Learning objectives:

1. Understand the history and technological basis of fundus multispectral imaging (MSI)
2. Become familiar with physiological/pathological fundus conditions utilizing MSI

History

1851 Invention of ophthalmoscope
   – Hermann von Helmholtz
1886 First in-vivo retinal photograph
   – Jackman and Webster
1911 Monochromatic ophthalmoscopy recommended
   – Ginestous
1913 Monochromatic ophthalmoscopy use
   – Vogt
1930 Filter to enhance retinal vasculature for FA
   – Kikai
1950s Excitation and barrier filters for FA
   – Several

MONOCHROMATIC IMAGING

Barrier/Excitation Filters

• Used in fluorescein angiography since 1950s
• Light enters short-wavelength (usually blue-green spectrum between 500-575 nm) ‘excitation’ filter onto fundus
• Returning light passes through longer wavelength (usually yellow-red spectrum between 600-715nm) ‘barrier’ filter onto camera’s image sensor
• Selectively enhances view of fluorescent dye that enters retinal and choroidal vasculature

History

1965 Spectral reflectance characteristics based on fundus structure and wavelengths
   – Behrendt and Wilson
1972 Red-free retinal nerve fiber layer analysis
   – Hoyt and colleagues
1970s-80s Further refinement of filters
1990s Digital fundus imaging evolution
2000s Scanning laser technology evolution
Present Continued evolution of technology
Barrier/Excitation Filters

- Discovered various fundus structures that naturally autofluoresced
- Certain tissues contain molecules called fluorophores
- Fluorophores emit longer light wavelength when stimulated by shorter light wavelength—without need for injected dye
- Most common autofluorescent ocular tissue: retinal pigmented epithelium (RPE)

Barrier/Excitation Filters

- Lipofuscin within each RPE cell autofluoresces
- A2-E (N-retinylidene-N-retinylethanolamine) is dominant fluorophore in lipofuscin—increases with age, abnormal metabolic load on RPE, RPE dysfunction
- Most common ocular disease related to A2-E: age-related macular degeneration (AMD)

Barrier/Excitation Filters

Other potentially autofluorescent ocular structures:
- Collagen and elastin in choroid blood vessel walls
- Macular lipofuscin pigment
- Hyaluronic acid in the vitreous
- Crystalline lens fibers
- Disc drusen

Barrier/Excitation Filters

Ocular diseases and autofluorescence

<table>
<thead>
<tr>
<th>OCULAR DISEASE</th>
<th>AUTOFLUORESCENCE FEATURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic disc drusen</td>
<td>Hyperfluorescence seen (if surface drusen)</td>
</tr>
<tr>
<td>Age-related macular degeneration (AMD)</td>
<td>Hyperfluorescence:</td>
</tr>
<tr>
<td></td>
<td>1. Macular drusen</td>
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<tr>
<td></td>
<td>2. RPE metabolic stress</td>
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<td></td>
<td>3. Choroidal neovascular membrane (wet AMD)</td>
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<tr>
<td></td>
<td>Hypofluorescence:</td>
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<tr>
<td></td>
<td>1. RPE atrophy/death</td>
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<tr>
<td>Central serous retinopathy (CSR)</td>
<td>Hyper/hypofluorescent “guttering” (fluid tracks) at/inferior</td>
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<tr>
<td></td>
<td>to macula</td>
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<tr>
<td>Plaqueul maculopathy</td>
<td>Hyper and hypofluorescent concentric rings indicating</td>
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<tr>
<td></td>
<td>RPE stress and RPE atrophy/death, respectively</td>
</tr>
<tr>
<td>Retinitis pigmentosa (RP)</td>
<td>Multifocal areas of scattered hypofluorescence (RPE atrophy)</td>
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<tr>
<td></td>
<td>with central hyperfluorescence (RPE stress)</td>
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<tr>
<td>Stargardt’s dystrophy</td>
<td>Macular hyper-/hypofluorescence with</td>
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<tr>
<td></td>
<td>hyperfluorescent ‘pisciform’ lesions in posterior pole</td>
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<tr>
<td>Vitreo-retinal fibrosis/tuft</td>
<td>Typically hyperfluorescent/variable</td>
</tr>
</tbody>
</table>

Green light

- 495-570nm
- Also called “Red-free” filter
- Used for several decades
- Excellent sharpness of anterior retinal structures
  - Internal limiting membrane, retinal nerve fiber layer
- Primary enhancement of vascular structures due to strong absorption by hemoglobin
  - Retinal vessels and hemorrhages appear dark
  - Retinal hole visualization may also be enhanced

Green light

- Also useful in evaluating anterior segment pathologies
  - Conjunctivitis
  - Episcleritis
  - Scleritis
- Often performed prior to fluorescein angiography as standard protocol
- Green monochromatic digital filters incorporated into retinal cameras
Structures/pathology with green light

<table>
<thead>
<tr>
<th>STRUCTURE/PATHOLOGY</th>
<th>DARKER WITH GREEN LIGHT</th>
<th>LIGHTER WITH GREEN LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal vessels</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinal hemorrhages</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Optic disc hemorrhages</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinal holes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinal pigment</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Retinal nerve fiber layer</td>
<td>+</td>
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<tr>
<td>Epiretinal membranes</td>
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<td>Optic disc drusen</td>
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<tr>
<td>Retinal exudates</td>
<td></td>
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<tr>
<td>RPE window defects</td>
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<tr>
<td>Cotton-wool spots</td>
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</tbody>
</table>

Green light

- Lesions within choroid typically less visible or invisible when using green light
- Due to shorter wavelength blocked at RPE level
- Allows determination as to which layer lesion is in – retina or choroid

Red light

- 620-750nm
- Bypasses retinal vasculature and RPE, making these more anterior structures less visible
- Choroidal vasculature (i.e. vortex veins) and any lesions in choroid more visible
- Choroidal nevi or choroidal melanomas are especially obvious when using this light
  - Usually appear darker, more defined
- Optic disc often ‘bleached-out’

Red light

- Interestingly, opacities in vitreous such as floaters may also be enhanced

Red light

- Due to utility of red light in evaluating choroid, used for indocyanine green (ICG) angiography
  - evaluates choroidal vasculature in more detail
- Like green light, red light used regularly with standard fundus photography via digital filters incorporated into retinal camera software

Structures/pathology with red light

<table>
<thead>
<tr>
<th>STRUCTURE/PATHOLOGY</th>
<th>APPEARANCE WITH RED LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortex veins</td>
<td>Darker or lighter, more defined (some exceptions)</td>
</tr>
<tr>
<td>Choroidal neovascular membranes</td>
<td>Darker or lighter, more defined (some exceptions)</td>
</tr>
<tr>
<td>Choroidal nevi/melanomas</td>
<td>Darker, more defined</td>
</tr>
<tr>
<td>Vitreous floaters</td>
<td>Darker, more defined</td>
</tr>
</tbody>
</table>
**Blue light**

- 450-495nm
- Often called Cobalt blue clinically
- Helps visualize corneal fluorescein staining
- Goldmann applanation tonometry
- Blue-green (490nm) light useful in better defining retinal nerve fiber layer (RNFL), internal limiting membrane (ILM) retinal folds and vitreo-retinal (V-R) adhesions.
- Anterior retinal structures more obvious

**Blue light**

- Blue light fundus evaluation limited by anterior media opacity
  - Corneal haze/edema
  - Lens opacities: i.e. nuclear sclerosis/other cataracts
- Topical fluorescein dye also fluoresces, reducing clarity of fundus image
- For retinal evaluation, should use blue light only with optimal media clarity and prior to any topical fluorescein use

**Structures/pathology with blue light**

<table>
<thead>
<tr>
<th>STRUCTURE/PATHOLOGY</th>
<th>APPEARANCE WITH BLUE LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal nerve fiber layer (RNFL)</td>
<td>Lighter, more apparent</td>
</tr>
<tr>
<td>Epiretinal membrane (ERM)</td>
<td>Lighter, more apparent</td>
</tr>
<tr>
<td>Retinal fold/cyst</td>
<td>Light and dark</td>
</tr>
<tr>
<td>Retinal vessels</td>
<td>Darker, more apparent</td>
</tr>
</tbody>
</table>

**Yellow light**

- 570-590nm
- Wratten #12 filter (used with Cobalt blue light)
  - Fluorescein anterior segment staining
- Excitation filter in fundus autofluorescence
- ‘Patient comfort’ condensing lenses
- Effectively filters out wavelengths below 480-490nm, reducing blue wavelengths visualized
- Possible benefit of short-wavelength filters, such as yellow tints, in reducing retinal toxicity

**Multispectral imaging (MSI)**

- Takes into account absorption spectra of tissues of retina and choroid
- Creates ‘slices’ of these tissues for selective viewing based on wavelength of light absorbed
- Involves passing series of monochromatic LED light from 450nm to 900nm across fundus
- Creating en face anterior-posterior image sections based on absorption depth of each particular wavelength of light
Multispectral imaging (MSI)

- Wide range of wavelength imaging
- Exceeds both conventional retinal camera imaging and human eye's visual spectrum

Multispectral imaging (MSI)

- Unlike optical coherence tomography (OCT), MSI relies on absorption and reflection of specific wavelengths of light by chromophores
- Chromophore = part of molecule that accounts for its color
- Main chromophore in fundus: hemoglobin
- Melanin and macular pigment also prominent chromophores in fundus

Main fundus chromophores

- Retinal hemoglobin
- Macular pigment
- RPE melanin
- Choroidal hemoglobin
- Choroidal melanin

Multispectral imaging (MSI)

- In general, longer wavelengths penetrate deeper layers of fundus
- Choroidal features more prominent as 600nm+ wavelengths reached
- Conversely, structures closer to ILM more prominent in 500nm and shorter wavelengths

Chromophore absorption and reflectance in fundus occurs at deeper layers with longer wavelength light
Multispectral imaging (MSI)

- This general wavelength/depth relationship somewhat simplistic, as absorption spectra of hemoglobin and melanin may have more than one peak
- Instrumentation accounts for these peaks via spectral analysis to give monochromatic ‘slice’
- Combining the monochromatic ‘slices’ via multispectral reflectometry can provide topographical map of fundus

Commercial instruments using MSI

- Carl Zeiss Meditec (Visucam 200 and 500)
  - Macular optical pigment density only
  - Software currently not available in US
- Topcon Medical (Retinal Functional Imager)
  - Currently not available in US
- Heidelberg Engineering (Spectralis models)
  - Multicolor scanning laser imaging
- Annidis (RHA)
  - up to 12 specific wavelengths per scan

CASE EXAMPLES

Normal fundus

- Increased detail of the vasculature and underlying RPE compared to conventional fundus photography

Macular pigment optical density

- Multispectral imaging that focuses on macular xanthophyll absorption spectra
- May allow better assessment of MPOD in conditions such as macular degeneration
- May assist in nutritional consultation
Macular degeneration
Greater detail of:
• Retinal pigment epithelial (RPE) changes
• Lipofuscin deposition
• Choroidal neovascular changes

Diabetic retinopathy
• Very fine resolution of microvascular changes in diabetic retinopathy
  – Microaneurysms (MAs)
  – Dot-blot hemorrhages
  – Venous beading (VB)
  – Intraretinal microvascular anomalies (IRMA)
  – Neovascularization
• Better resolution of laser treatment areas
• Oxy/deoxygenated hemoglobin perfusion maps

Glaucoma
• MSI can enhance certain glaucoma-related findings:
  – Splinter (Drance) hemorrhages
  – Alpha and beta-atrophy
• Hemoglobin-specific filters may permit better evaluation of vascular aspect of glaucoma
  – Oxy/deoxy hemoglobin contrast map
• Applicability to other neuropathies
Retinal vascular occlusion

Vascular occlusions may be seen with new clarity:
• Vein occlusions
• Artery occlusions
• Choroidal non-perfusion

Choroidal neovascular membrane

• Converse to identifying vascular occlusion
• MSI that focuses on oxygenated hemoglobin absorption spectra
• May allow better assessment of choroidal perfusion in conditions such as choroidal neovascular membranes (CNVMs)

Toxic retinopathy

Deep retinal and choroidal-focused imaging may:
• Increase understanding of toxic retinopathies:
  – Plaquenil (hydroxychloroquine)
  – Mellaril (thioridazine)
  – Talc
  – Others
• Monitor progression, and dosage/duration
• Help facilitate appropriate management
Non-Invasive Angiography

- Potential for angiography without contrast dyes
- Safer when patients may react to contrast dyes
- Can image oxygenated/deoxygenated hemoglobin
- Vascular perfusion on near cellular level
- Detailed retinal and choroidal vascular maps
- Vascular flowimetry through real-time analysis of oxygenated and deoxygenated blood absorption
- Detail may exceed what angiography can provide

Metabolic Imaging

- Esoteric but clinically-promising application
- Metabolic functional imaging
- Using near-infra-red (IR) imaging
- Blood flow, volume, oximetric changes below photoreceptors analyzed in real-time in response to visual stimuli
- Allows analysis of photoreceptor response
- Potentially provide information on various retinal conditions including occult disease

Side note...external biomarkers

Metabolic signature map of cross stimulus on macaque retina in vivo, showing the metabolic photoreceptor pattern (upper left), later signal of the axonal arches (upper middle), and receptor recovery (upper right). A time-course can be plotted (below) for the three stages, based on retinal reflectance changes (http://rfi.topconmedical.com/features/mfi.cfm)
Summary

- Like traditional fundus photography, optical coherence tomography (OCT), and fundus autofluorescence (FAF), multispectral imaging (MSI) will serve as a valuable diagnostic tool
- As greater refinements and interpretation of findings occurs, MSI should become a valuable, readily-accessible resource in better diagnosing and managing patients with ocular disease

THANK YOU!
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References