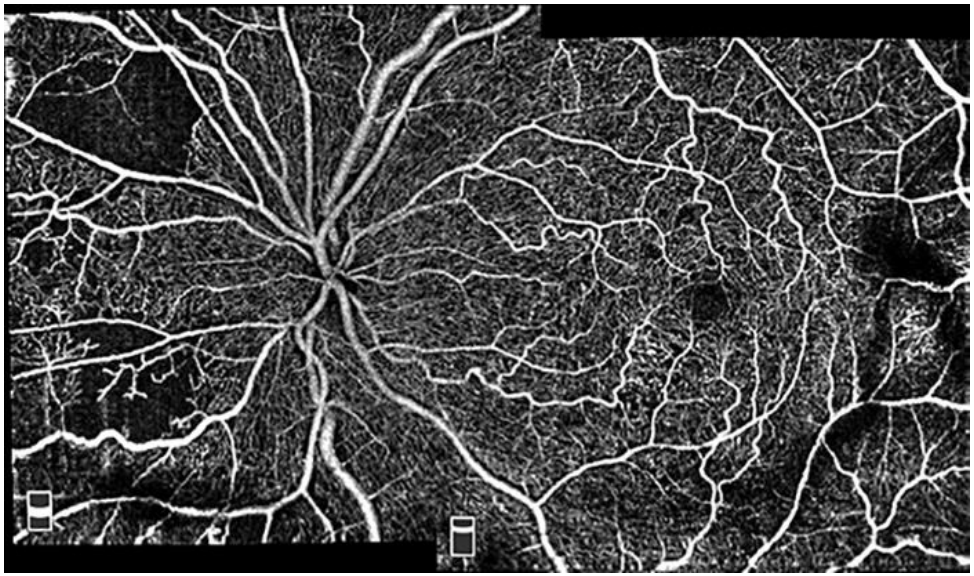
C. Deep Plexus of Inner Retina (the border of the INL and outer plexiform layer (OPL) are

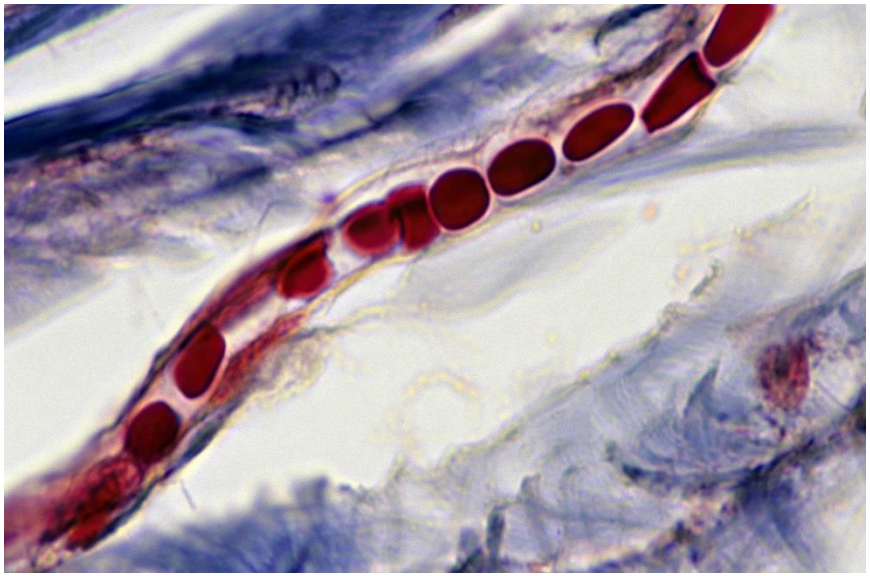
D. Outer Retina (OPL to Bruch's)

E. Choriocapillaris (10-µm slice directly below Bruch's membrane)

OCT Angiography: Principles

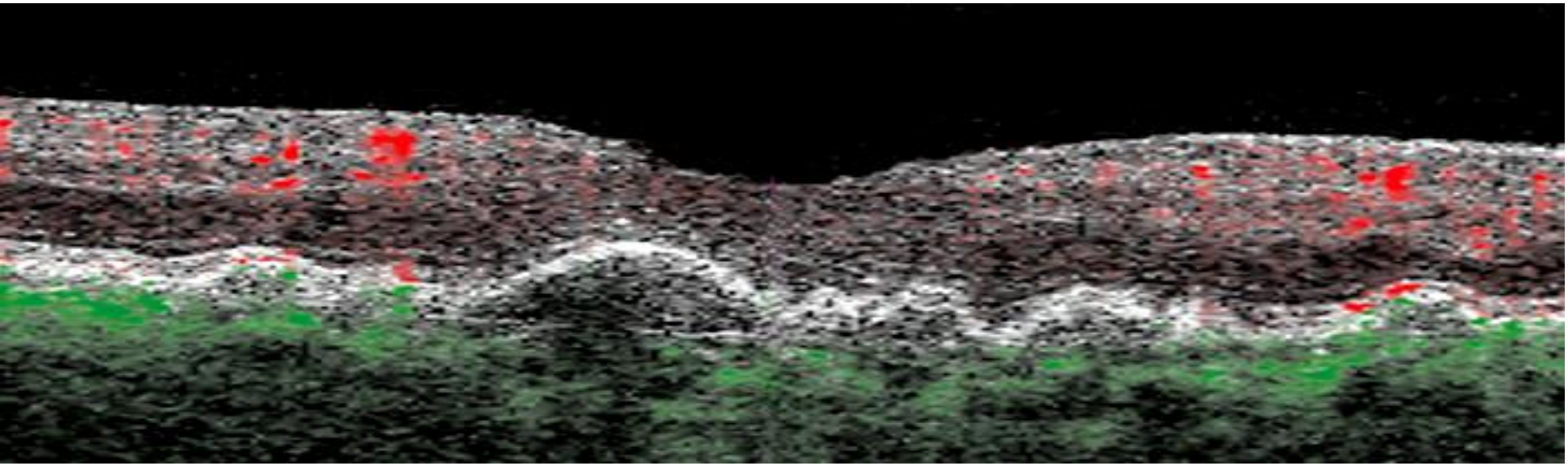
- Non-invasive and is dye-less- *Nix the Needle!*
- Uses the motion of red blood cells by analyzing the changes in reflectivity and light scattering from repeated B-scans performed in the same location
- Moving erythrocytes cause a time dependent backscattering of the OCT signal, which manifests as differences among the tens of thousands of repeated B scans
- Turns those pixels into high resolution images of capillary blood flow





Erythrocyte Flow on B-Scan

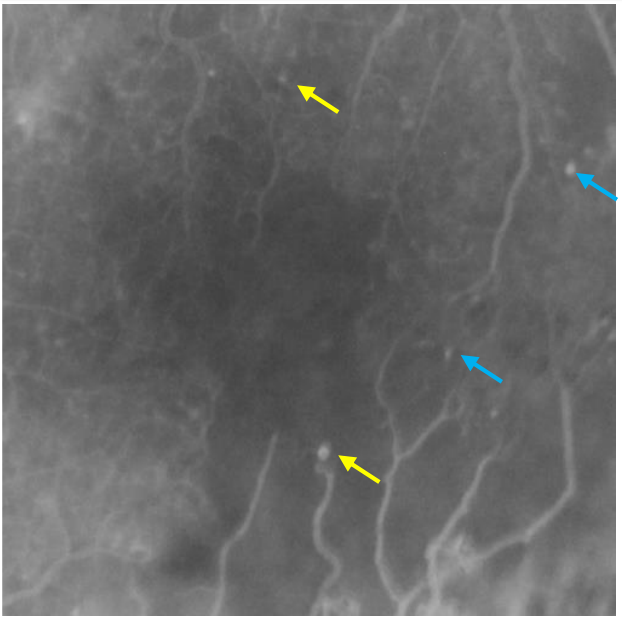
- Red : pre RPE flow
- Green : sub RPE flow

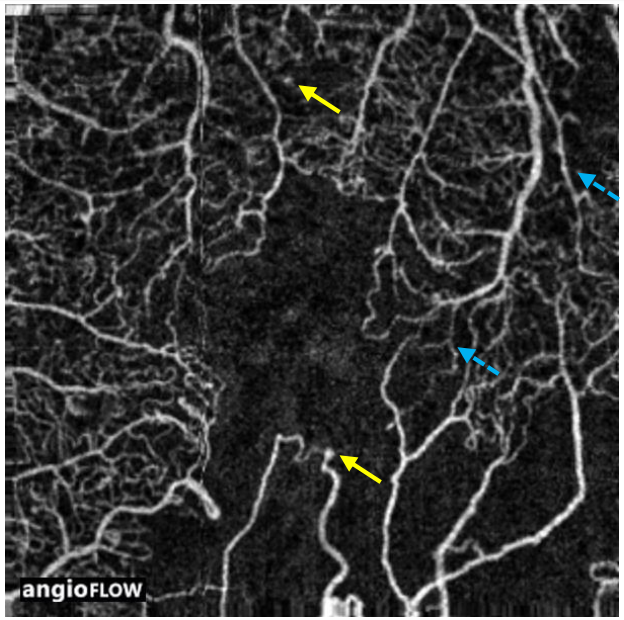


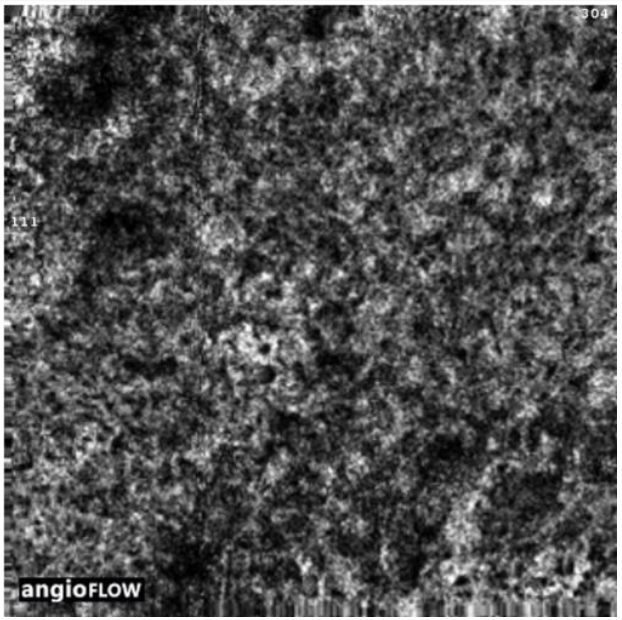
Flow, not Leakage
New growth of Vessels

Best Applications for OCTA

Capillary Drop Out

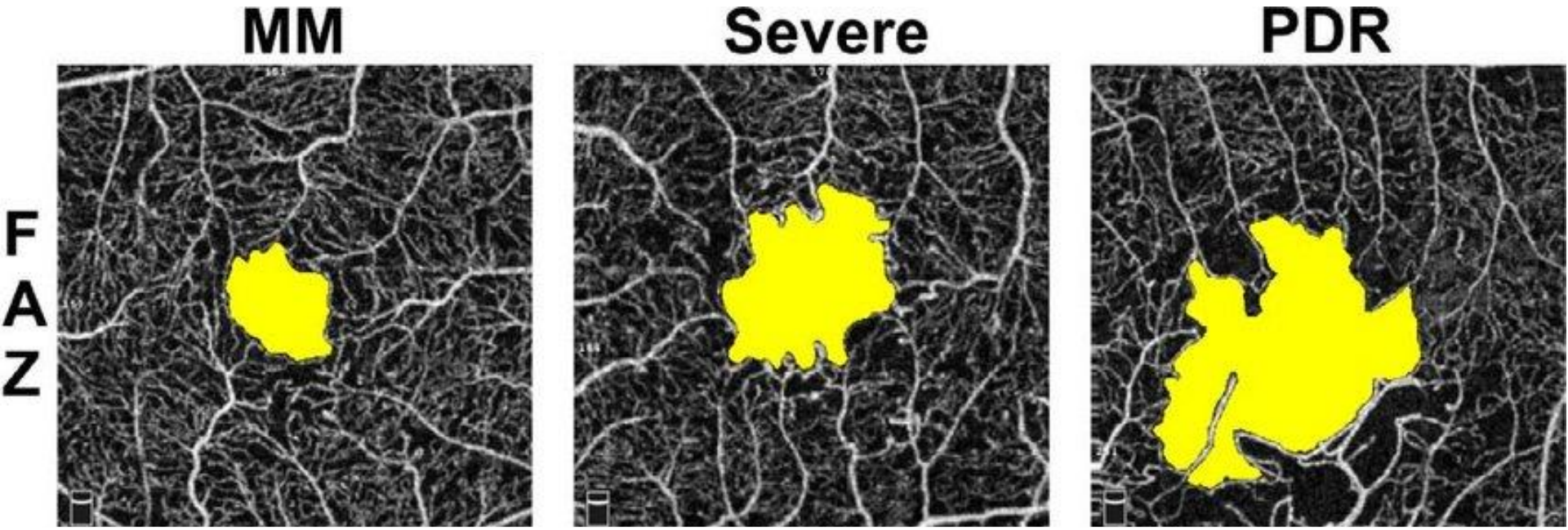




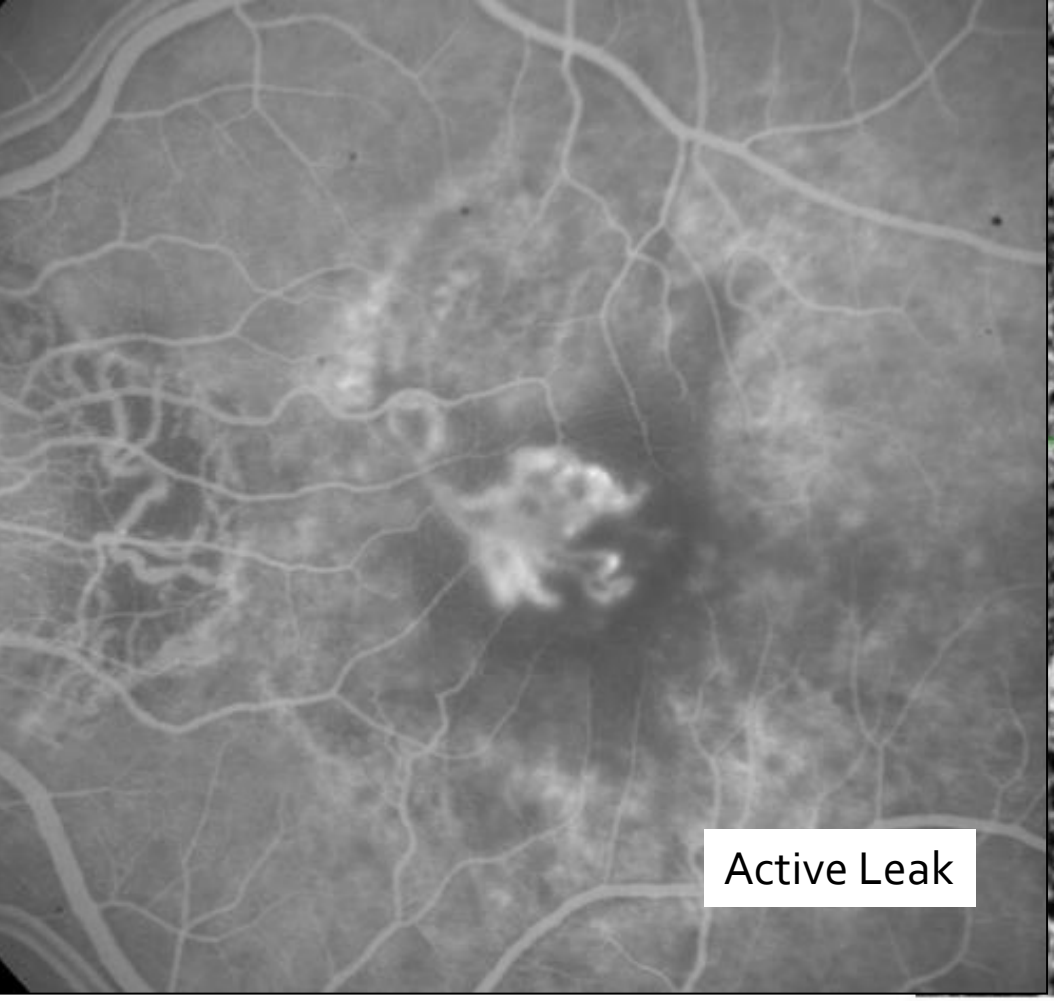


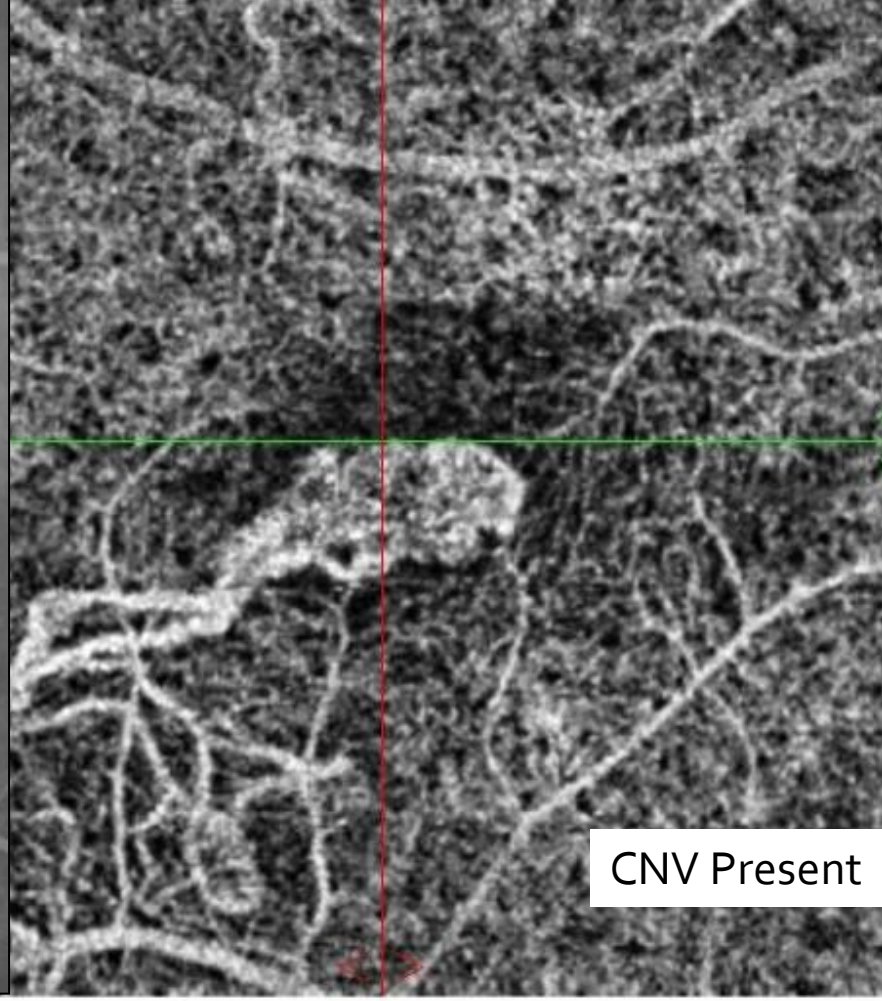
Yellow dashed lines: regions of non-perfusion
Radial peripapillary capillaries (RPC) only seen in OCTA

Enlargement of the FAZ



Research pints to evidence that patients with DR have larger FAX sizes as well as FAZ remodeling and subtle capillary non perfusion, not apparent



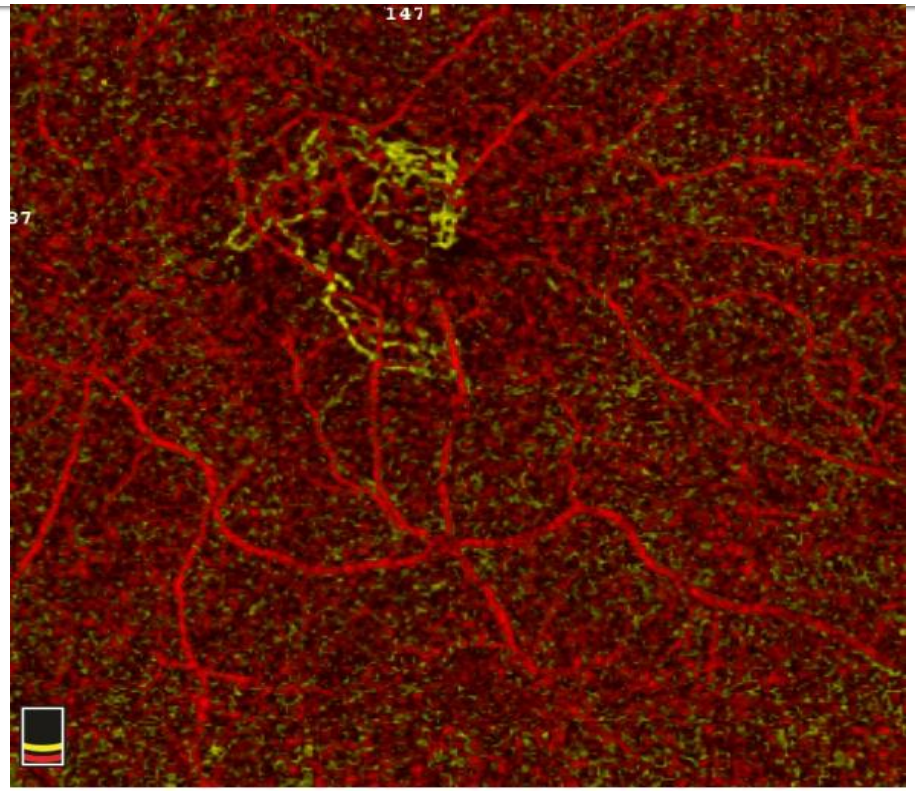


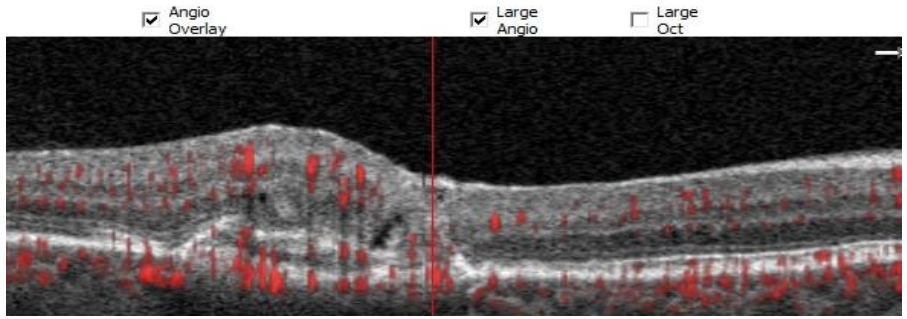
OCTA allows visualization of vasculature within the specific layers of the retina (in this case, choroidal) without the blurring and obscuring effects of staining and pooling observed on FA

Images courtesy of James Gilman, CRA

Impossible Venipuncture: OCTA

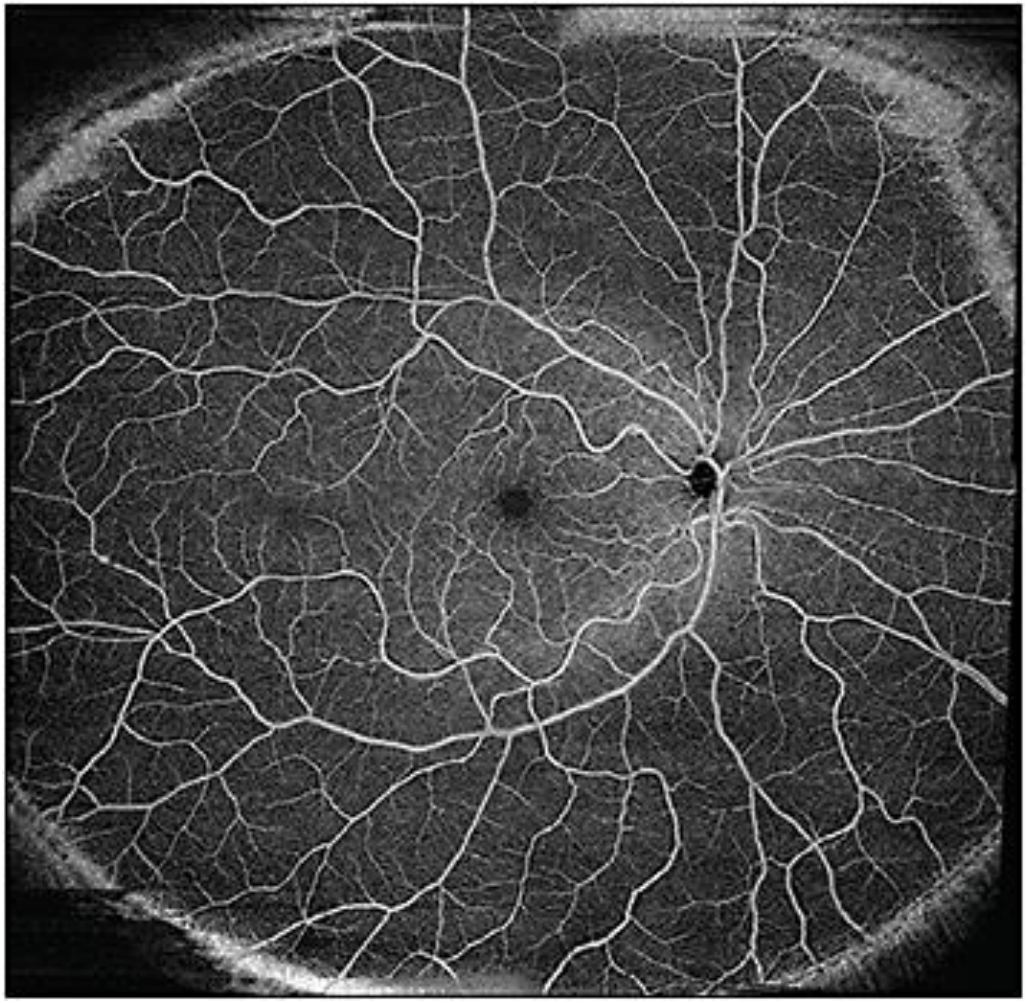
Obese patient with impossible venipuncture
Monocular- information imperative
Oral FA inconclusive- but! OCTA showed CNV

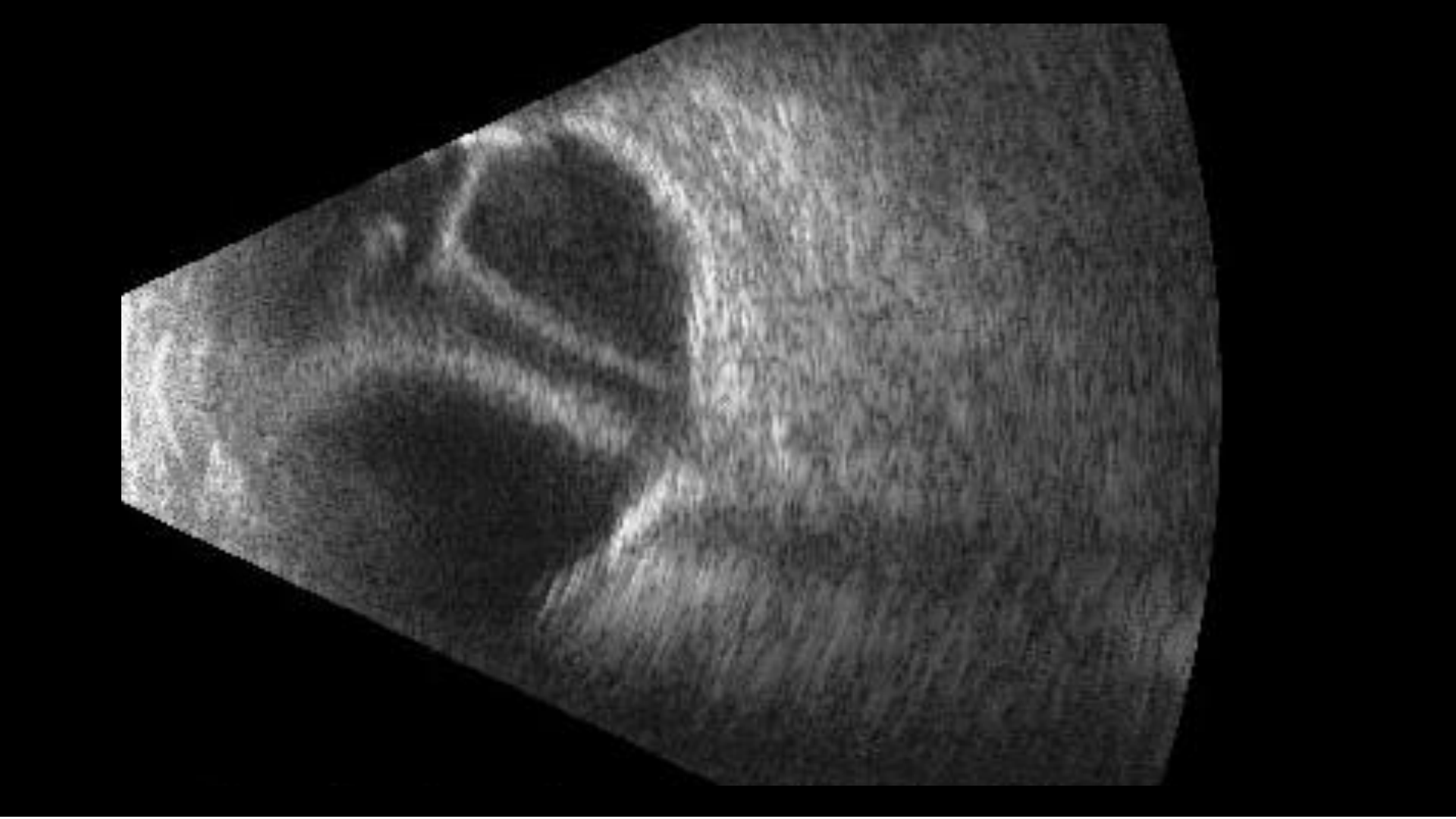




Swept Source OCTA (SS-OCTA)

- Montage of 12 mm x 12 mm scans from a normal eye obtained on the swept-source Zeiss Plex Elite 9000.
- Five 12 mm x 12 mm scans were obtained, one centered at the fovea, and the other 4 centered at the 4 quadrants around the fovea.
- The montaging of these 5 scans, by the Zeiss proprietary software, allows for visualization of a wider retinal field of view than that allowed by individual OCTA scan protocols

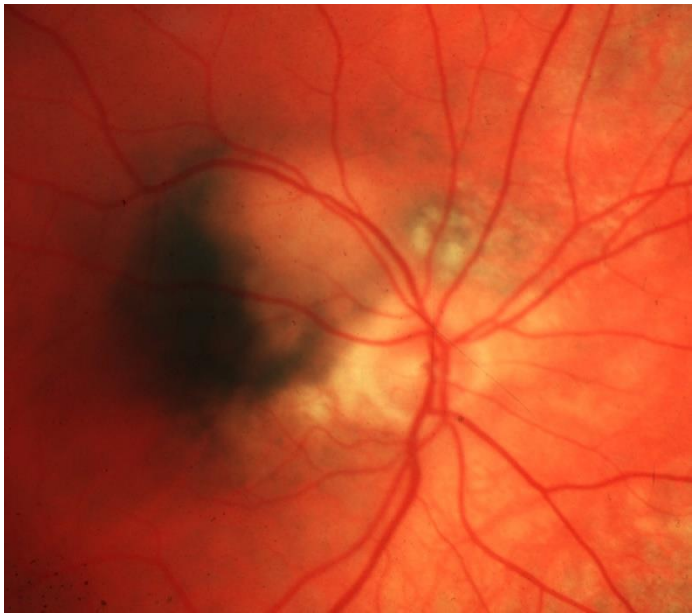




Diagnostic Ophthalmic Ultrasound

When you Can't See In, Use Sound

- When poor ocular media precludes good photography
- Can be used where OCT cannot: when pigment blocks OCT signal
- Useful as adjunct to OCT
- Better at imaging the far periphery of the retina
- Great for supplementary eval of the retina and choroid



Imaging Conclusions

- Advancements in these retinal imaging modalities continue to expand our ability to diagnose, monitor, and treat retinal diseases in our patients.
- **You! The imager, are a vital role in this process**
- Through the use of these ever evolving imaging modalities, often used in conjunction in some combination, we are able to screen for and detect diseases earlier than ever and quickly identify progression in disease that might require changes to treatment plans.

Ophthalmic Photographers' Society

"the Eye Imaging Experts"

www.opsweb.org

DeniceBars@gmail.com

EyeEducateU.com

I have loved being a ***Retinal*** *Imager for 43 years! I hope you do as well…*