Multispectral Imaging in Fundus Examination: a Primer
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COPE#: 38250-PS

Section: Retina and Posterior Segment

Course description:

Fundus evaluation technology has evolved exponentially over the last 150 years. Along with standard full-spectrum fundus photography, angiography, scanning laser polarimetry, and optical coherence tomography, multispectral imaging (MSI) shows great promise for enhanced evaluation of the fundus. This course serves to provide a background and clinical application of MSI for the optometrist.

Learning Objectives:

At the completion of this course, the attendee will be able to:

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

Introduction

The use of multispectral imaging (MSI) for fundus evaluation is a relatively newer technology as of the date of this article. The reader should be aware that the word ‘fundus’ is used to more inclusively describe retinal, optic nerve, and/or choroidal structures. MSI is capable of evaluation of any of these structures, and is not limited to retinal structures alone.

History

The advance of digital retinal imaging has evolved exponentially over the last decade. This has drastically improved the speed of image acquisition, analysis, and management for patients with retinal disease. Since the invention of the ophthalmoscope by Hermann von Helmholtz in 1851, and the first known in vivo retinal photograph by Jackman and Webster in 1886, clinicians have been able to view the inside of the eye with increasingly improved light and clarity (1, 2).

Over the last century, the use of monochromatic light was found to both enhance and suppress visualization of certain tissues within the eye (3). The first recommendation for the use of monochromatic light for ophthalmoscopic use was in 1911 by Ginestous in France (4), which was performed two years later on a practical level by Vogt in Germany (5). In 1930 Kikai discovered the use of a filter to enhance retinal vessels in fluorescein angiography, which would eventually evolve in the 1950s to the advent of retinal autofluorescence via excitation and barrier filter utilization (6, 7).

Researchers Behrendt and Wilson in 1965 discovered that the spectral reflectance (amount of light absorbed versus amount of light reflected) varied with different fundus structures, based on the wavelength of light used (8). The first practical clinical use of this technology occurred in 1972 when...
Hoyt and colleagues encouraged the use of red-free light to evaluate the retinal nerve fiber layer around the optic disc in patients with optic nerve disease (9). Further refinement of the specific wavelength filters and ophthalmoscope light source to view retinal and choroidal structures continued for the next three decades (1, 10, 11, 12).

It wasn’t until the 1990s that the potential of digital retinal imaging came to fore (13). The advent of the CCD (charged-coupled device) over film, as well as the ability to create various digital spectrum filters using the R-G-B-Y (red-green-blue-yellow) and grayscale digital processing capabilities, has provided clinicians with valuable information on retinal structures. Further software and hardware refinement has made digital imaging a clinical workhorse, particularly in the areas of diabetic retinopathy screening and telemedicine (14). In the 2000s, the advent of multispectral imaging (MSI) using scanning laser technology has resulted in unprecedented views of retinal, optic nerve, and choroidal structures (15).

**Monochromatic Imaging**

**Barrier/Excitation (Autofluorescence)**

The barrier/excitation method has been used in fluorescein angiography since the 1950s. Light is passed through a short-wavelength (usually blue-green spectrum between 500-585 nm) ‘excitation’ filter onto the fundus, and the returning light passes through a longer wavelength (usually yellow-red spectrum between 600-715nm) ‘barrier’ filter onto the camera’s image sensor. This method selectively enhances the view of the fluorescent dye that enters the retinal and choroidal vasculature.

It was soon discovered that various fundus structures autofluoresced – that is, certain tissues contained molecules called fluorophores that naturally emitted a longer wavelength of light when stimulated by a shorter wavelength of light – without the need for injected dye. The most common autofluorescent ocular tissue is the retinal pigmented epithelium (RPE), due to the lipofuscin within each RPE cell. The molecule A2-E (N-retinylidene-N-retinylethanolamine) in lipofuscin is the dominant fluorophore, and increases with age, abnormal metabolic load on the RPE, or RPE dysfunction. The most common ocular disease related to this fluorophore is age-related macular degeneration.

Other ocular structures that may show autofluorescence are collagen and elastin in choroidal blood vessel walls, macular lipofuscin pigment, hyaluronic acid in the vitreous, and the crystalline lens fibers. Some common ocular pathologies that may show distinctive autofluorescence patterns are summarized in Table 1. An example of fundus autofluorescence is shown in Figure 1.

<table>
<thead>
<tr>
<th>OCULAR DISEASE</th>
<th>AUTOFLUORESCENCE FEATURE</th>
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<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>
</tr>
<tr>
<td></td>
<td>2. RPE metabolic stress</td>
</tr>
<tr>
<td></td>
<td>3. Choroidal neovascular membrane (wet AMD)</td>
</tr>
<tr>
<td></td>
<td>Hypofluorescence:</td>
</tr>
<tr>
<td></td>
<td>1. RPE atrophy/death</td>
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Table 1: Common Ocular Pathologies That May Display Distinctive Autofluorescent Patterns
<table>
<thead>
<tr>
<th>Central serous retinopathy (CSR) - chronic</th>
<th>Hyper/hypofluorescent “guttering” (fluid tracks) at/inferior to macula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaquenil maculopathy</td>
<td>Hyper- and hypofluorescent concentric rings indicating RPE stress and RPE atrophy/death, respectively</td>
</tr>
<tr>
<td>Retinitis pigmentosa (RP)</td>
<td>Multifocal areas of scattered hypofluorescence (RPE atrophy) with central hyperfluorescence (RPE stress)</td>
</tr>
<tr>
<td>Stargardt’s dystrophy</td>
<td>Macular hyper-/hypofluorescence with hyperfluorescent ‘pisciform’ lesions in the posterior pole</td>
</tr>
<tr>
<td>Vitreo-retinal fibrosis/tufts</td>
<td>Typically hyperfluorescent/variable</td>
</tr>
</tbody>
</table>

Figure 1. Atrophic macular degeneration with standard color photo (left) and autofluorescence (right). Note the delineation of retinal pigment epithelium atrophy (dark areas) and areas of excess lipofuscin (light areas) in the autofluorescence picture.

Green

The use of green (‘red-free’) light in evaluating the retina has been quite common for several decades. Along with evaluating posterior segment structures, the green (540-570nm) wavelength range is useful in evaluating anterior segment pathology such as conjunctivitis, episcleritis and scleritis. Excellent sharpness of retinal structures are seen with green light, and the primary enhancement is seen with vascular structures due to the strong absorption of this wavelength by hemoglobin. As a result, retinal vessels appear dark, and hemorrhages are more easily seen when using green light (16). Other retinal changes such as epiretinal membranes or retinal holes may be better identified using green light.

Often green light photography is performed prior to fluorescein angiography, as standard protocol. Now, the green light is used regularly with standard fundus photography via digital filters incorporated into commercial retinal camera software (Figure 2).
It is important to know that lesions within the choroid are typically masked when using green light. Lesions such as choroidal nevi will be notably less visible, or invisible, when using green light, due to the shorter wavelength being blocked at the RPE level. This allows good determination as to which layer a lesion is in – retina, or choroid. Some common retinal structures and pathology that can be visualized better with green light are listed in Table 2.

Table 2. Common retinal structures and pathology that can be visualized better using green light

<table>
<thead>
<tr>
<th>STRUCTURE/PATHOLOGY</th>
<th>DARKER WITH GREEN LIGHT</th>
<th>LIGHTER WITH GREEN LIGHT</th>
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<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>
<td></td>
</tr>
<tr>
<td>Epiretinal membranes</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Macular drusen</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Optic disc drusen</td>
<td>+</td>
<td></td>
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<tr>
<td>Retinal exudates</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>RPE window defects</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cotton-wool spots</td>
<td>+</td>
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Red

Red (625-640nm) light, due to its relatively longer wavelength, is useful for evaluating the choroid. Use of this light bypasses the retinal vasculature and RPE, making these more anterior structures less visible. The choroidal vasculature and any lesions within the choroid then become more apparent. Lesions such as choroidal nevi or choroidal melanomas are especially obvious when using this light, appearing darker and more defined. Vortex veins and changes in the choriocapillaris may also be visible. Interestingly,
Opacities in the vitreous such as floaters may also be enhanced using red light. Figure 3 demonstrates the red light filter compared to standard retinal photography.

Figure 3. Standard retinal photograph (left) compared to red light photograph (right). Note the enhancement of choroid vasculature along with enhancement of a choroidal nevus (dark) in the superior arcade. The retinal vessels and optic disc are typically more ‘washed-out’ in appearance with red light.

Because of the utility of red light in evaluating the choroid, this light is used as a baseline for indocyanine green (ICG) angiography, which evaluates the choroidal vasculature in more detail. Like green light, the red light can used regularly with standard fundus photography via digital filters incorporated into commercial retinal camera software. Table 3 lists posterior segment structures and pathology better visualized with red light.

Table 3. Posterior segment structures and pathology that may be visualized better using 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

Eye doctors are very familiar with the use of blue light (often called Cobalt blue) to help visualize fluorescein staining of the cornea, and for Goldmann applanation tonometry. Blue-green (490nm) light is also useful in better defining the retinal nerve fiber layer (RNFL). This wavelength may also provide greater clarity of internal limiting membrane changes such as epiretinal membranes, as well as retinal folds and vitreo-retinal adhesions. Anterior retinal structures are more obvious with blue light.
The use of blue light for fundus evaluation is limited by anterior media changes, such as corneal haze/edema, and lens changes such as nuclear sclerosis or other cataracts. Any topical fluorescein dye will also fluoresce, reducing the clarity of the fundus image. As such, this light should ideally be used only when media clarity is optimal and prior to any topical fluorescein instillation. Table 4 lists retinal structures and pathology that may be better visualized with blue light.

Table 4. Retinal structures and pathology better visualized 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

Use of yellow light in funduscopic evaluation is typically for patient comfort rather than diagnostic purposes (17). The yellow light filters out wavelengths below 480-490nm, effectively reducing blue wavelengths from being visualized (18). Some studies infer a possible benefit in the use of short-wavelength filters, such as yellow tints, in reducing retinal toxicity (19). From a diagnostic standpoint, yellow filters are utilized as an excitation filter in fundus autofluorescence, as well as the deep yellow Wratten #12 filter commonly used in conjunction with the Cobalt blue light and topical fluorescein stain in contact lens and anterior segment evaluation (20).

Multispectral Imaging (MSI)

A relatively newer technology, multispectral imaging (MSI) takes into account the absorption spectra of tissues of the retina and choroid, and creates ‘slices’ of these tissues for selective viewing based on the wavelength of light absorbed. The method involves passing a series of monochromatic LED light from 450nm to 900nm across the fundus, creating en face anterior-posterior image sections based on the absorption depth of each particular wavelength of light (21). This wide range of wavelength imaging exceeds that of both conventional retinal camera imaging and the human eye’s visual spectrum (See Figure 4).
Unlike optical coherence tomography (OCT), MSI technology relies on the absorption and reflection of specific wavelengths of light by chromophores (the part of a molecule that accounts for its color). The main chromophore in the fundus is hemoglobin. Melanin and macular pigment are also prominent chromophores. A list of main fundus chromophores is shown in Table 5.

Table 5. Main chromophores of the fundus.

<table>
<thead>
<tr>
<th>CHROMOPHORE</th>
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</thead>
<tbody>
<tr>
<td>Retinal hemoglobin</td>
</tr>
<tr>
<td>Macular pigment</td>
</tr>
<tr>
<td>RPE melanin</td>
</tr>
<tr>
<td>Choroidal hemoglobin</td>
</tr>
<tr>
<td>Choroidal melanin</td>
</tr>
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</table>

In general, longer wavelengths penetrate deeper layers of the fundus; as a result, choroidal features become more prominent as the 600nm and longer wavelengths as reached. Conversely, structures closer to the internal limiting membrane (ILM) are more likely to be prominent in the 500nm and shorter wavelengths. Figure 5 illustrates this principle using a normal macula OCT cross-section.
This general principle is somewhat simplistic, as the absorption spectra of hemoglobin and melanin may have more than one peak. Instrumentation accounts for these peaks through spectral analysis.

Combining each of the series of monochromatic ‘slices’ via multispectral reflectometry results in a topographical map of the retina that can be evaluated by the clinician. At the time of this article there are four commercial ophthalmic companies that provide instruments that utilize MSI technology to varying degrees: Carl Zeiss Meditec (Visucam 200 and 500 models; macular optical pigment density only; software currently not available in the US), Topcon Medical (Retinal Functional Imager; currently not available in the US), Heidelberg Engineering (Spectralis models; multicolor scanning laser imaging), and Annidis (RHA model; up to 12 specific wavelengths per scan).

**MSI Case Examples**

MSI has the potential for more detailed imaging of numerous fundus conditions, including macular degeneration, glaucoma, diabetic retinopathy, retinal vasculopathies, inflammatory retinal diseases, and other conditions. Below are examples.

**Normal fundus**

Retinal vasculature can be enhanced with multispectral imaging, revealing more detail (Figure 6).
Figure 6. Conventional fundus photo (left) and MSI fundus image (right) of same eye. Note the increased detail of the vasculature and underlying RPE (Source: http://www.heidelbergengineering.com/us/products/spectralis-models/imaging-modes/multicolor/).

Macular pigment optical density

Multispectral imaging that focuses on macular xanthophyll absorption spectra (Figure 7) may allow better assessment of MPOD in conditions such as macular degeneration, as well as for nutritional consultation.
Figure 7. Macular pigment optical density (MPOD, middle and right) measured in a normal eye (left). Distribution and quantification of the pigment density (right) can be obtained (Source: http://meditec.zeiss.com/meditec/en_de/products/ophthalmology/retina/diagnostics/fundus-imaging/visucam-K200.html#MPOD%20Determination).

Macular degeneration

Greater detail in the areas of retinal pigment epithelial changes, lipofuscin deposition, and choroidal neovascular changes may be enhanced with multispectral imaging. An example is shown in Figure 8.

Figure 8. Conventional fundus photo (left) and MSI deep red fundus image (right) of same eye in a patient with non-exudative AMD. Note the deeper pigmentary macular changes that are not visible in the conventional photo (Source: http://www.annidis.com/index.php?q=amd-5).

Diabetic retinopathy

Multispectral imaging allows very fine resolution of microvascular changes in diabetic retinopathy, as well as better resolution of laser treatment areas (Figure 9).
Figure 9. Multispectral imaging (MSI) of neovascularization of the disc (NVD, left) and neovascularization elsewhere (NVE, right - arrows) with scattered photocoagulation scars in two patients with diabetic retinopathy. The definition of the vascular changes is enhanced with MSI (Source: Heidelberg Engineering)

Glaucoma

While multispectral imaging can enhance certain glaucoma-related findings such as splinter (Drance) hemorrhages and beta-atrophy, the hemoglobin-specific filters may permit better evaluation of the vascular component of glaucoma (Figure 10).
Retinal vascular occlusion

Vascular occlusions may be seen with new clarity using multispectral imaging – for vein occlusions, artery occlusions, as well as areas of choroidal non-perfusion. Figure 10 illustrates one example.

Choroidal neovascular membrane
Conversely to identifying areas of vascular occlusion, multispectral imaging that focuses on oxygenated hemoglobin absorption spectra may allow better assessment of choroidal perfusion in conditions such as choroidal neovascular membranes (CNVMs). Figure 12 shows an example.

Figure 12. Fundus photo (right) and oxyhemoglobin contrast map (left) of patient with a choroidal neovascular membrane (CNVM) in the macular area. (Source: http://www.annidis.com/index.php?q=choroidal-neovascular-membrane).

Toxic retinopathy

Deep retinal and choroidal-focused imaging, such as in Figure 13, may increase our understanding of toxic retinopathies such as Plaquinil (hydroxychloroquine) retinopathy, as well as help facilitate appropriate management with these patients.

Figure 13. Wide-field right eye (right) and standard-field left eye (left) fundus multispectral images of a patient with Mellaril (thioridazine) toxicity. (Source: Heidelberg Engineering).
Non-Invasive Angiography

Perhaps the most powerful application of MSI is the potential for angiography without the need for contrast dyes. Since MSI can focus on both oxygenated and deoxygenated hemoglobin, vascular perfusion mapping is possible on a near cellular level (22). This allows both 1) highly detailed retinal and choroidal vascular maps, as well as 2) vascular flowmetry, due to real-time analysis of oxygenated and deoxygenated blood absorption (23, 24).

In situations where patients may react to contrast dyes such as fluorescein sodium or indocyanine green, the use of MSI may provide a safer alternative for patients with diabetic retinopathy, exudative macular degeneration, retinal vascular occlusions, normotensive glaucoma, or other retinal or choroidal vascular diseases. Images can be viewed as a real-time sequence similar to angiography, and the detail may exceed what angiography can provide (25). Examples of this technology are shown in Figures 14 and 15.

Figure 14. Left - capillary perfusion map obtained MSI. Red blood cells (RBCs) serve as the absorptive source. MSI focuses on and analyzes a series of RBC motion signals to reveal microvasculature, often in greater detail than with contrast agent. The Foveal avascular zone (FAZ) is easily determined in this healthy retina. Right – Blood flow velocity map. MSI can identify the movement of clusters of blood cells, resulting in a real-time flowmetry analysis of arterioles, venules, and capillaries. (Source: http://www.opt-imaging.com/rfi_description.asp#)
Figure 15. Fluorescein angiogram (left) and capillary perfusion map obtained with MSI (right) of a patient with diabetic retinopathy. Note the greater definition of microaneurysms (yellow arrows) with the MSI image. In addition, a vascular loop and shunt (green and orange arrow) is identified with the MSI image, but not with the fluorescein angiogram image (Source: http://rfi.topconmedical.com/features/cpm.cfm)

Metabolic Imaging

A more esoteric but clinically-promising application of MSI is in the area of metabolic functional imaging. Using near-infra-red (IR) imaging, blood flow, volume, and oximetric changes below the photoreceptors can be analyzed in real-time in response to visual stimuli (Figure 16). This allows analysis of photoreceptor response, which gives a functional assessment of retinal health (26). This technology may in the future potentially provide information on various retinal conditions such as macular degeneration, retinitis pigmentosa, toxic retinopathies, and other occult retinal diseases.
Figure 16. 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 (Source: http://rfi.topconmedical.com/features/mfi.cfm).

Summary

Like traditional fundus photography, optical coherence tomography (OCT), and fundus autofluorescence (FAF), multispectral imaging (MSI) will play a greater role in the non-invasive assessment and management of ocular disease. As greater understanding of MSI in the interpretation of various diseases occurs by clinicians, the technology should become a valuable, readily-accessible resource in better diagnosing and managing patients with ocular disease.

References

3. George TW, Miller NR. Monochromatic (red-free) photography and ophthalmoscopy of the peripapillary retinal nerve fiber layer