

## On Different Wavelengths: The Spectrum of Retinal Imaging

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## On Different Wavelengths: The Spectrum of Retinal Imaging

- Wavelengths
- Monochromatic Photography
- Filters
- Angiography
- cSLO
- Fundus Autofluorescence

## Wavelength

- Physical distance between the crests of energy waves in the electromagnetic spectrum.
- Expressed in nanometers (nm).
- Determines color.



## Wavelength Specific Imaging

- Monochromatic Photography
- Fluorescein Angiography
- ICG Angiography
- Fundus Autofluorescence
- IR Reflectance

## Monochromatic

- **mon'•o•chro•mat'ic** *adj.*
  1. Having or appearing to have only one color.
  2. Of or composed of radiation of a single wavelength.

## Monochromatic Fundus Photography

- The practice of imaging the ocular fundus with monochromatic illumination to enhance visibility of various fundus structures or pathologies.



## Photographic Principles

- The use of contrast filters to alter subject tones in black-and-white photography.
- The increased scattering of light at shorter wavelengths.



## Contrast Filters

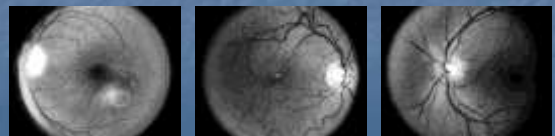
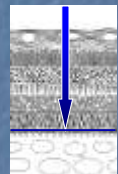
- Alter the tonal rendition of different subject colors by introducing brightness differences between colors that would normally reproduce as similar tones of gray.
- Contrast filters selectively limit the range of wavelengths reaching the imaging plane.

## Monochromatic: Common Uses

- “Red-free” photos before fluorescein angiography.
- Retinal nerve fiber layer photography.
- Choroidal lesions.

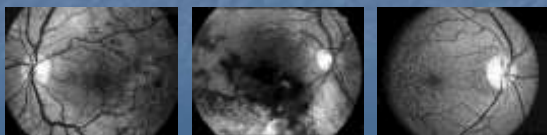
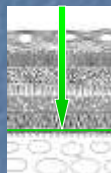
## Short (Blue/Cyan) Wavelengths

- RNFL
- Epiretinal membranes
- Retinal folds, cysts



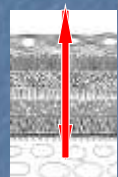
## Medium (Green) Wavelengths

- Retinal vasculature
- Hemorrhages
- Drusen
- Exudates



## Long (Red) Wavelengths

- RPE disturbances
- Choroidal ruptures
- Choroidal nevi
- Melanomas



## Absorption Filters

- Organic dyes coated on acetate or glass.
- Selectively absorb certain wavelengths while transmitting others.
- Most common type of filter for general photographic use.
- Broad bandwidth and curved gradients.

## Absorption Filters

- Gelatin filters
  - Easy to cut to fit aperture of fundus camera filter sliders.
- Glass filters
  - More durable than gel filter.
  - Will not warp.



## Interference Filters

- Multiple thin coatings of materials with known refractive indices.
- The difference in refractive index between layers creates 'interference' and rejection of specific wavelengths.
- Efficient, linear gradients, capable of narrow bandwidth.
- Necessary for modern angiography.

## Common Filters

- Available with most fundus cameras:
  - Green (red-free): 530-560nm
  - Blue-green fluorescein exciter: 490nm
  - Red filter standard on ICG capable cameras: 615-640nm

## Digital Imaging

- Monochrome digital sensor (analogous to B&W film).
- Color sensor set for monochrome capture.
- Color capture through filters, with post capture conversion to grayscale.
- Full color capture with software color separations.

## Multiple Monochromatic Renditions

- Photo essay approach - single monochromatic renditions don't tell the whole story.
- Multiple renditions can provide additional clinical information.
- Provide a full color photo as a baseline reference.

## Fluorescence Imaging

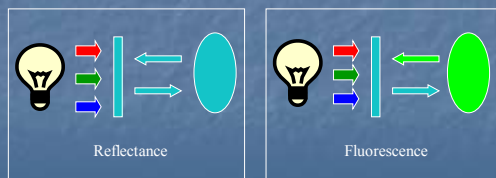
- Fluorescein angiography
- ICG angiography
- Fundus autofluorescence

## Fluorescence

- Fluorescence occurs when susceptible molecules (fluorophores) absorb electromagnetic energy, temporarily exciting them to a higher energy state which 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).



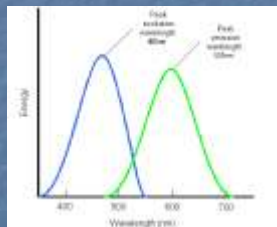
## Fluorescein Synthesized, 1871

- Fluorescein sodium is a fluorescent dye synthesized from petroleum derivatives resorcinol and phthalic anhydride.
- Synthesized by Adolf Baeyer in 1871.
- Baeyer received the Nobel Prize in 1905.



## 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.



## Indocyanine Green (ICG)

- First used as a dye in the manufacture of Kodak Wratten filters.
- Used for cardiac output and liver function studies.
- Low fluorescence efficiency compared with fluorescein.
- Poor results in early attempts with infrared films.

## ICG

- Peak excitation: 805nm
- Peak emission: 835nm



Ophthalmic Photography. Saine & Tyler, 2002

## ICG/Near Infrared Wavelengths

- Longer wavelengths allow better penetration through blood and RPE, allowing visualization of the choroidal vasculature.

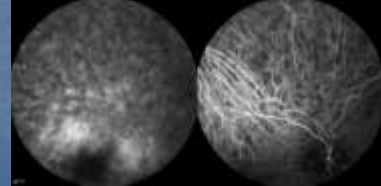


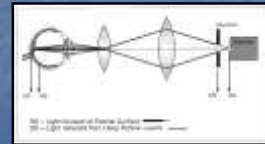
Image courtesy of Ethan Priel, FOPS

## 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.



Ophthalmic Photography. Saine & Tyler, 2002

## Spectralis HRA

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

## Scanning Laser Ophthalmoscope

- Confocal imaging reduces the effects of short wavelength scatter in the ocular media and confounding AF from crystalline lens.
- Oversampling improves contrast and reduces noise. Example single image vs. oversampled
- Need example of fundus camera and SLO red free image through cataract. ?? Confounding AF from crystalline lens?

## Fundus Autofluorescence (FAF)

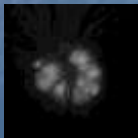
- Fundus autofluorescence (FAF) is a diagnostic imaging technique for documenting the presence of fluorophores in the human eye.
- Fluorophores are chemical structures that possess fluorescent properties when exposed to light of an appropriate wavelength.

## Fundus Autofluorescence (FAF)

- The term “autofluorescence” is used to distinguish fluorescence that can occur naturally from 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 (FAF)

- 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 (FAF)

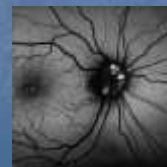
- The current use of FAF imaging centers mostly on documenting the deposition of lipofuscin in the RPE.
- Lipofuscin is a fluorescent pigment that accumulates in the RPE as a metabolic byproduct of cell function.
- Lipofuscin deposition normally increases with age, but may also occur from RPE cell dysfunction or an abnormal metabolic load on the RPE.

## 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

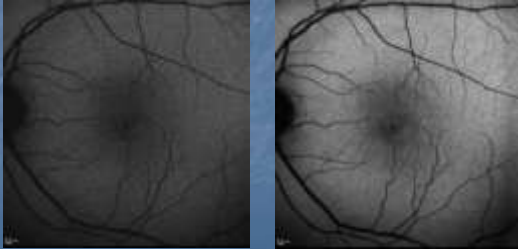
## cSLO FAF

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



## cSLO FAF Oversampling

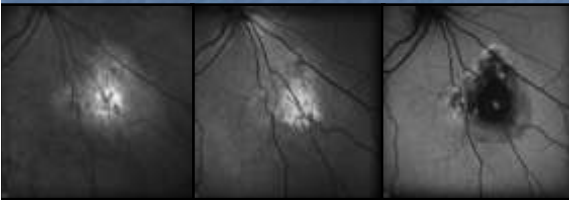
- Smooths noise and increases exposure



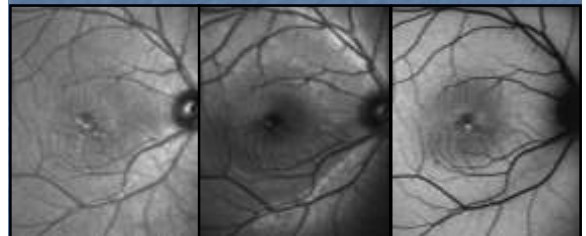
## cSLO FAF

- 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.

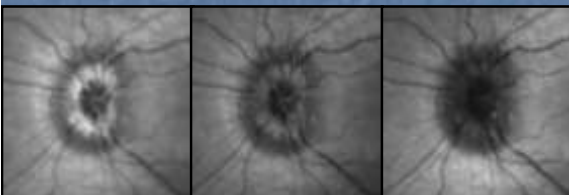
## Spectralis IR/RF/FAF



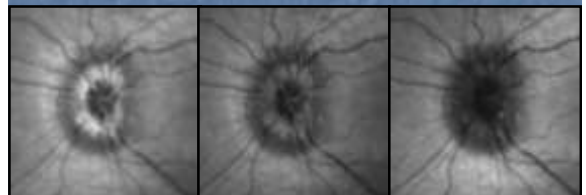
## Spectralis IR/RF/FAF



## Wavelength Difference?



## Confocal SLO Focus Shift



All taken at IR Reflectance wavelength (820nm)

## Monochromatic Wavelengths

- Monochromatic
  - Short (blue-green) 450-490 nm
  - Medium (green) 520-560 nm
  - Long (red) 610-625 nm

## Angiography Wavelengths

- Fluorescein angiography
  - Excitation 480-490 nm
  - Transmission 520-530 nm
- ICG angiography
  - Excitation 805 nm
  - Transmission 835 nm

## cSLO Wavelengths

- Spectralis cSLO
  - Fluorescein & FAF excitation 488 nm
  - ICG excitation 790 nm
  - IR reflectance 820 nm

## FAF Wavelengths

- Fundus Autofluorescence
  - cSLO excitation 488 nm
  - cSLO transmission 521 nm
  - Fundus camera excitation 580 nm
  - Fundus camera transmission 580 nm
  - Spaide excitation 535-585 nm
  - Spaide transmission 605-715 nm

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