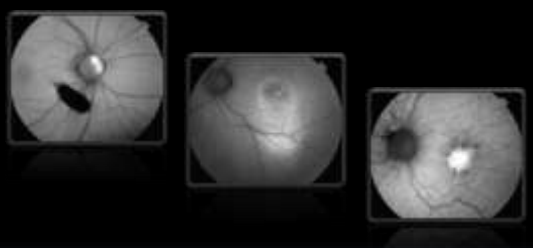


FUNDUS AUTOFLUORESCENCE

Timothy J. Bennett, CRA, OCT-C, FOPS
Penn State Eye Center
Hershey, PA

- I have no financial or proprietary interest in the subject matter of this presentation.

Fluorescence Imaging

- Fluorescein angiography
- ICG angiography
- Fundus autofluorescence

Fundus Autofluorescence (FAF)

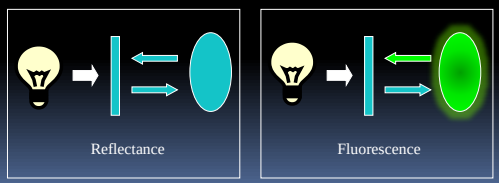
- Fundus autofluorescence (FAF) is a diagnostic imaging technique for documenting the presence of fluorophores in the human eye.
- Fluorophores are chemical structures that possess fluorescent properties when exposed to electromagnetic energy of an appropriate wavelength.

Fluorescence

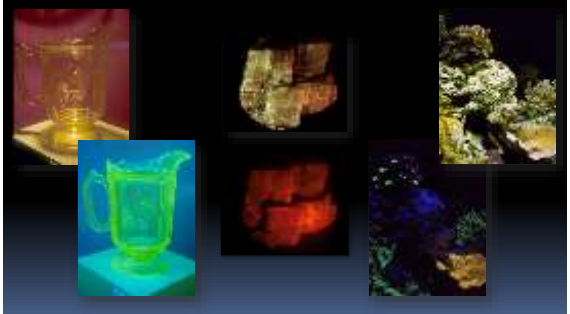
- When fluorophores absorb light of a particular wavelength, they are temporarily excited to a higher energy state.
- Triggers the emission of light at wavelengths longer than the excitation source.

Fluorescence

- Emission occurs only as long as the fluorescent subject remains illuminated by the exciting source (10^{-8} seconds).

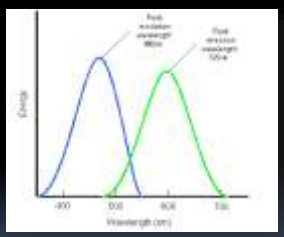


Fluorescence



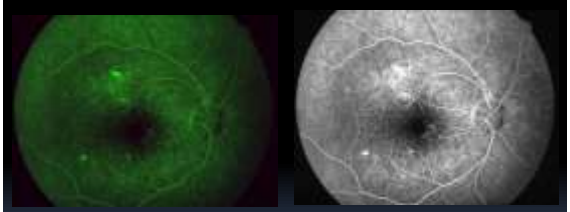
Fluorescein

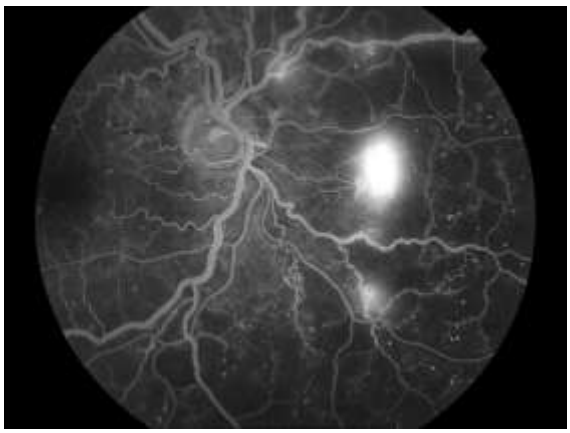
- Absorbs blue light, with peak absorption and excitation occurring at wavelengths between 465-490nm.
- Fluorescence occurs at the yellow-green wavelengths of 520 to 530nm.



Exciter and Barrier Filters

FA: Color → Grayscale



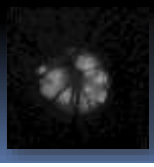


Fundus Autofluorescence

- The term "autofluorescence" is used to distinguish fluorescence that can occur naturally vs. fluorescence that is derived from administration of fluorescent dyes.
- Optic nerve drusen, astrocytic hamartomas, lipofuscin pigments in the retina, and the aging crystalline lens are all believed to exhibit natural fluorescence.

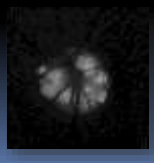
Fundus Autofluorescence

- Procedures for documentation of highly fluorescent entities such as optic disc drusen have been employed for years with varying degrees of success using a fundus camera with fluorescein excitation and barrier filters.



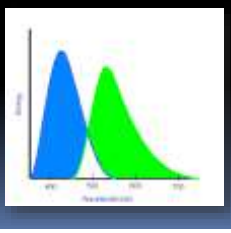
Fundus Autofluorescence

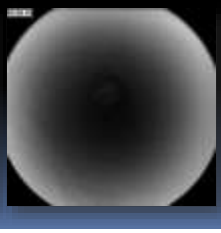
- Required high ISO films push-processed to increase sensitivity.
- Results were inconsistent and often confounded by pseudofluorescence.



Pseudofluorescence

- Occurs as a result of crossover in the spectral transmission curves of the exciter and barrier filters.



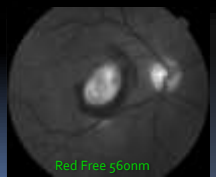


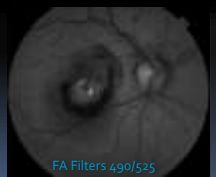
Pseudofluorescence

- Crossover can be the result of mismatched or aging filters. Modern interference filters rarely exhibit significant crossover unless they have deteriorated.

Pseudofluorescence

- Reflectance from bright fundus structures will not be fully blocked by the barrier filter.





Auto vs. Pseudo Fluorescence

- *Are optic disc drusen exhibiting autofluorescence, pseudofluorescence or reflectance?* Barry C, Singh J, Constable IJ. J Ophthalmic Photography 2000;22:32-37

Fundus Autofluorescence

- The current use of FAF imaging centers mostly on documenting the deposition of lipofuscin in the retinal pigment epithelium (RPE).

Renewed Interest in AF

- Autofluorescence imaging of lipofuscin first became practical with the implementation of confocal scanning laser technology.


Distribution of fundus autofluorescence with a scanning laser ophthalmoscope

Fundus Autofluorescence

- Lipofuscin is a fluorescent pigment that accumulates in the RPE as a metabolic byproduct of cell function.
- Lipofuscin deposition normally increases with age, but may also occur from RPE cell dysfunction or an abnormal metabolic load on the RPE.

Fundus Autofluorescence

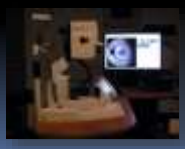
- There are as many as ten different fluorophores found in lipofuscin.
- The dominant fluorophore in lipofuscin is A2-E, a compound consisting of two vitamin A molecules and ethanolamine.
- A2-E possesses toxic properties that may interfere with normal RPE cell function.

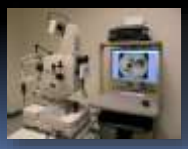
Fundus Autofluorescence

- FAF imaging is particularly challenging due to low levels of fluorescence and variability in the amount of lipofuscin present depending on age, health of the RPE, and disease process.
- Requires a more light-efficient method than the traditional technique used for imaging disc drusen.

Fundus Autofluorescence

- There are two different digital technologies currently used to capture fundus autofluorescence images:
  - cSLO
  - Modified fundus camera.



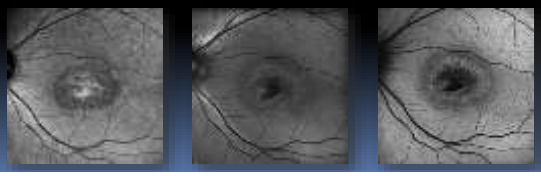


Fundus Autofluorescence

- Both systems require significant amounts of light and high gain settings to achieve adequate exposure, and are susceptible to unwanted noise that can interfere with image detail.
- Noise is false pixel data that occurs from poor signal-to-noise ratios and the amplification needed to record fluorescence.

Scanning Laser Ophthalmoscope

- The confocal scanning laser ophthalmoscope (cSLO) is an instrument that can be used for several retinal imaging modalities including fluorescein angiography, ICG angiography and fundus autofluorescence.

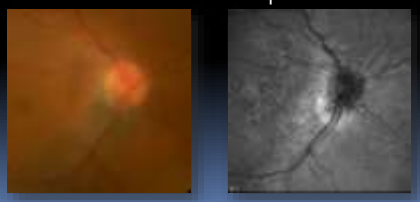


Scanning Laser Ophthalmoscope

- A monochrome laser scans across the fundus in a raster pattern to illuminate and record successive elements of the retina, point-by-point at speeds up to 24 milliseconds.
- The laser delivers a very narrow wavelength band allowing for efficient excitation of fluorescence.

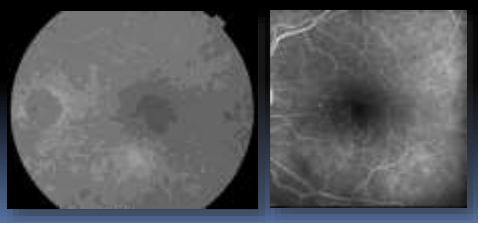
Scanning Laser Ophthalmoscope

- A confocal aperture positioned conjugate to the focal plane of the retina blocks non image-forming light from reaching the sensor to minimize scatter and improve contrast.



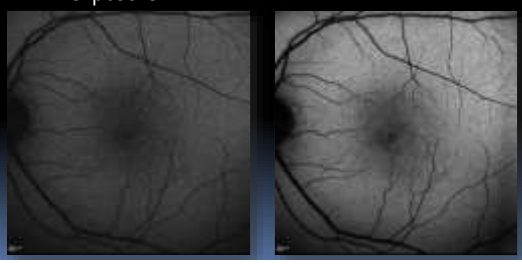
Scanning Laser Ophthalmoscope

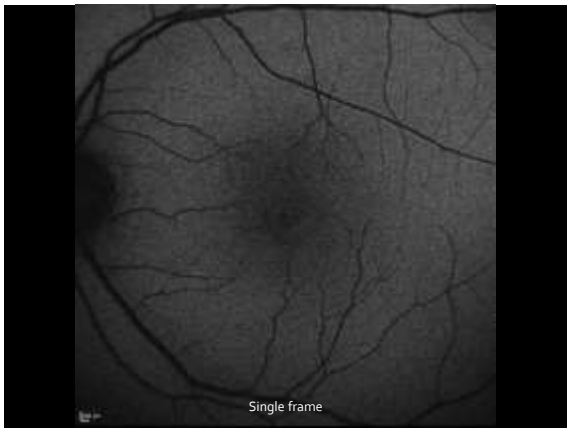
- Confocal imaging reduces the effects of short wavelength scatter in the ocular media and confounding AF from the crystalline lens.

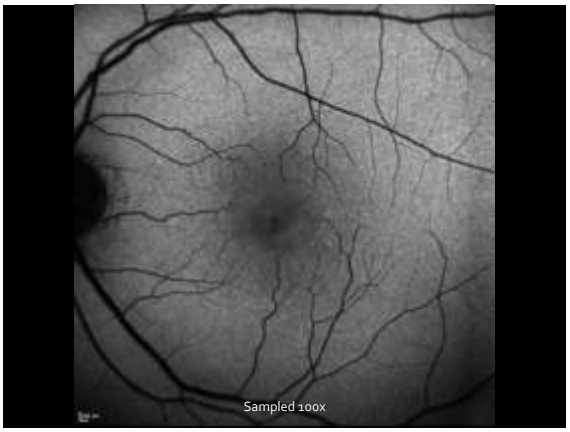


cSLO FAF Sampling

- Sampling smoothes noise and increases exposure.



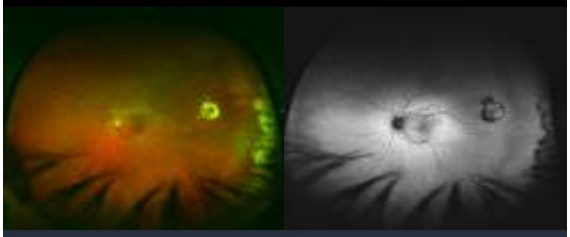




SLO: Spectralis HRA

- FA excitation and blue reflectance (red free)
  - 488nm solid state laser
- ICG excitation
  - 790nm diode laser
- IR Reflectance
  - 820nm diode laser

SLO: Optos



Optos images courtesy Jessica Carnevale, CRA

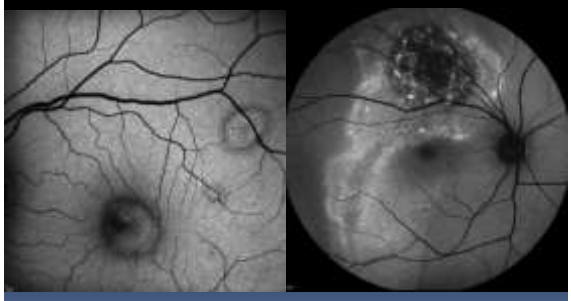
cSLO FAF

- The cSLO uses an excitation wavelength of 488 nm and a wide band-pass barrier filter at 521 nm, the same settings used for fluorescein angiography.

cSLO FAF

- FAF imaging must be done before angiography if both procedures are performed with a cSLO on the same visit.
- Even the slightest amount of intravenous fluorescein will compromise the effectiveness of cSLO FAF.
- Residual topical fluorescein may also interfere with FAF.

cSLO FAF



Modified Fundus Camera FAF

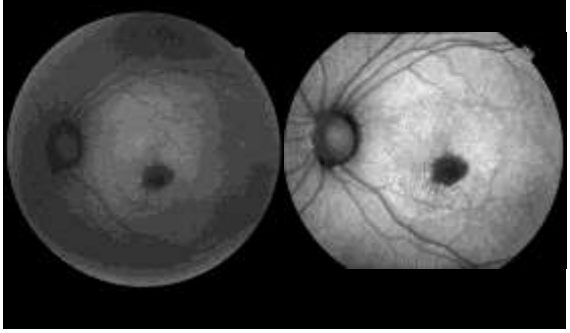
- More recently (2003), digital fundus-camera based systems have been developed for autofluorescence imaging.

Fundus Autofluorescence and Age-related Macular Degeneration

Modified Fundus Camera FAF

- Utilizes high-sensitivity monochrome digital sensors with different filter combinations than used for angiography.

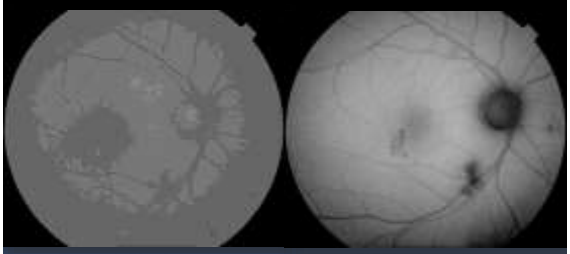
Modified Fundus Camera FAF



Modified Fundus Camera FAF

- The digital fundus camera technique first described by Spaide employs an excitation filter centered at 580 nm and a barrier filter centered at 695 nm.
- These wavelengths are shifted toward the red end of the spectrum to avoid unwanted short-wavelength autofluorescence from the crystalline lens.

Modified Fundus Camera FAF



Fluorescein Filter Set 490/525
Pseudofluorescence?

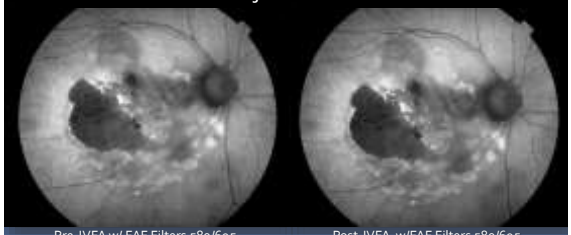
FAF Filter Set 580/695
Autofluorescence of Lipofuscin

Fundus Camera FAF

- FAF imaging can be conducted either before or after fluorescein angiography with fundus camera based systems.
- The FAF excitation wavelength of 580 nm causes minimal excitation of fluorescein and the barrier filter centered at 680 nm blocks the emission peak of fluorescein (520 nm).

Modified Fundus Camera FAF

- Longer wavelengths allow FAF to be done before or after injection of fluorescein.



Pre-IVFA w/ FAF Filters 580/695 Post-IVFA w/FAF Filters 580/695

Modified Fundus Camera FAF

- Longer wavelengths allow FAF to be done before or after injection of fluorescein.

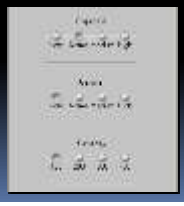
Fluorescein Filters 490/525 FAF Filters 580/695

Exposure/Noise

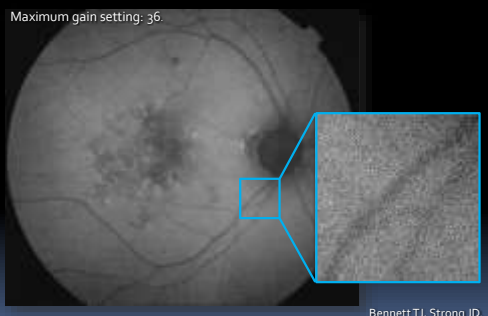
- Sampling or image averaging is not currently available for fundus camera systems.
- The challenge is trying to achieve a balance between adequate exposure while minimizing noise.
- Careful use of gain settings is essential.

Gain

- Amplifies signal to increase light sensitivity.
- Analogous to ISO in film.
- Increasing gain also increases noise.

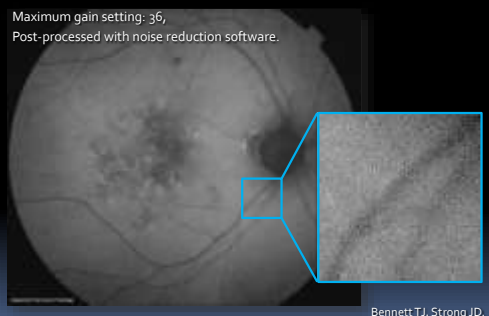


Gain



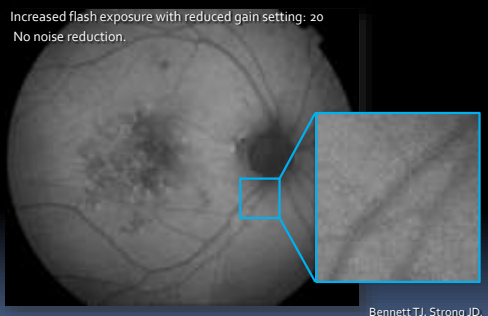
Bennett TJ, Strong JD.
Journal of Ophthalmic Photography, Supplement Summer, 2007

Gain



Bennett TJ, Strong JD.
Journal of Ophthalmic Photography, Supplement Summer, 2007

Gain



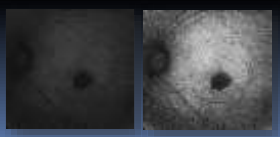
Bennett TJ, Strong JD.
Journal of Ophthalmic Photography, Supplement Summer, 2007

Modified Fundus Camera FAF

- The default camera controls for FAF typically place the gain near the maximum setting in order to record low-level fluorescence.
- There may be very little room for lowering gain to reduce amplifier noise and still maintain sufficient exposure.

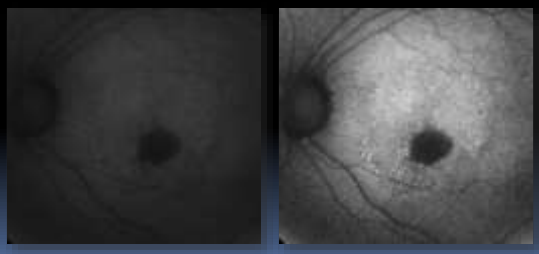
Modified Fundus Camera FAF

- If the gain setting is too low, underexposure can occur resulting in dark, low-contrast photographs.
- Enhancement of underexposed images to improve brightness and contrast will increase noise in a manner similar to increasing gain.



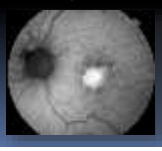
Post Capture Enhancement

- Increasing contrast exaggerates noise.



Gain

- In very low-light situations where high gain is used, like fundus autofluorescence:
  - Set the fundus camera for maximum light transmission and flash output.
  - Control exposure using gain to ensure lowest possible gain setting to reduce noise.

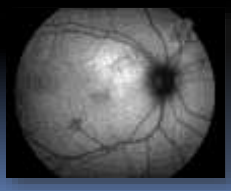


Maximizing Transmission

- All controls should be set for maximum light transmission and flash output.
- Light transmission may be best at the widest-angle setting in some variable angle fundus cameras.
- If the fundus camera is equipped with an illumination diaphragm it should be set to the largest aperture.

Maximizing Transmission

- When light transmission is maximized, eyes with significant accumulation of lipofuscin can be imaged with reduced gain settings while still maintaining adequate exposure.



Maximizing Transmission

- In the absence of significant accumulation of lipofuscin, underexposure can still occur in some widely dilated eyes with clear media.
- Young patients.

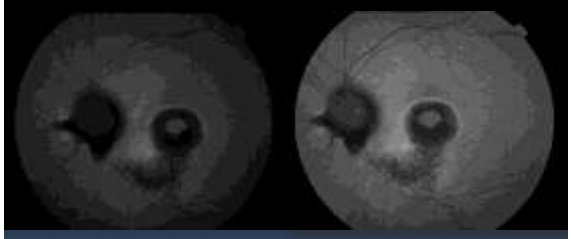
New "Spaide" Filters

- New, more efficient filter sets that significantly improve light transmission are now commercially available for some fundus-camera based systems.
- The patented Spaide filters are only available for Topcon Imagenet systems, but other manufacturers may also have 2nd generation filter sets.

New "Spaide" Filters

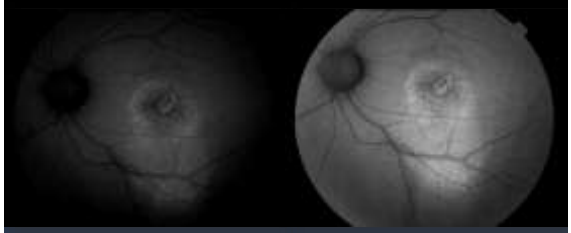
- The excitation filter has a band-pass range of about 535-585 nm and the barrier filter has a band-pass range of about 605-715 nm.
- Avoids excitation of both the crystalline lens and fluorescein, improves light transmission, and reduces noise.
- FAF imaging can be done either before or after fluorescein angiography.

New "Spaide" Filters



Original FAF Filters: Gain 36, 300ws | New Spaide Filters: Gain 16, 300ws

New "Spaide" Filters

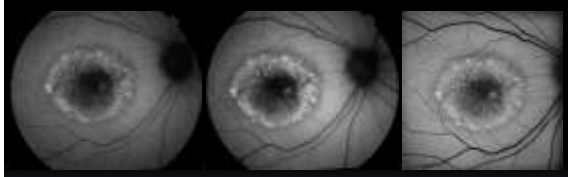


Original FAF Filters: Gain 36, 300ws | New Spaide Filters: Gain 16, 300ws

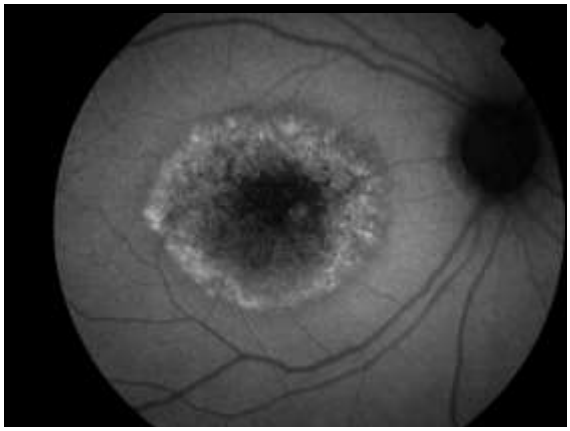
FAF Wavelengths

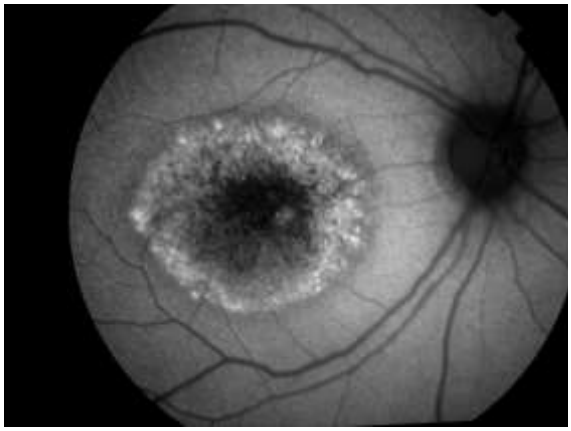
- cSLO
  - Exciter: 488 nm
  - Barrier: 521 nm (short cutoff/wide bandpass)
- Original Spaide filters:
  - Exciter: 580 nm
  - Barrier: 695 nm
- New proprietary "Spaide" filters:
  - Exciter: 535-585 nm
  - Barrier: 605-715 nm

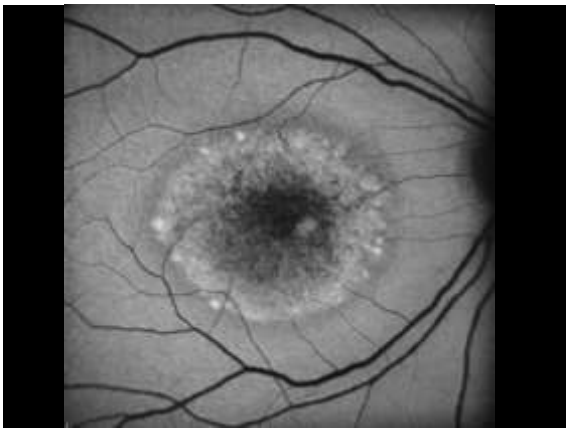
FAF Wavelengths

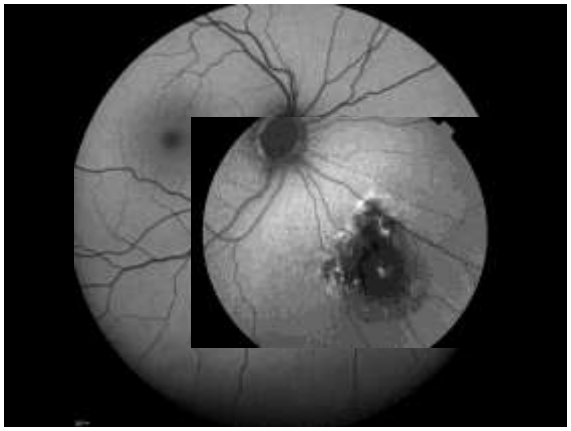


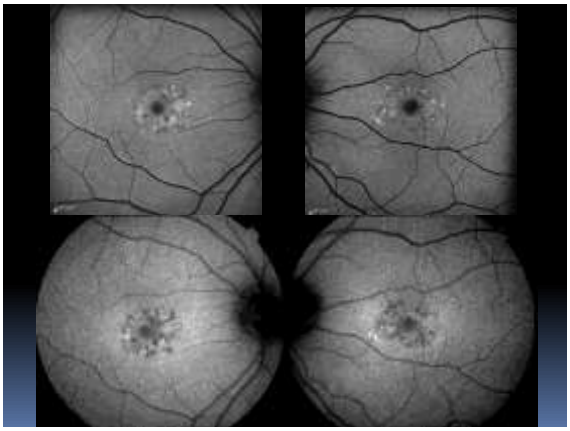
Original FAF Filters 580/695 | New "Spaide" Filters 535-585/605-715 | Spectralis SLO 488/521









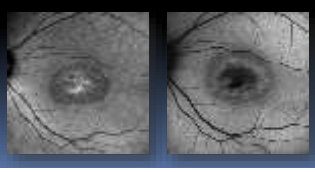


Instrument/Camera Technique

- Proper camera technique is necessary to obtain quality FAF images.
- Target the focus to the level of the retinal pigment epithelium, which is where the majority of autofluorescence typically occurs.
- Once images are captured at this focus level, the camera can be refocused on different layers when autofluorescence is detected in other retinal structures.

Instrument/Camera Technique

- cSLO: If viewing in IR prior to FAF, focus will need to be adjusted to account for the shorter wavelengths of the blue laser (488 vs 820 nm).
- Turn the focus knob approximately ¼ turn clockwise.

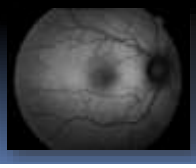


Instrument/Camera Technique

- Exposure can be improved with optimal axial alignment of the illuminating beam within the center of the dilated pupil.
- The ability to move the beam within the pupil to avoid prominent media opacities also helps.
- Maximum pupillary dilation will allow even illumination and exposure.

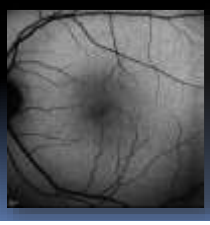
FAF Findings

- The optic nerve, retinal blood vessels, and the fovea normally appear dark against a variable background of fluorescence from the RPE.
- The absence of the RPE at the optic nerve head causes it to appear dark.



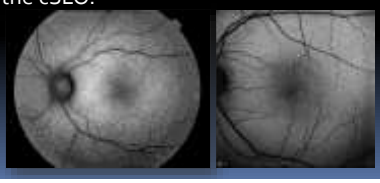
FAF Findings

- Retinal vessels block both the excitation and emission of fluorescence from the underlying RPE and also appear dark.



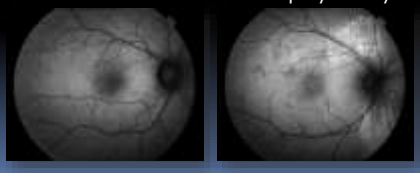
FAF Findings

- The density and morphology of pigment in the fovea causes absorption of the excitation wavelengths making the fovea appear darker than the surrounding macula, especially with the cSLO.



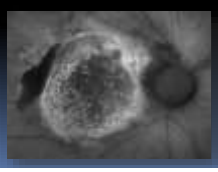
Findings/Interpretation

- Autofluorescence imaging is effective because it can detect metabolic changes in a cell monolayer, the retinal pigment epithelium, making it useful in conditions where the health of the RPE plays a key role.



Findings/Interpretation

- Hyperfluorescence is a sign of increased lipofuscin accumulation, which may indicate degenerative changes or oxidative injury.
- Hypofluorescence usually indicates missing or dead RPE cells.

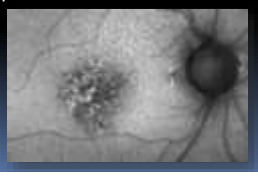


Documentary vs. Diagnosis

- Documentary:
  - Geographic atrophy
  - Pigmentary changes (RP, ICSC…)
- Diagnostic:
  - Early detection of bullseye/retinal toxicity
  - Progression of geographic atrophy
  - Buried disc drusen
  - Macular hole
  - ICSC activity/leakage?

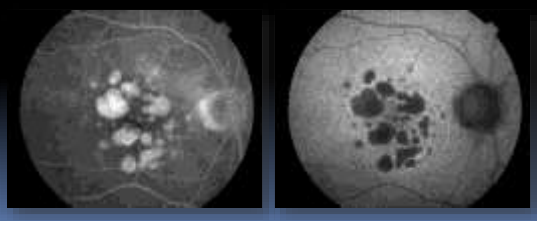
Diagnostic Applications

- The role of lipofuscin in the pathogenesis of macular degeneration is currently unknown, but increased autofluorescence may precede development or progression of geographic atrophy in ARMD.

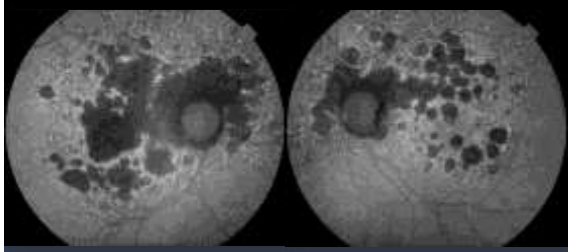


Diagnostic Applications

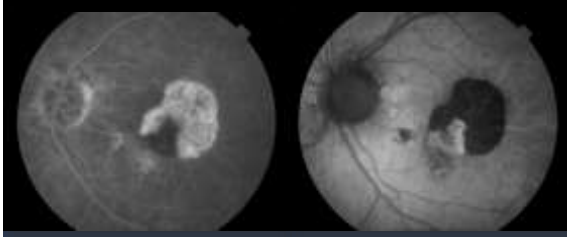
- Geographic atrophy that appears as a "window defect" in fluorescein angiography will appear dark in autofluorescent imaging.



Geographic Atrophy

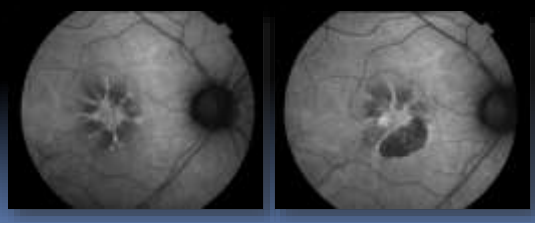


RPE Rip



Diagnostic Applications

- Serial FAF imaging can be used to track progression of geographic atrophy.

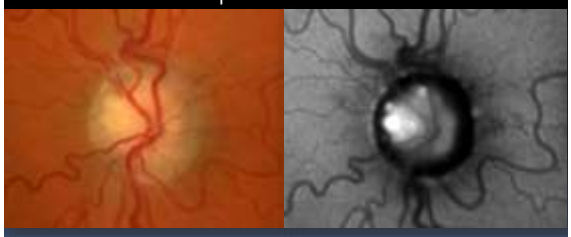


Macular Hole

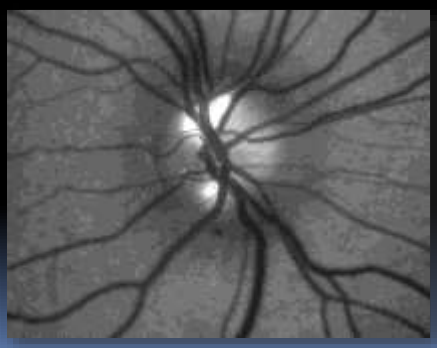
- Loss of overlying retinal tissue reveals "bare" hyperfluorescent RPE.

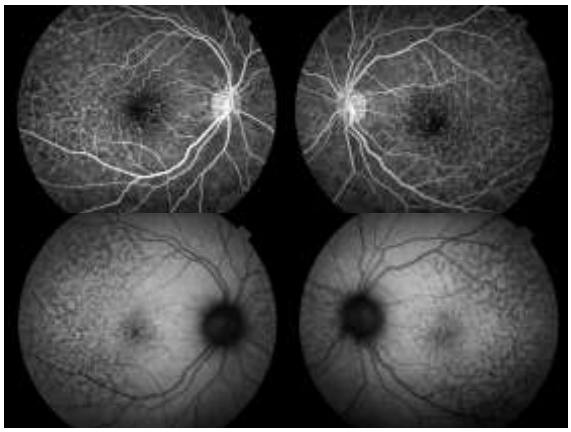
Disc Drusen

- Focused on optic nerve rather than RPE.

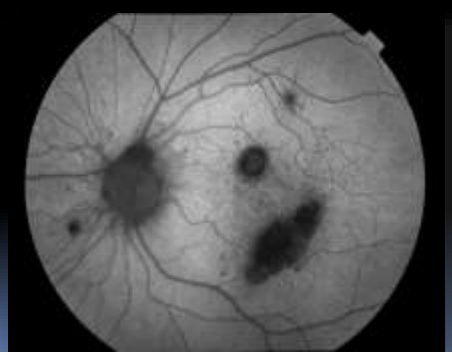


Disc Drusen

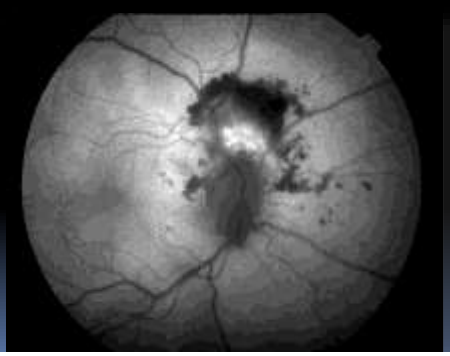




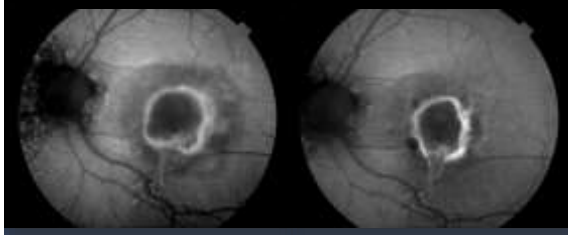
Ocular Histoplasmosis



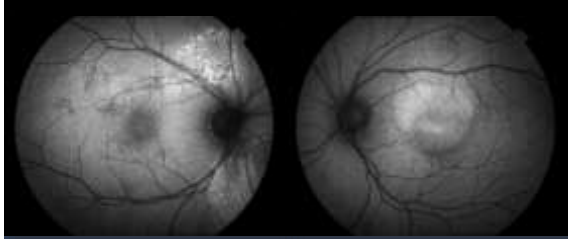
Active SRNVM



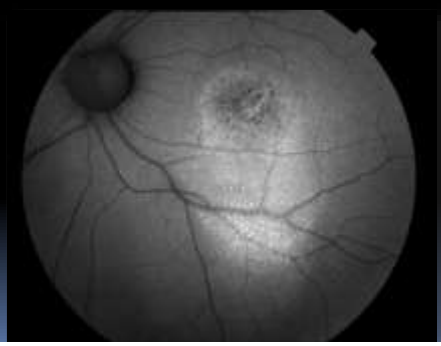
ARMD Turbid Fluid



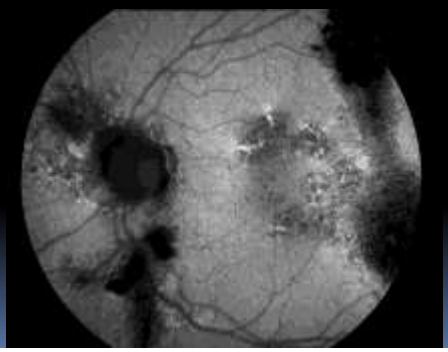
ICSC

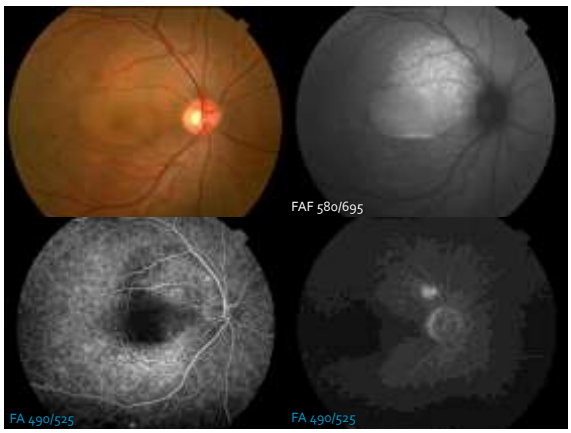


ICSC Turbid Fluid

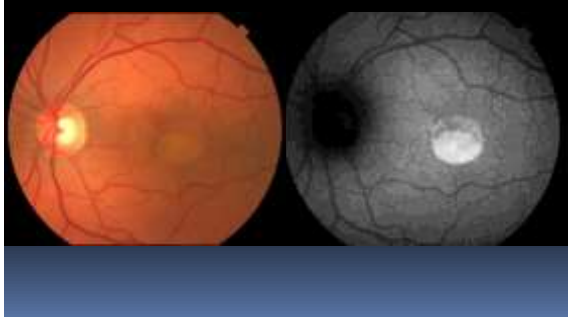


Chronic ICSC

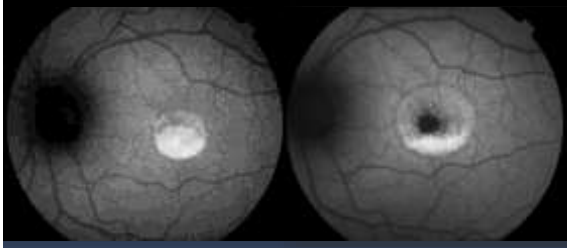




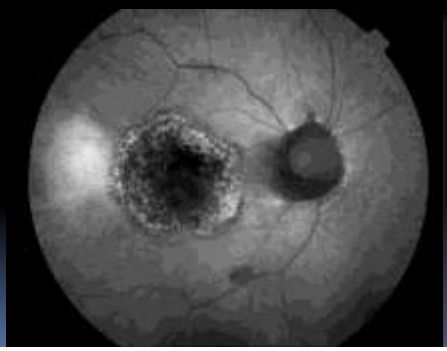
Vitelliform Lesion



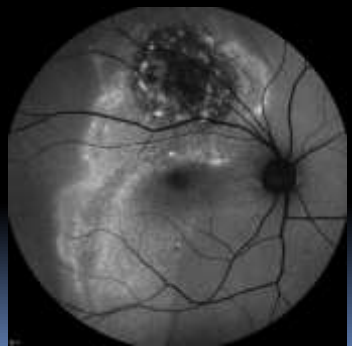
Vitelliform Lesion



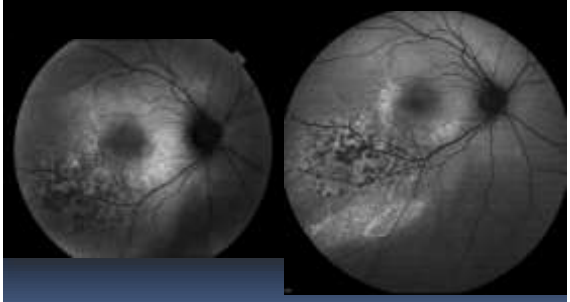
Inflammatory Disease?



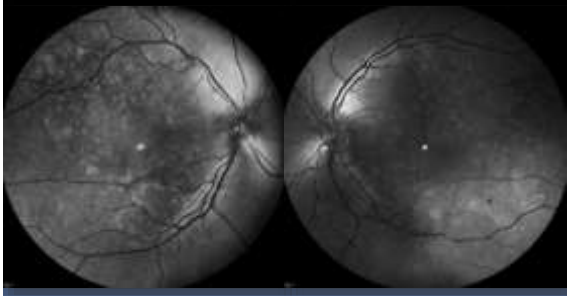
Choroidal Melanoma



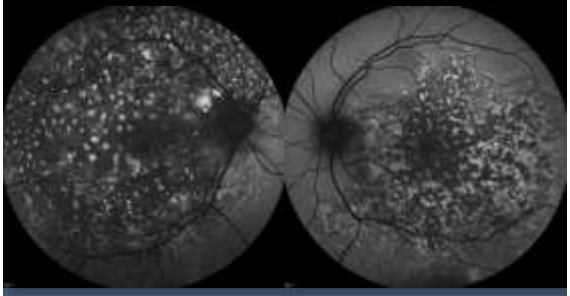
Metastatic Lesion



Metastatic Lesion

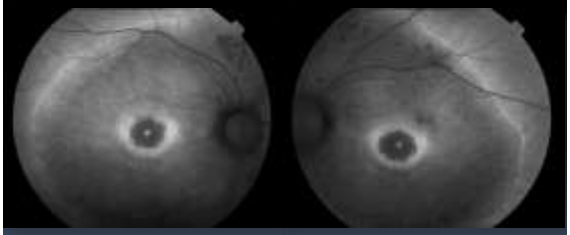


Metastatic Lesion

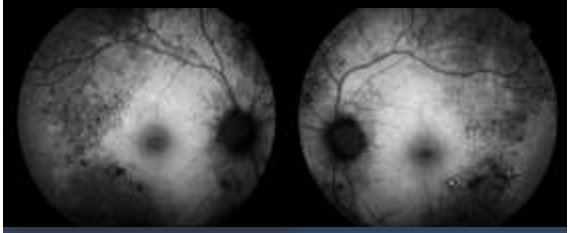


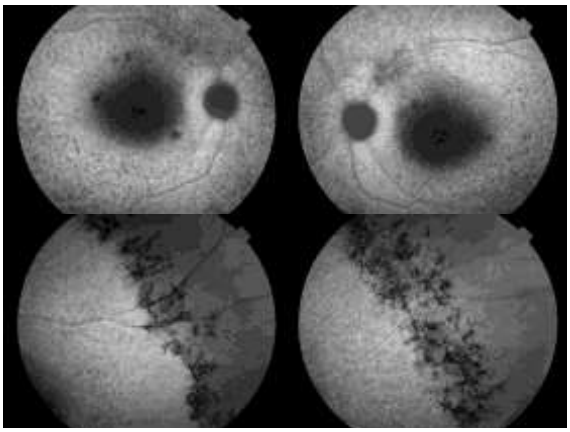
Cone Dystrophy

Retinitis Pigmentosa

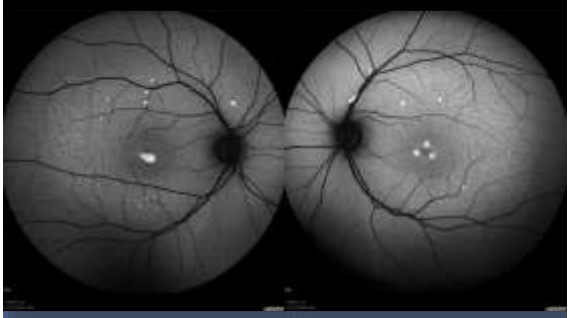


Retinitis Pigmentosa

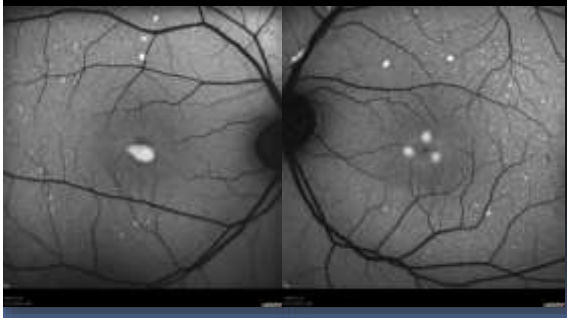




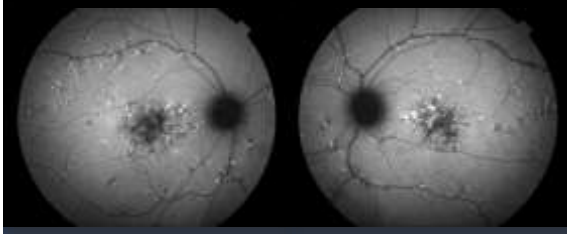
Stargardt Disease



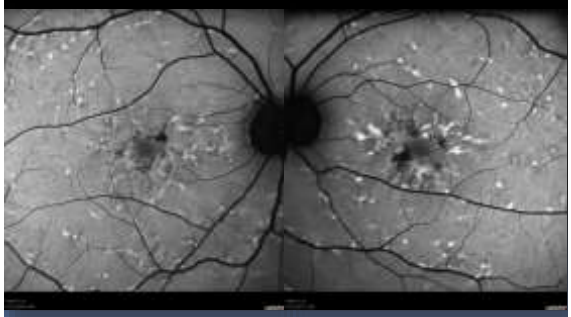
Stargardt Disease

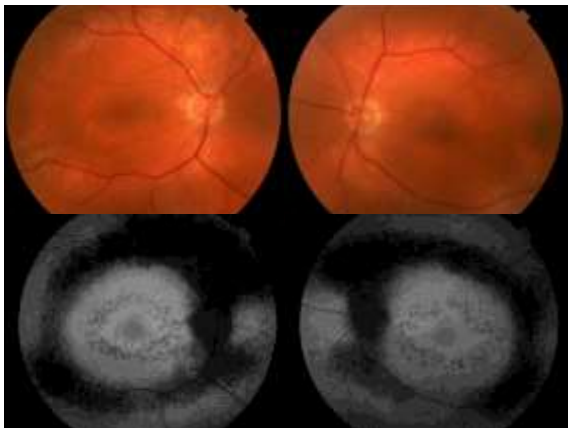


Stargardt Disease

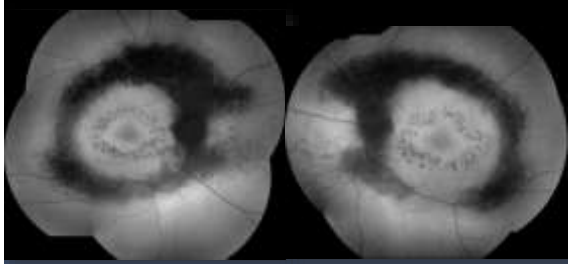


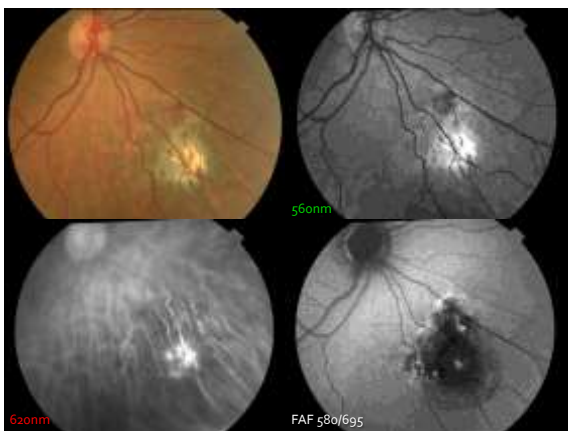
Stargardt Disease





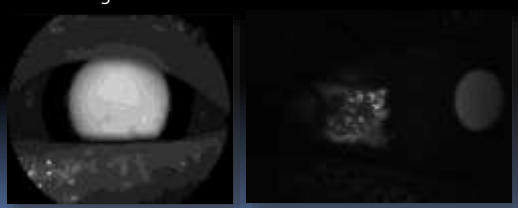
Chloroquine Toxicity





Autofluorescence

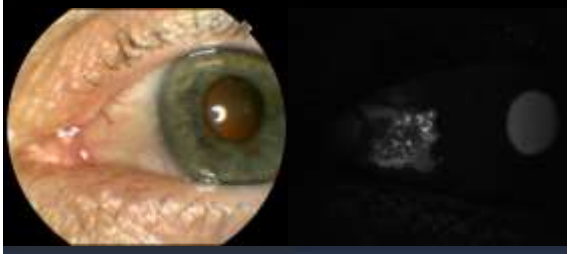
- Anterior Segment Findings
  - Aging crystalline lens
  - Pingueculae



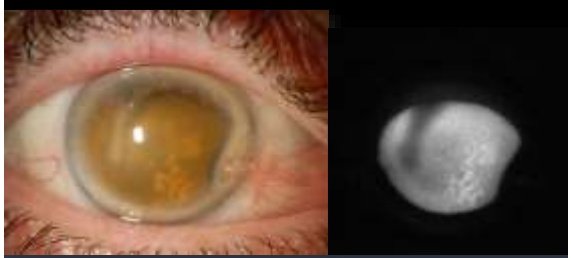
Autofluorescence

Br J Ophthalmol 2009;93:396–399.

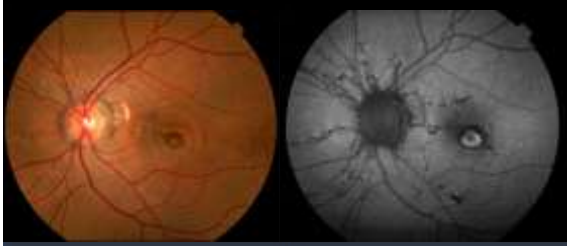
AF of Pinguecula

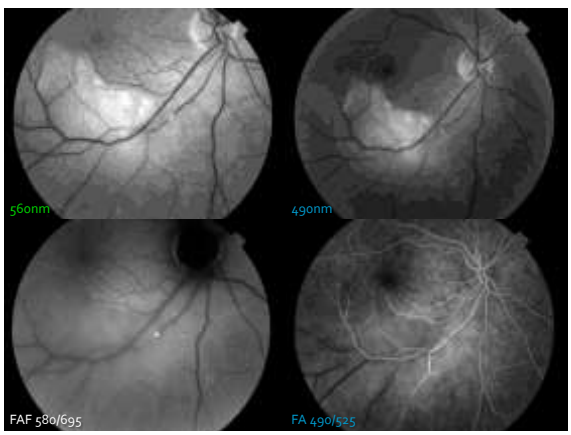


AF of Hemosiderin/Hemoglobin



AF of Blood/Hemoglobin?





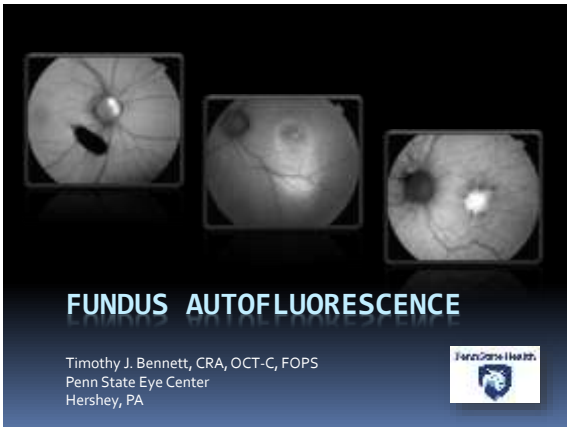
Thank You!

Amy Anderson
Education Manager
Evan Jacobsen
Director of Information Technology

IJCAHPO
INTERNATIONAL JOINT COMMISSION ON ALLIED HEALTH PERSONNEL IN OPHTHALMOLOGY

Questions?

- email: timbennett@eye-pix.com
- http://eye-pix.com/fundus-autofluorescence



FUNDUS AUTOFLUORESCENCE

Timothy J. Bennett, CRA, OCT-C, FOPS
Penn State Eye Center
Hershey, PA