

Innovation in Diagnostic Retinal Imaging: Multispectral Imaging

Multiple-wavelength imaging of the retina and choroid.

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Multispectral imaging (MSI) technology is used in various applications from airborne mapping to astronomical imaging to extract detailed information from distant views.¹ The term refers to imaging systems that use a number of nonoverlapping discrete spectral bands, or slices, to highlight certain features within the field of view. MSI has been used in a variety of medical applications including dentistry, dermatology, and histopathology.² An emerging and advanced application of MSI is its use in visualizing the entire span of the posterior pole of the eye from the internal limiting membrane (ILM) through to the choroid, highlighting the retinal pigment epithelium (RPE).³

MSI TECHNOLOGY

The RHA digital imaging ophthalmoscope (Annidis) combines advanced MSI technology with intuitive software, providing a 45° noninvasive en face view of the retina and choroid for early detection and diagnosis of a variety of ocular pathologies such as age-related macular degeneration (AMD), diabetic retinopathy, and glaucoma. The image output is the result of collecting and combining spectral information that highlights specific anatomic and metabolic signatures to assist in disease detection and management. Examples of these signatures include melanin stacking in geographic atrophy (GA) and hemoglobin oxygenation in choroidal neovascular membranes (CNVM).

The instrument utilizes safe, discrete, light emitting diodes (LEDs) across a wavelength range from 520 nm (green) to 940 nm (infrared) to progressively examine the layers of the retina and choroid; the longer wavelengths penetrate deeper into the structures of the eye.⁴ Automated spatial and spectral filters further combine wavelengths to enhance the system's flexibility and specificity for better visualization and differential diagnosis, accentuating small details that might not otherwise be visible. Each narrow band spectral slice represents successive images of the fundus as the targeted and deliberately selected LED light sources differentially reflect, scatter, and absorb deeper into the posterior pole, enhancing differential visibility of the retinal and choroidal features. MSI has a role in aiding the clinician with the identification, interpretation, diagnosis, and management of ocular pathology via spectral dissection. The RHA is designed as a platform to support ongoing improvements as medical innovations emerge.

LIMITATIONS OF WHITE LIGHT IMAGING

Traditional fundus imaging provides a visual representation of a clinical observation with minimal diagnostic value.⁵ Diagnostic fundus imaging technologies, such as optical coherence tomography (OCT) and MSI, are vital in aiding clinicians in the diagnosis, management, and understanding of retinal and choroidal diseases. Early and subtle ocular pathologies are often difficult to isolate,

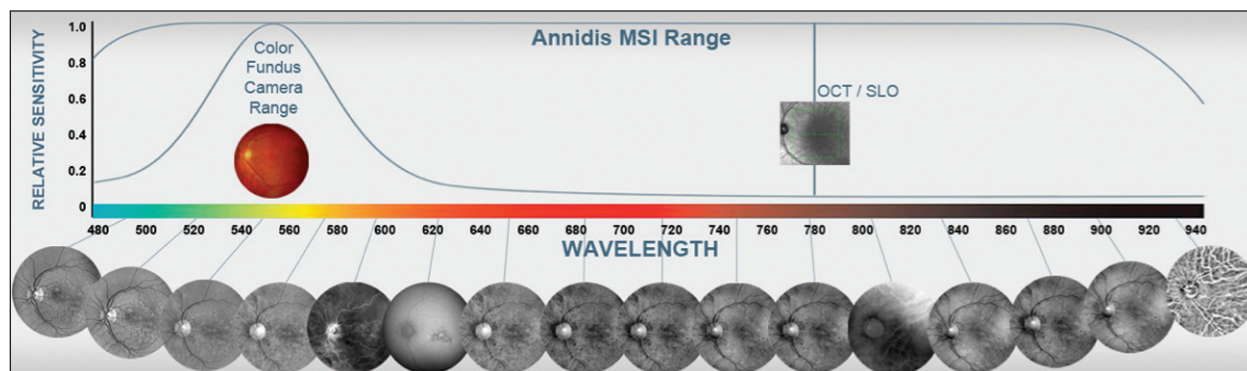


Figure 1. A bell shaped blue curve represents the sensitivity of the human eye and the spectrum used by traditional digital fundus cameras with a peak at 555 nm. The result is 1 en face view of the retina. MSI creates progressive spectral slices through the retina from the ILM to the choroid. These are represented with color lines starting at 520 nm and going to 940 nm. The result is a composite series of monochromatic en face fundus images providing enhanced visualization of the posterior pole.

identify, and interpret, and this is even more evident when pathologies overlap within the retina, as in the case of comorbidity. In addition, macular pigment, rod and cone pigments, the lens, and blood all restrict the ability to see and image deeper retinal structures when white light digital photography is used, ultimately limiting the clinician's ability to detect, differentiate, and further investigate pathologies as they develop.

Figure 1 shows a diagram of the visible and near infrared portions of the spectrum relative to the response of the human eye, which is illustrated by the bell-shaped curve peaking at 555 nm and falling rapidly to either side, versus the spectral range of MSI. Conventional fundus photography uses white light and broad color filters in a limited range from 480 nm to 600 nm to match the sensitivity of the human eye. This restricts the discrimination of the reflected light to the blue, green, and red channels within the curve and provides a replica of what is seen with the human eye. Structures that fall outside of the sensitivity curve cannot be readily observed.

WAVELENGTH-STRUCTURE CORRELATION

The carefully selected spectral bands used by MSI are targeted to the clinically relevant structures and metabolic characteristics of the retina and choroid, particularly the ocular chromophores melanin and hemoglobin, as well as the fluorophore lipofuscin (due to the fluorescent nature of its primary component, A2E [N-retinylidene-N-retinylethanolamine]).⁶ The RHA uses narrow spectral bands and a combination of illumination processes including direct feature backscatter, retroillumination with feature silhouetting, and transmission imaging in which the incident light travels through the sclera, rather than through the globe, to produce a series of spectral images.

Melanin, which absorbs long wavelength red and

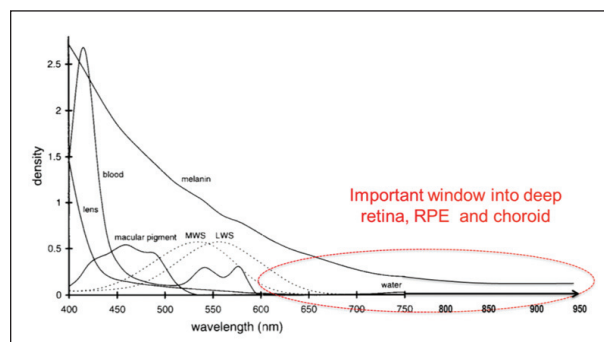


Figure 2. Absorption spectra for the major absorbing elements of the eye. Note that some of the spectra change with relatively small changes in wavelength. Maximizing the differential visibility requires utilizing small spectral slices. Melanin is the dominant absorber beyond 600 nm, which is outside the eye's sensitivity range.⁷

infrared light, falls within the wavelength spectrum used by traditional fundus cameras, but in the wavelength range from 450 nm to 600 nm it is obscured by other dominant components of the eye including the lens, hemoglobin, zeaxanthin, lutein, and rhodopsin, to name a few.⁷ Beyond 600 nm, melanin is the dominant retinal pigment. Figure 2 shows the absorption spectra for various retinal components.⁸ The spectral sensitivity of these structures changes quite rapidly, such that a broad band of a particular wavelength may have reduced sensitivity to subtle variations. MSI essentially narrows the band of light used in order to maximize the differential visibility of the absorbing chromophore or fluorophore as a biomarker of disease. This, in turn, enhances the visualization of that particular retinal structure.

A short wavelength broadband light source LED with a median of 550 nm (green) increases the visibility of the

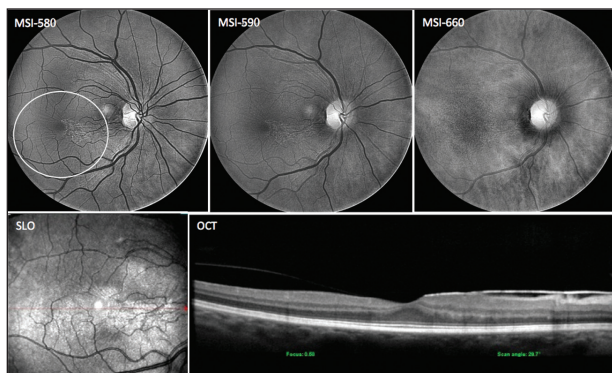


Figure 3. Vitreoretinal traction is highly visible using noninvasive MSI technology. An ERM is seen and represents glial cell proliferation between the ILM of the neurosensory retina and the posterior hyaloid membrane of the vitreous. Striae in the ILM (white circle) are seen in the shorter (580 nm and 590 nm) narrow band slices but are no longer visible in the long wavelength (660 nm) image. SLO and OCT images are included for comparison.

anterior retinal layers, primarily the ILM, which is almost transparent when viewed with a conventional white light source. This wavelength band matches the conventional red-free spectrum and is absorbed by the retinal pigment and blood vessels, providing a dark background against which specular reflections and scattering in the anterior fundus are enhanced. This wavelength improves the visualization of vitreoretinal interface disorders including retinal folds, cysts, epiretinal membranes (ERM), vitreomacular traction (VMT), vitreomacular adhesion (VMA), and macular holes.⁹

Figure 3 shows an ERM representing glial cell proliferation between the ILM of the neurosensory retina and the posterior hyaloid membrane of the vitreous. Striae in the ILM are seen in the shorter (580 nm and 590 nm) narrow band slices but are no longer visible in the long wavelength (660 nm) image.

"Using MSI, longstanding tractional forces associated with VMTs have been observed to cause disruption of the melanin in the underlying RPE; this RPE injury may be an indication for timely intervention and is currently being investigated," according to Pravin Dugel, MD, a retinal specialist at the Retinal Consultants of Arizona.

Medium wavelength, very narrow band light sources at 580 nm and 590 nm (yellow and amber, respectively) are tailored for metabolic monitoring of the anterior to mid retina. These wavelengths are also absorbed by blood but are partially reflected by the retina. These light sources enhance the visibility of the retinal vasculature and nerve fiber layer and help to identify hemorrhages, drusen, lipids, edema, exudates, and neovascularization.

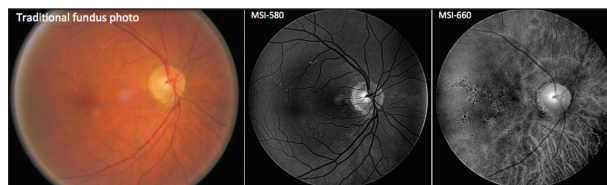


Figure 4. The traditional fundus photograph does not provide detail about the depth of the pathology. MSI-580, on the other hand, distinctly shows soft drusen, while MSI-660 shows extensive melanin stacking along the borders of the lesion, potentially indicating the direction of the next disruption.

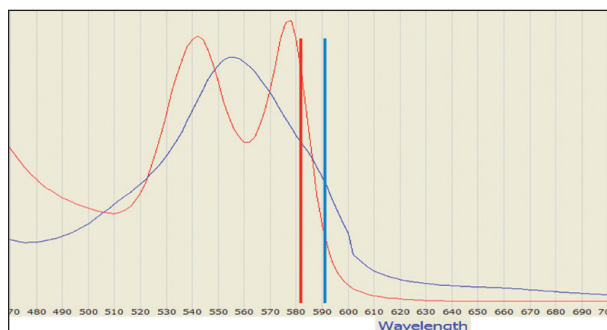


Figure 5. Absorption spectrum of retinal oxygenated (red curve) and deoxygenated (blue curve) hemoglobin. The red line indicates the MSI 580 nm probe, and the blue line indicates the MSI 590 nm probe.

With longer wavelengths, 620 nm to 740 nm (red and deep reds, respectively), retinal pigmentation is enhanced, allowing the deep retinal structures and superficial choroid to become visible by removing the effects of short wavelength scatter. This is the peak emission range for the direct observation of melanin in the RPE, which is spectrally revealed without interference from the inner retinal layers. The shorter red wavelengths reveal intraretinal and subretinal hemorrhages and photoreceptor degradation, while the deep red wavelengths may show neurofibromas, nevi, and melanomas.¹⁰

Figure 4 shows an example of extensive melanin stacking associated with intermediate dry AMD. The traditional fundus photograph does not provide detail about the depth of the pathology. MSI-580, on the other hand, distinctly shows soft drusen, while MSI-660 shows extensive melanin stacking along the borders of the lesion, potentially indicating the direction of the next disruption.

The longest MSI wavelengths cover the range from 760 nm to 940 nm (infrared). At these wavelengths, the retinal pigment appears progressively more transparent, revealing the underlying choroid. Infrared light is useful for imaging congenital hypertrophy of the RPE, CNVM, choroidal ruptures, neurofibromas, nevi, and melanomas.

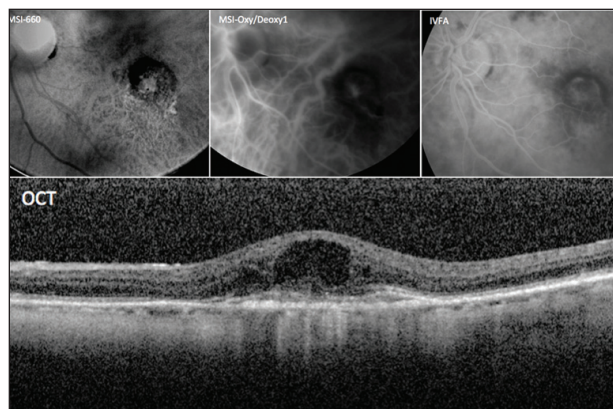


Figure 6. The MSI-660 reveals melanin stacking along the borders of the lesion. The oxy/deoxy contrasted map shows an area of hyperreflectance indicating CNVM perfusion that distinctly resembles the adjacent IVFA image. An OCT image is included for comparison.

OXY/DEOXYHEMOGLOBIN MAPPING

The high oxygen demands of the retina and the relatively sparse nature of the retinal vasculature are thought to contribute to the retina's vulnerability to vascular disease. In addition, because a large proportion of retinal diseases have a vascular component, any disruption in oxygen supply to the retina may be a critical factor.¹¹ The 580 nm and 590 nm wavelength images may be combined to enhance the differential contrast of the retinal vasculature based on hemoglobin oxygenation level in the vessels and the retina. This is illustrated in Figure 5, and the images are referred to as retinal oxy/deoxyhemoglobin contrasted maps (MSI-Oxy/Deoxy1). These maps may be used to support the examination of retinal blood flow, vascular health, neovascularization of the retina and choroid, retinal ischemia due to retinal vein or artery occlusions, ischemic optic neuropathy, and other disease processes that affect retinal blood flow.

An example of a CNVM with macular edema is shown in Figure 6. MSI-660 reveals melanin stacking along the borders of the lesion. The oxy/deoxy map shows an area of hyperreflectance indicating CNVM perfusion that distinctly resembles the adjacent intravenous fluorescein angiogram (IVFA) image. An OCT image is included for comparison.

Similarly, the 760 nm and 810 nm wavelength images may be combined to create a choroidal oxy/deoxyhemoglobin contrasted map (MSI-Oxy/Deoxy2). Figure 7 illustrates the spectral absorption of the choroidal vasculature. This map supports examination of the choroid for CNVM, chorioretinal scars, nevi, and melanomas. "These oxy/deoxy maps are akin to noninvasive multispectral angiography and provide similar imaging data to IVFA," Dr. Dugel said.

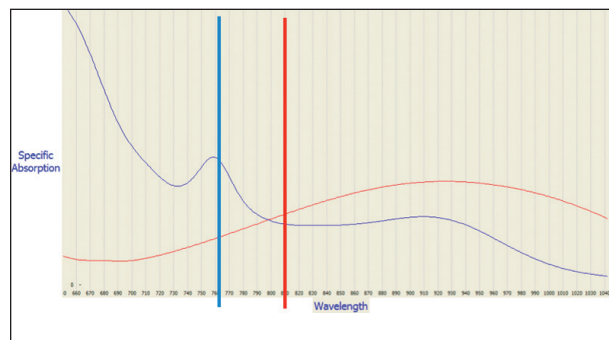


Figure 7. Absorption spectrum of choroidal deoxygenated (blue curve) and oxygenated (red curve) hemoglobin. The blue line indicates the MSI 760 nm probe while the red line indicates the MSI 810 nm probe.

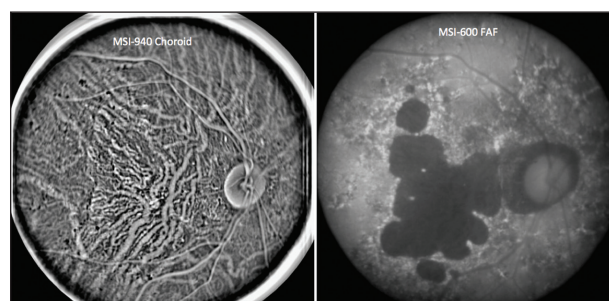


Figure 8. This MSI-940 choroid image shows the underlying choroidal vasculature through a large window defect. Melanin stacking, visible around the borders of the GA, may be predictive of the direction of the next area of atrophy. MSI-FAF shows central hypoautofluorescence in the area of RPE atrophy with surrounding areas of hyperautofluorescence, indicating active degeneration.

MSI FUNDUS AUTOFLUORESCENCE

Fundus autofluorescence (FAF) is used to document the presence of fluorophores such as lipofuscin in the eye. Visible light may be used to fluoresce lipofuscin at different wavelengths including short (488 nm), medium (530 nm to 580 nm), and long (600 nm).¹² The main challenge is that the image quality in the macular area is limited with short wavelength FAF, primarily due to lens scatter and the macular pigment absorption of blue light, making it difficult to distinguish normal from abnormal tissue. Long wavelength FAF performed using the RHA can penetrate through the macular pigment to reveal hyper- and hypoautofluorescent lesions.

MSI-FAF is generated by an absorption and reemission process in which the retina is illuminated with an average wavelength of 600 nm. A red filter blocks the strong signal from the retina in this wavelength region of the spectrum and passes the light back in the 660 nm region, thus depicting the fluorescence pattern of lipofuscin

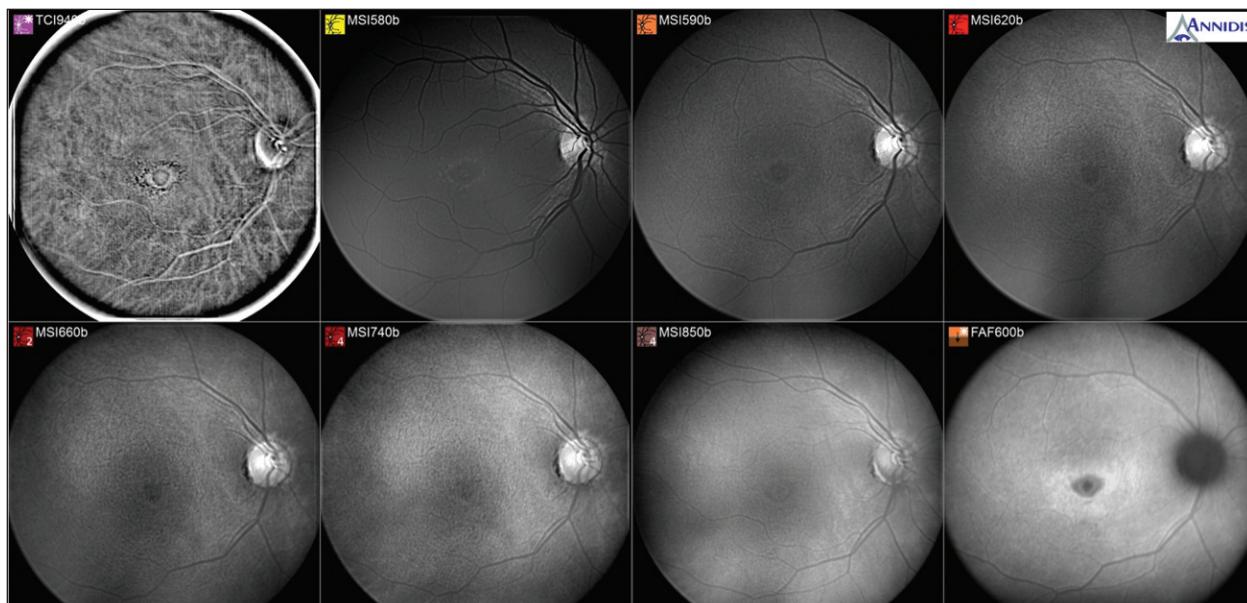


Figure 9. Eight sequential MSI images are shown. Parafoveal flecks are evident surrounding the atrophic macula in the short wavelength MSI-580. More diffuse RPE alterations surrounding the flecks are identifiable on the MSI-660, while the MSI-940 choroidal image highlights both the central lesion and the parafoveal flecks. The MSI-600 FAF reveals concentric rings of hypo- and hyperautofluorescence.

accumulation in AMD, Stargardt disease, and vitelliform dystrophy. Hypoautofluorescence can be seen in dry AMD, GA, hydroxychloroquine toxicity, and scars associated with panretinal photocoagulation.

CHOROIDAL IMAGING

The RHA can capture transmission images of the choroid by projecting 940 nm light into the sclera, which acts as a leaky waveguide carrying the light to the back of the eye.¹³ The light returns through the pupil to the camera in a single pass, providing an enhanced image of the choroidal vasculature without the use of invasive dyes. The result is a high resolution en face spectral image of the choroid showing the vascular network and indications of the choriocapillaris that strongly resemble indocyanine green angiography. This noninvasive imaging technique can be used to identify CNVM, deep chorioretinal scars, nevi, and melanomas.

Figure 8 shows an MSI-940 choroidal image of GA, showing the underlying choroidal vasculature. Melanin stacking visible around the borders of the GA may be predictive of the direction of the next area of atrophy. MSI-FAF shows central hypoautofluoresces in the area of RPE atrophy and surrounding areas of hyperautofluorescence indicating active degeneration.

MSI CLINICAL VALUE

MSI of the retina and choroid represents a major advance in ocular diagnostics. The discrete LEDs and filters used in

MSI enhance visualization of vital fundus structures. The result is a photo essay of monochromatic en face spectral slices that allows clinicians to examine the retina for early morphologic changes that are not generally visible clinically or with traditional fundus imaging modalities. Given the important role of the RPE in the pathogenesis of AMD and other retinal pathologies, examination of this layer with MSI may prove highly valuable at every stage of disease.¹⁴

According to Dr. Dugel, “AMD affects multiple planes within the retina and choroid, including the RPE, Bruch membrane, and the subretinal space. With spectral slicing, the clinician may be able to characterize the various forms of drusen more acutely and monitor changes as the disease progresses. Melanin in the RPE, lipofuscin accumulation, CNVM, and GA can all be more readily identified, which is essential for risk management in AMD patients.”

Eight sequential MSI images of an eye with bull's eye maculopathy are shown in Figure 9. Parafoveal flecks are evident surrounding an atrophic fovea in the short wavelength MSI-580. More diffuse RPE alterations surrounding the flecks are identifiable on the MSI-660, while the MSI-940 choroidal image highlights both the central lesion and parafoveal flecks. The MSI-600 FAF reveals concentric rings of hypo- and hyperautofluorescence. According to Bailey Freund, MD, a retinal specialist at Vitreous-Retina-Macula Consultants of New York, “The Annidis RHA offers a broad range of MSI capabilities, including an array of views of the fundus, particularly the

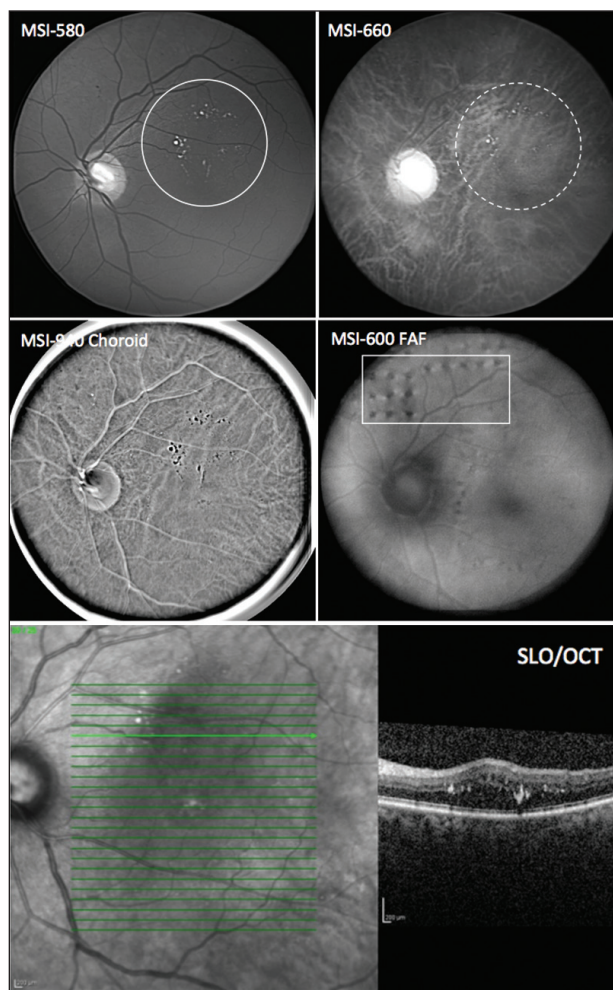


Figure 10. Multiple dot-and-blot hemorrhages and hard exudates (white circle) are seen with MSI-580. There is a large area of diabetic macular edema (DME) centrally, as evidenced by the elevated blurry area (dashed white circle) surrounded by hard exudates in the MSI-660 image. The MSI-940 image shows extensive damage to the choroidal structure. Focal grid laser scars appear as hypoautofluorescent central islands surrounded by hyperautofluorescent borders on the MSI-FAF (white square). An SLO/OCT is included for comparison.

RPE and choroid, that may not be available with OCT or indocyanine green angiography.”

When vital substructures overlap, retinal pathologies may be difficult to interpret clinically. This is the case particularly in diabetic retinopathy. Superficial hemorrhages from retinal vasculature damage can obscure the view of smaller or less visible hemorrhages arising from deeper vessels that may run perpendicular in the deep layers of the retina. Likewise, these hemorrhages may also obscure the view of the nerve fiber layer or lipid exudates that routinely deposit deeper in the outer plexiform

layer. MSI can aid clinicians in visualizing and tracking the full extent of disease. This is illustrated in Figure 10. Multiple dot-and-blot hemorrhages and hard exudates are seen with the shorter MSI-580 wavelength. There is a large area of diabetic macular edema centrally, as evidenced by the elevated blurry area, surrounded by hard exudates in the MSI-660 image. MSI-940 shows extensive damage to the choroid. Focal grid laser scars appear as hypoautofluorescent central islands surrounded by hyperautofluorescent borders on the MSI-600 FAF.

CONCLUSION

MSI demonstrates benefits in clinical utility for a host of retinal diseases and disorders, allowing the eye care professional to detect and identify pathology earlier in the disease process to prevent debilitating vision loss. ■

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