

Techniques of Fundus Imaging

Amit B. Jain, Vadivelu Jaya Prakash and Muna Bhende

Shri Bhagwan Mahavir
Vitreoretinal Services,
Sankara Nethralaya

Correspondence to:
Vadivelu Jaya Prakash
Associate Consultant,
Shri Bhagwan Mahavir
Vitreoretinal Services,
Sankara Nethralaya
Email: drvjp@snmail.org

Introduction

Ophthalmology is a specialized field of medicine, where we can directly visualize the pathology and treat accordingly. Imaging of fundus helps in documenting the disease process which further helps in classifying, treating and following up effectively. Fundus imaging helps in diagnosing various ocular and systemic disorders like diabetic retinopathy, hypertensive retinopathy, age-related macular degeneration, glaucoma, subacute bacterial endocarditis, leukemia, systemic malignancy with ocular metastasis etc. and thus helps in effectively managing the condition. This review focuses on various fundus imaging modalities such as digital fundus photography, autofluorescence, infrared reflectance, etc. making a note of recent advances in fundus imaging technology. The history of fundus imaging dates back to early 20th century, but it has advanced as time has progressed. With the advent of digital imaging, the film-based imaging is almost an obsolete technique now. Table 1 summarizes the major differences in film-based and digital imaging.

Fundus imaging can be defined as a two-dimensional image of a three-dimensional retinal tissue captured using reflected light. Fundus imaging can be categorized into contrast and non-contrast modalities as shown in Table 2.

Discussion in this review will be limited to non-contrast techniques of fundus imaging along with recent advances. A detailed discussion on FFA and ICG is beyond the scope of this review. The list of available machines mentioned is by no means exhaustive and does not indicate any commercial interest of the authors.

Red free fundus photography

It is a technique of monochromatic retinal imaging which uses green contrast filters to modify individual tones in monochrome images and the increased scattering of light at shorter wavelengths. Various fundus structures can be better visualized by limiting the spectral range of the illuminating source.³

Green light enables excellent contrast and view of fundus as the peak spectral sensitivity of the human eye falls in the green-yellow portion of the spectrum. Less scatter compared to shorter wavelengths, results in a better view even in media opacities. Retinal vasculature and nerve fiber layer (RNFL) are best seen in red free photography, thus helping in better identification of hemorrhages, drusen, microaneurysms, RNFL defects and exudates. For this reason, green filter 'red-free' photos are routinely taken as baseline images before fluorescein angiography.⁴

Table 1 Comparison of digital fundus and film-based fundus imaging¹

	Digital	Film-based
Resolution	1000 pixels	10000 pixels (in ideal conditions)
Stereoscopic viewing	Available	Available
Image manipulation	Easy	Difficult
Processing and duplication time	Seconds	Hours
Lesion measurement	Irregular tracing made easy	Best fit circles
Transmission	Worldwide via internet (instantaneous)	Manual delivery of copies (slow)
Cost	Installation cost high	Installation cost less

Table 2 Various imaging modalities based on use of contrast

Contrast	Non-contrast
Fluorescein angiography (FFA)	1. Red-free photography
Indocyanine angiography (ICG)	2. Color fundus photography
	a Stereo fundus photography
	b Digital fundus photography
	c Confocal scanning laser ophthalmoscopy (cSLO)
	3. Fundus autofluorescence
	4. Infrared reflectance
	5. Hyperspectral retinal imaging
	6. Adaptive optics SLO

Color fundus imaging

A. Stereo-imaging

Diagnostic information can be greatly improved by creating a visual sense of depth by laterally shifting the fundus camera a few millimeters between sequential photographs. The illuminating beam of the fundus camera falls on opposite slopes of the cornea creating a cornea-induced parallax.⁴ This results in pseudo three-dimensional image formation from an ordinary two-dimensional fundus photographs. Dedicated stereo display software is available for accurate alignment of digital stereo images.⁴

B. Digital fundus photography

Carl Zeiss Company in 1926 introduced the first commercially available fundus camera which provided a 20° field of view of the retina.⁵ Traditionally used fundus cameras (Fig. 1) provide 30° to 45° field of view which can subsequently be increased by mosaic or montage patterns.

Imaging beyond 50° is called wide-field photography and the recent development came with the introduction of 'ultrawidefield' photography. These include the Pomerantzeff camera,⁶ the Retcam (Clarity Medical Systems, Inc., Pleasanton, CA, USA), the Panoret-1000™ camera (Medibell Medical Vision Technologies, Haifa, Israel), the Optos® camera (Optos PLC, Dunfermline, UK), and the Staurenghi lens⁷ (Ocular Staurenghi 230 SLO Retina Lens; Ocular Instruments Inc, Bellevue, WA, USA) etc. allowing 100° to 200° view of fundus. This allows for simultaneous evaluation of the peripheral and central retina without causing any patient discomfort.

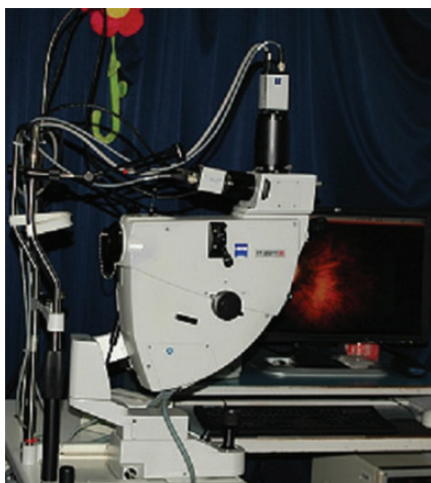


Figure 1. Zeiss FF 450 plus IR colour fundus camera which provides good quality colour fundus images with optical zoom of three magnification levels (20°, 30° and 50°) and FFA along with the option of adding ICG and FAF to its software.

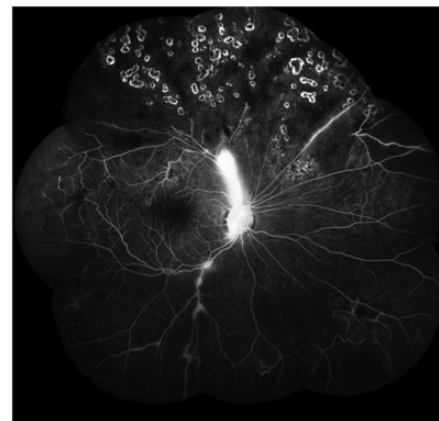


Figure 2. Montage of FFA of the right eye of a patient with vasculitis showing neovascularisation at the disc along with extensive capillary nonperfusion areas and laser marks superiorly. Note the enlarged view obtained by montage technique (approximately 75°).

C. Confocal scanning laser ophthalmoscopy imaging

Some of the newer imaging techniques use the principle of confocal scanning laser ophthalmoscopy (cSLO). Instead of a bright flash of white light as in traditional photography, cSLO uses laser light to illuminate the retina. Confocal imaging acts by a detailed point-by-point scanning of the entire field by a focused laser beam and then capturing the reflected light through a small confocal pinhole, which in turn suppresses any scattered or reflected light outside the focal plane that could blur the image. This results in a sharp, high-contrast image of an object layer located within the focal plane.^{8,9}

The advantages of using cSLO over traditional fundus photography include improved image quality, suppression of scattered light, and patient comfort through less bright light, three-dimensional imaging capability, video capability, and effective imaging of patients who do not dilate well. Examples of cSLO-based ultrawidefield imaging (UWFI) systems include the Optos® camera (Optos PLC, Dunfermline, UK), and Spectralis® (Heidelberg Retina Angiograph (HRA 2), Heidelberg Engineering, Germany).

The main limitation in wide-field imaging is the difficulty in projecting a curved surface on a flat two-dimensional plane (Greenland effect) and software are being developed to address this. Research is also going on to determine whether retinal surface area can be precisely measured in square millimeters.¹⁰

Fundus autofluorescence

Fluorescein angiography uses fluorescein dye to delineate retinal vascular and associated

pathology, the basic nature of dye to cause fluorescence helps in the diagnostic ability. The human eye itself has few components which fluoresce without any external addition of dye. Imaging of such auto-fluorescing substances has given an altogether new dimension to diagnostic imaging. Fundus autofluorescence imaging takes advantage of the intrinsic autofluorescence of lipofuscin, normally found in RPE cells accumulated as a result of phagocytosis of the constantly shed photoreceptor outer segments. RPE dysfunction leads to an imbalance between lipofuscin formation and clearance resulting in excessive accumulation of lipofuscin.¹¹ Higher the lipofuscin content of the RPE cell, the more autofluorescent it will appear on FAF. Atrophic (dead) RPE cells appear severely hypoautofluorescent as they do not contain lipofuscin, whereas viable cells have a varying content of lipofuscin and accordingly, a varying degree of hyperautofluorescence.

Modified fundus cameras use a single flash with excitation spectrum of 535–585 nm to record a single image whereas confocal-based systems use continuous scanning with an excitation spectrum of 488 nm resulting in recording of multiple images with averaging to obtain a final image of high resolution. Overall image quality and contrast are maximized with the cSLO's multiple image acquisition and mean image calculation.¹²

FAF imaging gives information about the metabolic changes at the level of the RPE and helps to identify areas that may be at high risk for the development of geographic atrophy or CNV. FAF imaging has helped us with better understanding of various diseases, classification, prognostication, and follow up in conditions like ARMD, CSR, Stargardt's disease, Leber's congenital amaurosis and Serpiginous choroiditis.^{11,13–17}

Infrared reflectance

A newer variant of fundus photography, infrared reflectance (IR) imaging uses infrared light instead of white light for illumination. Red wavelengths

are longer than the other colored wavelengths of the visible spectrum making them less prone to absorption by blood and melanin. This results in greater amount of light (reflectance) being available to form an image as compared to shorter wavelengths in the visible spectrum, and hence constitute better tool for imaging structures deeper to the RPE.^{18,19}

IR is a non-invasive en face imaging technique which helps in visualizing sub-retinal pathology.¹⁸ IR imaging has several advantages over flash fundus photography. The invisibility of infrared light makes it better acceptable for imaging children and light-sensitive patients. As the penetration of infrared light is better, it acts as a useful imaging tool in patient with media opacities like dense cataract. IR imaging may offer better visualization of epiretinal membranes, cystoid macular edema and deeper structures compared to fundus photography and red-free imaging. IR was found to be superior to standard color fundus photography in screening for neovascular AMD.¹⁹

Hyperspectral retinal imaging

It is a novel, non-invasive imaging technique for ocular diagnosis using the spectroscopy principle of retinal oximetry, which exploits the different spectral characteristics of oxygenated vs deoxygenated hemoglobin to assess tissue perfusion. This enables evaluation of functional characteristics of tissue health. Its role is important in microvascular disorders like diabetic retinopathy, sickle cell disease, ARMD.^{20,21}

Adaptive optics SLO

The optical properties of the normal eye result in a point spread function width approximately the size of a photoreceptor. Standard fundus cameras have the shortcomings of inability to visualize individual cells/structure because of the various aberrations of the visual system. Adaptive optics uses mechanically activated mirrors to correct the wavefront aberrations of the light reflected from

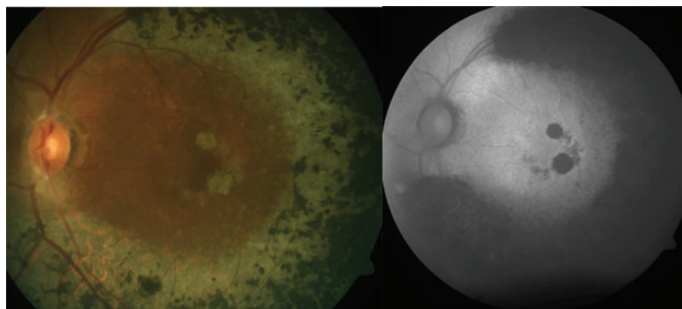


Figure 3. Left eye fundus photograph of 30 year old man with retinitis pigmentosa showing extensive bony spicules reaching almost upto the macula along with some atrophic changes. FAF image of the same eye clearly showing the demarcation between the viable (normal autofluorescence) and the atrophic areas (hypoautofluorescence).

the retina and thus allows individual photoreceptors to be imaged.²² Recent studies have shown the ability of adaptive optics-based system to image microscopic blood vessels and the cone photoreceptor mosaic. Its utility has been shown in identifying reduced cone photoreceptors in Retinitis pigmentosa.²³

Three commercially available machines which incorporate some of the above recent imaging modalities are discussed further.

Optos®

The first and one of the most widely used commercially available ultra-widefield systems is the Optos noncontact camera. The Optos system utilizes an ellipsoid mirror to produce images with approximately 200° field of view, providing an image of more than 80% of the retina in a single capture.²⁴ The Optos provides various high-resolution imaging systems with multiple software options catering to specific requirements. Also instead of white light, Optos incorporates low powered laser wavelengths that scan simultaneously. This allows viewing of the retinal substructures in their individual laser separations. The red free view scans the sensory retina up to the pigment epithelial layer whereas the choroidal view scans layers from the RPE to the choroid.²⁵ Optos also provides ultra-widefield, high-resolution angiography, enabling a simultaneous central and peripheral view of the retina. Thus the entire retinal vasculature can be imaged during the dye transit. This is particularly useful in conditions affecting periphery first like retinal vasculitis. Recent studies also support the added advantage of peripheral imaging in diabetic retinopathy, vascular occlusion, choroidal mass, Uveitis, choroidal dystrophies, retinal detachment, pediatric retinal conditions and many more disorders.^{26–34} Wessel et al.²⁶ reported that UWFI visualizes 3.2 times more retinal surface area than the conventional seven standard fields. Also in 10% of eyes, UWFI diagnosed peripheral retinal pathology not seen on traditional fundus imaging.²⁶ Optos has wide range of ultra widefield devices depending upon the various features it provides. While the Optos 200Dx and Optos 200C provides features like color fundus photography, ophthalmoscopy along with red free and choroidal imaging, Optos Daytona has the added feature of FAF. Optos 200Tx goes further with the addition of FFA and the newly introduced Optos California includes ICG in addition to the all above-mentioned features thus making it feasible for ultrawidefield multimodal imaging.²⁵

Despite its ability to produce dynamic widefield fundus images, the Optos Optomap® has several limitations. It requires a skilled technician to obtain peripheral images. Also unlike traditional fundus photography, Optos does not allow a

retinal view before taking the image, due to the ellipsoid mirror used for focusing the confocal ophthalmoscope. Even though it provides a very wide field of view, it is still unable to view the entire retina in one shot and is prone to miss the anterior retinal findings. The extent of imaging of the superior and inferior peripheral retina is less as compared to nasal and temporal periphery.³⁵ Also, there is a significant distortion and decreased resolution of the extreme temporal and nasal peripheral retina.²⁹ A method for correcting this peripheral distortion, when evaluating the retinal area and making measurements, has been described.²⁹ Artifacts related to structures anterior to retina like eyelashes, implanted intraocular lens, vitreous opacities are known to occur in Optos causing degradation of the quality and extent of imaging.³⁶

Spectralis

Heidelberg Spectralis (Fig. 2) has enhanced the role of fundus imaging by combining the spectral domain OCT with confocal SLO resulting in enhanced anatomical details, improved reproducibility and automatic rescan at same site at follow-up. Simultaneous multimodal imaging (Fig. 3) with FAF, infrared reflectance, FFA and ICG is also possible. Previously, the Heidelberg Spectralis® provided a 25° and 35° field of view of the retina, with the possibility of a 55° noncontact lens attachment (HRA). A different model (HRA +OCT) combines the features of HRA with spectral domain OCT and EDI OCT, thus providing an efficient tool for multimodal imaging of fundus. More recently, Heidelberg Engineering (Heidelberg, Germany) has developed a noncontact ultra-widefield angiography module for the Spectralis® and Heidelberg Retina Angiograph (HRA 2) with 105° field of view.^{37,38} Even though

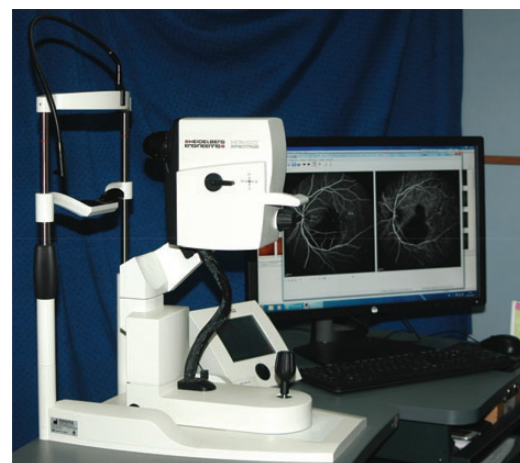


Figure 4. Spectralis by Heidelberg Engineering showing simultaneous FFA and ICG (multimodal imaging).

the field of view is less when compared to Optos, on qualitative analysis, the high-contrast, undistorted and evenly illuminated images of Spectralis

are found to be better and more informative than that taken by Optos.²⁴ Also image capturing is easy in Spectralis because of the use of infrared

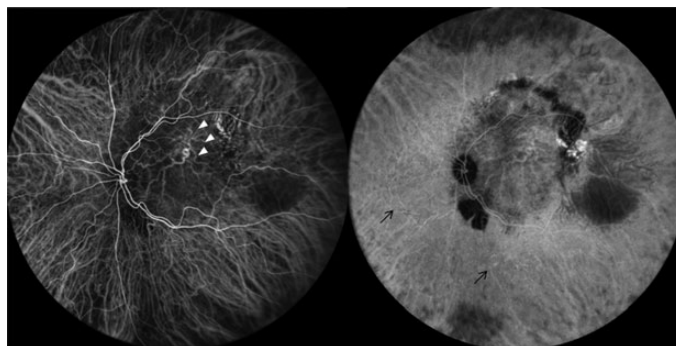


Figure 5. Spectralis widefield ICG imaging done by a 150 degree non contact lens system shows a network of polypoidal vessels (arrow heads) suggestive of IPCV. The late phase of ICG shows multiple choroidal folds (black arrows) in periphery that could have been missed on a routine ICG.

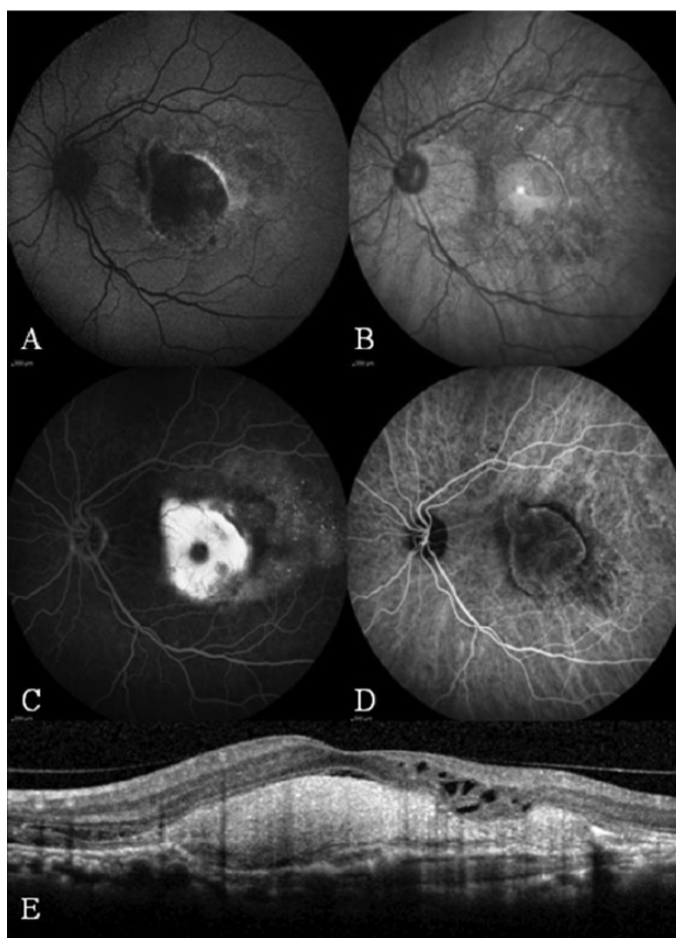


Figure 6. Multimodal imaging of a patient with Left eye CNVM done on Spectralis. A. FAF shows presence of hypoautofluorescent centre surrounded by a hyperautofluorescent margins. B. Infrared reflectance shows an isoautofluorescent centre with hyperautofluorescent margins. C. Late phase of FFA shows staining at the centre along with leakage at the margins suggestive of active neovascular membrane which can be appreciated as a distinct, well demarcated membrane on ICG (D). Spectral domain OCT (E) shows dense subretinal fibrin deposition with irregular RPE Bruch complex. Note presence of subretinal fluid and cystoid spaces suggestive of activity.

light for focusing the confocal ophthalmoscope (instead of ellipsoid mirror as in Optos), one can view the retina before taking an image.³⁹ In addition, Staurengi contact lens can be used in conjunction with the Spectralis® to provide up to 150° field of view in a single shot.^{7,39} A recent study has shown utility of Spectralis in imaging infant fundus along with FFA to look for any retinal diseases.⁴⁰ One limitation of Spectralis is that instead of true color images, pseudo color images are displayed, which may not represent the exact clinical picture.

RetCam

RetCam is an advanced, wide-field digital imaging system which provides good quality of ophthalmic visualization and photo documentation with great ease. It has been found to be an effective tool for immediate evaluation of pediatric ocular pathology. The newer-generation RetCam 3 provides further high-resolution digital images and can be shared electronically with an ophthalmologist or a physician and can be tracked longitudinally over time. RetCam 3 is equipped with a family of interchangeable lenses that provides a wide range of image options allowing the physician to view both anterior and posterior ocular pathology.^{41,42} Retcam-3 has five changeable lenses: 130° (pediatric retina and adult anterior chamber), 120° (pediatric and young adult), 80°(high contrast pediatric and adult), 30° (high magnification) and Portrait (external imaging).⁴³ The major use of RetCam has been in screening, classification and follow-up of pediatric conditions like retinopathy of prematurity (ROP) (Fig. 7), Retinoblastoma (Fig. 8), Coats disease (Fig. 9) etc. It helps in identifying the appropriate intraocular group of retinoblastoma, assessing treatment response, regression patterns and to detect any recurrence. It also

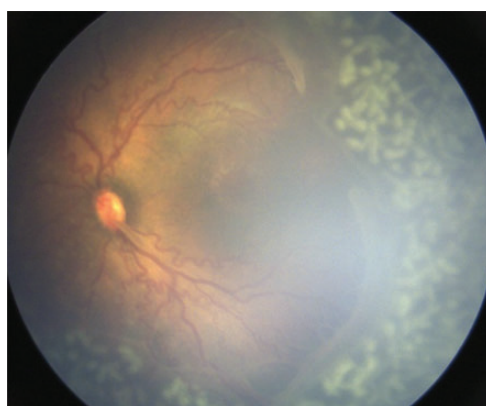


Figure 7. RETCAM image of Lasered Stage 2 Zone I ROP with plus disease—left eye showing tortuous vessels at posterior pole. Note presence of ridge just temporal to macula and lasered avascular zone anteriorly.

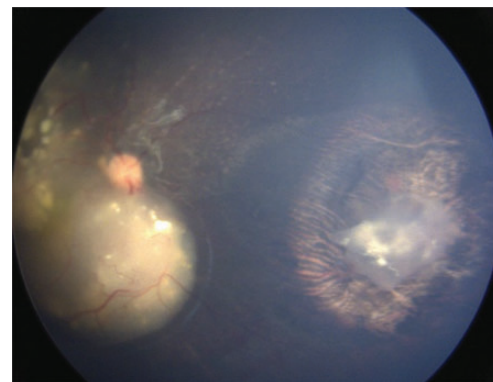


Figure 8. RETCAM image of right eye of a child showing Intraocular Retinoblastoma undergoing treatment. Note the active tumour involving macula with calcification and another regressing tumour nasally with surrounding chorioretinal atrophy and central fish flesh appearance. RETCAM helps in documenting the treatment response by serial imaging.

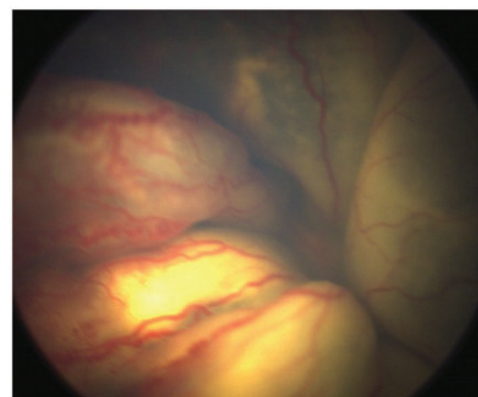


Figure 9. RETCAM image of right eye of a young boy showing bullous exudative retinal detachment with presence of dense subretinal exudates and telangiectatic vessels confirming the diagnosis of Coats disease.

allows screening of premature infants at risk for ROP and has been shown to be an invaluable tool in resident training, parent education and photo documentation of ROP.⁴⁴ RetCam 3 can also be equipped with an optical light source to perform fluorescein angiography which may be helpful in differentiating between diseases like Coats and Retinoblastoma. RetCam has gained great success as a screening imaging tool in various national and international tele-ophthalmology projects such as KIDROP (Karnataka Internet-Assisted Diagnosis of Retinopathy of Prematurity), and SUNDROP (Stanford University Network for Diagnosis of Retinopathy of Prematurity).^{45,46} RetCam in addition to fundus imaging can also be used to image and document the angle and other

anterior segment abnormalities like iris nevus, pigment dispersion/pseudoexfoliation, neovascularization etc.⁴³

Conclusion

Different retinal diseases require different imaging modalities for better understanding and management. The field of fundus imaging is developing rapidly and several new and exciting technologies are on the horizon which will cater to the needs of an ophthalmologist. This will help us in evolving our understanding of various disease entities thus helping us in better management of our patients.

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