Fundus Autofluorescence and its PRACTICAL applications: Retina Beyond the Color

Jeffry D. Gerson, O.D., F.A.A.O
Shawnee, KS
jgerson@hotmail.com

Disclosure
- I have worked with/consulted for or received honoraria from the following:
  - Allergan, B&L, Kemin, Maculogix, Nicox, Optos, Optovue, Thrombogenix, VSP & Zeavision

Start to think about this....

What is my job this morning?
- Get you pumped up about an exciting new technology
- Keep you awake while doing it
- That being said....

Getting pumped up...

Coffee
- Do you drink coffee?
  - Over 50% of North Americans
  - Is this important?
    - Coffee is leading source (by far) for antioxidant intake in the US diet!!
  - Neither coffee nor caffeine intake were associated with early AMD per BDES
  - Beware:
    - COFFEE and DOUGHNUT Maculopathy

1. As reported by American Chemical Society Blog
Coffee

- Do you drink coffee?
  - Over 50% of Americans drink coffee

- Is this important?
  - Coffee is leading source (by far) for antioxidant intake in the US diet! \(^\text{1}\)

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- Beware:
  - COFFEE and DOUGHNUT Maculopathy \(^\text{2}\)

  As reported by American Chemical Society (Aug)


2 Cohorts
- 41,736 men Hx Professionals Flp Study – 18 years
- 86,214 women Nurse’s Hx Study - 24 years

Results

<table>
<thead>
<tr>
<th>Intake</th>
<th>Men</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 cup / month</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>1 c/m – 4 cups/w</td>
<td>1.02</td>
<td></td>
</tr>
<tr>
<td>5-7 cups / week</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>2-3 cups / day</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>4-5 cups / day</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>&gt; 6 cups / day</td>
<td>0.74</td>
<td></td>
</tr>
</tbody>
</table>

P<0.001 for trend and independent of caffeine intake

Who Has:

Once you have a histologic section done, you are not likely to have a good outcome!!

But what is the ultimate in an instrument showing you what you couldn’t otherwise see?

FUNDUS BEYOND THE COLORS!!

Who has:

Clinical exam
  - OLD (and still crucial)

Traditional photography
  - OLD, and nothing more seen than clinically

Digital widefield photography
  - Better documentation of more

OCT
  - Revolutionary

Fundus Autofluorescence
  - Will (again) revolutionize how we see the retina

How do we see what we see?

FUNDUS BEYOND THE COLORS!!
What is Fundus Autofluorescence (FAF)?

Autofluorescence (af) provides information on the RPE and overlying photoreceptors.

Current Clinical Applications:
- Method of non-invasive, quantitative, objective diagnosis
  - Visualization of characteristic changes
  - Confirmation of suspected diagnosis
  - Detection of phenotypes: What is it really??
- Monitoring disease progression

Angioid Streaks

Autofluorescence (AF)
- Captures a response from molecules in the RPE
- Can be captured by scanning laser or filters attached to fundus camera
- Spectralis by Heidelberg
- Cannon
- DAYTONA by Optos
- The only source of widefield FAF

Fundus Autofluorescence (FAF)
- A “new” form of imaging taking advantage of optics/wavelength capture to assess retinal disease
- More commercially available now than in past, and frequently in ophthalmic literature and gauge for clinical studies

*Ref: typical parafoveal ring of hyperfluorescence
# Evaluation of FAF

<table>
<thead>
<tr>
<th>Hypo FAF</th>
<th>Hyper FAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>➢ Reduction in RPE lipofuscin density</td>
<td>➢ Excessive RPE lipofuscin accumulation</td>
</tr>
<tr>
<td>➢ Increased RPE melanin content, e.g. RPE hypertrophy</td>
<td>➢ Occurrence of fluorophores anterior or posterior to the RPE cell monolayer</td>
</tr>
<tr>
<td>➢ Absorption from extracellular material/cells/liquid anterior to the RPE</td>
<td>➢ Lack of absorbing material</td>
</tr>
<tr>
<td></td>
<td>➢ Optic nerve head drusen</td>
</tr>
</tbody>
</table>

# Simply stated

- **Hyper-autofluorescence**: Bright is dying
- **Hypo-autofluorescence**: Dark is Dead

Exemplary Cases of Hyper and Hypo af

- **Hyper af**: Bright is dying
- **Hypo af**: Dark is Dead

Interpreting AF Images

- The normal RPE yields a slightly granular grey AF glow in contrast to the optic disc and retinal blood vessels which appear black.
- **Hyperautofluorescence**: Identifies sick cells that result in a bright white signal.
- **Hypoautofluorescence**: Dead cells give off no signal which appear dark grey or black.
- Ultra-widefield AF reveals abnormalities which cannot be viewed with other imaging modalities.

Daytona - Autofluorescence

![Daytona - Autofluorescence Image]
How is Optos AF imaging different than established AF methods?

- Optomap AF images are obtained using the green wavelength (532nm) Heidelberg’s scanning laser ophthalmoscope uses a blue wavelength (488nm). Fundus cameras can also obtain green illuminated AF images by using a Spaide filter.
- Optomap AF images are captured using only one image whereas competitors often use multiple images (HRA – 20-100 images averaged in post processing). Images can be captured undilated, and the imager does not need to use another laser to focus the image. For example, on the Heidelberg SLO, the imager has to initially focus utilizing the green laser and then switch to the blue laser to acquire the AF image.
- Due to the poor reaction of lipofuscin to the low light, images taken with the Staurenghi lens do not provide an accurate representation of the natural autofluorescence signal.

Structure and function: FAF and OCT (or just FAF…)

Autofluorescence: Lipofuscin

- Is it structure or function?
  - It is a measure of functional ability of the RPE: compliments OCT
- Measuring lipofuscin can be invaluable in AMD
  - Early devel, sensitive measure if change/progress of GA
- Tends to be more AF in dystrophies than AMD

How might this relate to FAF?

What Novel Information Does Autofluorescence Give Us?

AF Abnormalities have Myriad Etiologies

The Vitamins Mnemonic is helpful to remember possible etiologies of AF abnormalities
**BACKGROUND**

In which disease does AF offer a diagnostic or therapeutic advantage?

- Age-related macular degeneration – the primary cause is thought to be the premature dysfunction of the retinal pigment epithelium (RPE) – therefore areas affected by AMD appear very dark
- Geographic atrophy – large dark areas often surrounded by bright halos
- Inherited retinal diseases: Stargardt’s, Retinitis Pigmentosa – dark patterns surrounded by areas of bright disruption
- Optic nerve head drusen – will appear bright white over the disc
- Pigmentary retinopathy – very dark spots throughout the retina
- Multifocal central serous retinopathy – will show as spotted areas of decreased FAF signal (dark) with broad patches of increased FAF signal (light)
- Tumors – will show both areas of light and dark depending on stage of growth
- Inflammatory diseases (VKH syndrome, neurosyphilis, tuberculous chorioiditis) – areas will appear very dark with striking areas of increased FAF signal (light).

**Autofluorescence**

- Captures a response from molecules in the RPE
- Can be captured by scanning laser or filters attached to fundus camera
- Spectralis by Heidelberg
- DAYTONA by Optos

**Some FAF examples…**

**Optic Nerve Head Drusen**

**GA: color and OCT, but…**

Would FAF have a role here?
GA is a most common cause of vision loss
• New treatments in pipeline to treat: some are oral and topical
• How can we tell if they are working or not?
• Recent study showed some correlation between OCT and FAF, but FAF more consistent between observers

Progression of GA
The FUTURE
• At least 16 new therapies are under development to help slow or stop progression to wet AMD

Peripheral AMD changes???
• Where do changes happen first in AMD
• Could it be in periphery?
• Correlating data may prove to allow earlier detection of AMD (and other diseases)

Reykjavik Eye Study
• 573 patients imaged at 12 year time period
• Color and AF imaging
• The peripheral retina was analysed and the study found:

In 77% of patients, AMD related changes were visualised outside of the area of the Topcon photos

Case 6: Optomap® Color Fundus Image of Left Eye
Case 6: Optomap® Fundus Autofluorescence Image of Left Eye
Case 6: Optomap® plus with Resmax Color Fundus Image of Left Eye
Case 6: Optomap® plus with Resmax FAF Image of Left Eye
Case 6: Optomap® plus with Resmax Green Separation Fundus Image of Left Eye

*Courtesy of the Reykjavik Eye Study

At higher magnification the underlying choroidal vessels are more easily visualized within the macular lesion.
Note the hypo AF zone in the macula is surrounded by an area of hyper AF. “Hot spots” corresponding to the surrounding hyper AF area indicate an increase in both metabolic activity and lipofuscin.
More recent literature on FAF and AMD

- 3 distinct FAF patterns identified
  - Granular, Mottled and Nummular
  - 90% concordance between eyes
- More FAF in neovasc AMD vs dry vs normal
  - 86% vs 73% vs 16%
  - OR wet AMD: 12.7 and Dry: 6.2 vs normal
  - Older age and female also associated w more FAF
- Associations found between:
  - Granular FAF and peripheral drusen
  - Mottled FAF and RPE depigmentation

Tan et al. Peripheral FAF in AMD. Ophth. 6/2013

FAF examples

- A: Peripheral drusen
- B. Granular FAF
- C. RPE atrophy
- D. Nummular FAF
- E. Periph RPE depig and finde drusen
- F. Mottled FAF at arrow, and fine FAF at drusen

FAF from AREDS2

OPERA: Optos Peripheral Retina (study within AREDS2)
Looked for AF abnormalities in 3 zones: central, to vortex, and outside vortex
Percentage w abnormalities by zone: 94/83/50%
Some difference between superior and inferior periphery, but not significant
Abnormalities in both advanced and non-advanced eyes


Same appearance and vision as fellow eye

What Could FAF tell us?
Central Serous (ICSC)

- FAF of chronic ICSC
- OCT of same pt: 20/20 vision

2 f/u visits

Improvement at f/u

Another ICSC

- Another pt w recurrence based on OCT
- What does FAF show that FA does not?

Routine plaquenil check.
Hx SLE and associated liver dysfunction
Hx of Dry Eyes
Meds Include: Naproxen, and Plaquenil 200 mg qd X 2 Years-
Cumulative Dose 146,000 mg
BCVA 20/20 OU
What is going on and What Should I do?
PAF images reveal a perifoveal ring of hypofluorescence in each eye.

Case 5: Optomap® FAF OD

New Guidelines per AAO:
- Risk increases sharply to 1% at 5-7 yrs or cumulative dose of 1000 g (usual dose 400 mg/d HCQ or 250 mg/d CQ)
- New screening guidelines include baseline exam and then annually at every 5 yrs
- Objective tests: mfERG or FAF or SDOCT
- Subjective test: 10-2
- Fundus exam still important, but findings are generally late stage

Recommendations by: Marmor et al. Ophthalmology 2/11
What does RP really look like?

RP: 20/20- vision OU: impressive FAF!

Another RP: 20/25-, symptomatic

RP macula: Early macular changes

Debbie…a patient for over 10yrs

- Vision approximately 20/100 and stable for years
- Always wondering if vision will get worse
- Some difficulties w job, but nobody at workplace knows of visual difficulties
- Drives w Biopic and has for years

What is the dx and can we tell if she is getting worse?

Stargardt’s Dystrophy
Rod/Cone dyst

What do you see here besides Asteroid Hyalosis???

FAF: At least you can see there is an ONH and vasculature

Long-term patient Choroidal osteoma

Is he getting worse?

What is the pathology? ONH swelling?
A normal appearing retina in a 25 year old complaining about a new visual problem that has slowed his reading. VA is 20/20 or 6/6 in each eye.

**Retinal Toxicity Invisible to Ophthalmoscopy (Plaquenil, Accutane)**

Only the AF image reveals an abnormality in the right eye.

**Bull’s Eye Maculopathy**

Left eye of same patient reveals a bull’s eye maculopathy which is present with AF but not with standard imaging. The left eye reveals a perifoveal ring of hypo AF.

**Early retinal degeneration** (not observed with standard fundus photography)

13 yo Hispanic female with no visual complaints and 20/20 VA both eyes.

**Diagnosis: Early Retinitis Pigmentosa with Bull’s Eye Maculopathy (invisible to Ophthalmoscopy & Fundus Photography)**

This is the same 13 yo Hispanic female.
Technology is fun! Take advantage of it! Your patients deserve it!!

Jeffry Gerson, O.D., F.A.A.O.
jgerson@Hotmail.com

Capture modes
- Non-mydriatic ultra-widefield autofluorescence imaging of the retina using fundus autofluorescence and green laser light (532nm)
- Visualization of RPE changes
- Through 2mm pupils
- Comfortable for the patient and gentle on the eye
- Both color imaging and autofluorescence can be carried out in one workflow

Non-mydriatic ultra-widefield autofluorescence imaging of the retina using fundus autofluorescence and green laser light (532nm)

Visualization of RPE changes

Through 2mm pupils

Both color imaging and autofluorescence can be carried out in one workflow

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Ultra-Widefield Autofluorescence Imaging

- Optos Ultra-Widefield devices image up to 200° of the retina in one capture - not multiple scans which requires frame averaging
- Green laser wavelength (532nm) is used for visualising fundus autofluorescence.
- To compare with a blue laser, at 488nm the wavelength is absorbed by the macular pigment and also induces emission from lipofuscin which is the main fluorophore in the RPE. At 532nm, there is little absorption by the macular pigment and this gives a purer lipofuscin signal.

Images courtesy of Doheny

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Autofluorescence Imaging

- Fundus Autofluorescence (af) has been developed over the last 10 years and is a non-invasive method of studying the fluorescent properties of lipofuscin* in the RPE
- AF imaging gives information over and above conventional imaging techniques such as fundus photos, Fluorescein Angiography or OCT.
- It allows visualisation of the metabolic changes at the level of the RPE and helps to identify areas that may be at high risk (e.g. of developing geographic atrophy or CNV).

* Lipofuscin is a mixture of autofluorescent pigments that accumulate in the RPE as a by-product from the incomplete degradation of photoreceptor outer segments.

Autofluorescence Imaging (Cont’d)

- AF imaging is easily repeatable, without risk and is also very suitable for children.
- In certain cases, af shows changes that are still invisible with regular ophthalmoscopy resulting in the early detection of any changes and diseases.
- The possibilities and limitations of AF are currently being explored, but soon now AF can replace FA when dealing with dry retinal changes (no vessels involved).

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Disc Drusen - Revealed with af (with Congenital Tortuosity)
**Buried Disc Drusen - Revealed with af (with Congenital Tortuosity)**

**How are AF images captured on the current device?**

- Once the AF imaging mode is selected, the AF image is captured in the same way as an Optos color image, however instead of red and green light combined, just green light is swept across the fundus. Light coming back from the retina in AF imaging comprises green reflectance light and autofluorescence light which is in the red spectrum. AF images on Optos devices are therefore detected on the red detector, and viewed on the red color channel.

**How is AF useful for disease diagnosis and observation of progression?**

- AF can easily identify areas of dead (black) or dying tissue (white) from areas of normal tissue (grey). Disease diagnosis is possible by identifying characteristic patterns only seen in that disease.