Review Article

Clinical applications of the retinal functional imager: A brief review

Chaitra Jayadev, Nimesh Jain, Ashwin Mohan, Naresh K Yadav

The advances in treating blinding conditions often depends on the development of new techniques that allows early detection, treatment, and follow-up of the disease. Functional changes often precede structural changes in many retinal disorders. Therefore, detecting these changes helps in early diagnosis and management, with the intention of preventing permanent morbidity. The Retinal Functional Imager (RFI) is a non-invasive imaging system that allows us to assess the various functional parameters of the retina. The RFI quantitatively measures the retinal blood-flow velocity, oxygen saturation, metabolic demand and generates a non-invasive capillary perfusion map that provides details similar to a fluorescein angiography. All of these parameters correlate with the health of the retina, and are known to get deranged in retinal disease. This article is a brief review of published literature on the clinical utility of the RFI.

Key words: Capillary perfusion maps, non-invasive retinal imaging, retinal functional imager

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Functional changes most often precede structural changes in many retinal disorders and detecting these changes aids in understanding pathogenesis, early diagnosis and timely management. Currently available non-invasive imaging modalities allow assessment of structural changes in various retinal vasculature diseases in great detail. However, understanding the pathogenesis and functional sequelae in depth is the first step in developing new treatment modalities to target the disease in the early stages. The retinal function imager (RFI) has the potential to detect such functional changes in the retinal vasculature much before structural changes can be detected clinically or on conventional imaging. It is non-invasive and provides easy, repetitive, qualitative and quantitative imaging parameters, which include:

(1) Blood-flow velocity
(2) Non-invasive capillary perfusion maps (nCPM)
(3) Blood oximetry
(4) Metabolic state (metabolic demand).[1]

Principle

The RFI can determine the blood flow velocity and the nCPM by tracking erythrocyte flow. It measures the variation in the reflected light with respect to the wavelength and assesses the relative concentration of haemoglobin chromophores in both the vessels and capillary background for information about the intravascular oxygen content.[2,3]

When viewed with the green wavelength of the visible spectrum, haemoglobin within the red blood cells provides a natural, high-contrast chromophore (at wavelength between 530-590 nm) for tracking blood flow.[4-6] Cross-correlation match between the moving blood cell patterns within an image series gives a direct measure of the velocity. The RFI can measure velocities not only for the first order but the second and tertiary branches of the main retinal vessels (both arteries and veins) as well. Flow velocity changes across a large number of arterioles and venules can be detected simultaneously. The red blood cells appear dark under green light and are arranged randomly along the blood column. This creates a light and dark pattern along the vessel which is better appreciated with the movement of red blood cells along the blood column.

A single capture acquires a "series" of 8 monochrome standard fundus images. This sequence of 8 frames can be presented in the form of a movie to track the motion of individual clusters of red blood cells or even a single red blood cell [Fig. 1]. Though the most direct method of quantifying flow velocity is to manually measure the spot-by-spot distance moved per frame interval, a path-constrained cross correlation technique is used for automated flow velocity quantification [Fig. 2a and b].[1] A negative value in the blood flow velocity map indicates flow away from the heart, whereas a positive value indicates blood flow towards the heart. Landa *et al.* have shown in their study using RFI in normal individuals that the arterial blood flow velocity is in the range of 3.7 to 5.8 mm/sec and that of veins is from 3.0 to 4.5 mm/sec depending on the calibre of vessels.[6] They also found a direct correlation between venous blood flow velocity and central retinal thickness.[6]

Department of Vitreo-Retina, Narayana Nethralaya Eye Institute, Rajajinagar, Bengaluru, Karnataka, India

Correspondence to: Dr. Chaitra Jayadev, Narayana Nethralaya, 121/C, Chord Road, 1st R Block, Rajajinagar, Bengaluru - 560 010, Karnataka, India. E-mail: drchaitra@hotmail.com

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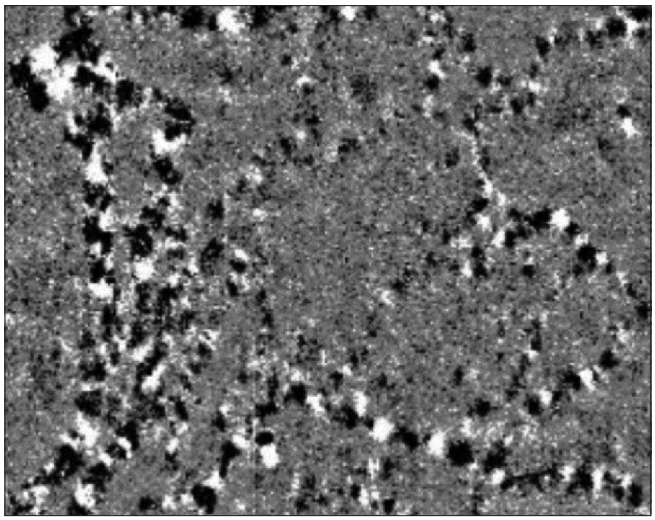

Figure 1: Differential images: Black spots are erythrocytes or erythrocyte clusters; White spots or gaps represent absence of erythrocytes. The direct nature of measurement allows quick assessment of any gross abnormalities in blood flow

Technical Specifications

The RFI system combines digital fundus imaging and functional optical imaging with various other enhancements/parameters and a standard data storage facility. The RFI has two main systems - the RFI 3000 and the 3005. The 3000 has blood flow velocity and nCPM. The 3005 aditionally has multispectral imaging for retinal oximetry and metabolic function imaging. This system works with a minimum pupil diameter of 6mm.

Rapid sequential imaging

A 60 Hz, 1024 × 1024 pixel digital imaging system with a stroboscopic flash lamp system takes images of the fundus at rates high enough to reduce the inter-frame retinal motion and follow red blood cells moving at up to 20 mm/sec. This range is most sensitive for secondary and tertiary order vessels, and provides high resolution, region specific flow information. Further analysis of the red blood cells' movement in the retina generates capillary non-perfusion maps that complement the blood flow velocity measurements.

Rapid delivery of illumination of sufficient intensity to permit low-noise imaging

In each series, 8 consecutive flashes with an inter flash interval of less than 20 millisecond are delivered to a subject, generating 8 red-free images in under a second. Multiple series of 8 frames are obtained from each session. The captured 8-frame sequences can be presented as a movie.

Multi spectral imaging (rapid changes in illumination wavelength)

This is performed for oximetric measurement. The filter wheel can have upto 8 filters which can switch at an interval of 30 ms. This allows multiple wavelength image acquisition with minimal eye movement.

Stimulus generator

It uses visual patterned stimulus with a specified pattern, frequency and duration.

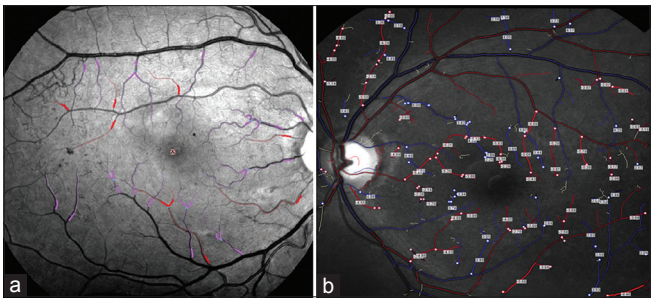

Figure 2: (a): Arteries (red) and veins (violet) that were manually selected for quantification. (b): Blood velocity map - measured velocities in veins (positive values) and in arteries (negative values) are presented in millimetres per second (value + SD). The value is an average of measurements from three combined series

Clinical Applications

Direct visualization of retinal blood flow, without the use of an intravenous dye injection and its associated complications,[7] opens up many new diagnostic possibilities for various disease pathologies associated with alterations in the blood flow velocity of retinal capillaries, arterioles and venules. Different collateral vascular patterns in normal as well as in the diseased retina can be studied with the help of the RFI system. Landa *et al.* in showed four different patterns of collateral circulation in the retina (looped pattern, vertical pattern, H-shaped pattern and cilioretinal-retinal collateral pattern).[8] The use of RFI has been demonstrated in conditions like diabetic retinopathy, hypertension and other retinal vascular disorders.[9]

The movement of red blood cell clusters in the RFI imaging system is distinct and complemented with less motion blur allowing the clarity of the smallest of vessels to be on par with that of the large vessels. The resulting map non-invasively gives us finer details of vascular anatomy that is not visible clinically or with most conventional imaging devices [Fig. 3].

Capillary perfusion map (CPM)

After image acquisition, various parameters are analysed to detect the motion of red blood cells. Microvasculature tracing is based on motion contrast rather than reflectance contrast. The recently improved algorithm for CPMs now generates images providing much finer details of the retinal microvasculature than the corresponding conventional fundus fluorescein angiography (FFA) images [Fig. 4].

Hence, the most significant advantages of CPMs over FFA for macular imaging are: (1) non-invasiveness, (2) higher resolution, (3) repeatability, and (4) potential to calculate blood flow velocity.[8] Conventional FFA also cannot delineate between normal and diseased capillaries, which can be done on the RFI using the blood flow velocity [Fig. 5]. Due to the better resolution, CPMs provide greater details of microaneurysms in diabetic retinopathy, including non-leaking microaneurysms that are rarely seen with FFA. Adequate details of the vascular frond morphology of neovascularization and reasonable delineation of areas of capillary non-perfusion can be achieved with the RFI.[9]

Optical coherence tomography angiography (OCTA) is another non-invasive technology that utilizes the flow of red blood cells as an intrinsic contrast agent to generate flow signals allowing for visualization of vascular networks without the

need of dye injection. It acquires repeated OCT B scans at the same location to detect motion. An important advantage of OCTA is segmentation allowing for abnormalities in different retinal layers to be detected. The advantage of RFI remains a higher resolution and field of view when compared to OCTA [Figs. 6 and 7].

Blood flow velocity

Clinical studies with the RFI have shown a significantly altered blood velocity in patients with non-proliferative diabetic retinopathy compared to healthy controls.[10] In patients with early diabetes and no diabetic retinopathy, the RFI detected an increased in the blood flow velocity and in the size of the foveal avascular zone compared to controls.[11] Such changes in blood flow velocities and early detection of ischaemic areas in the initial course of the disease can help in early diagnosis as well as customizing treatment.

In AMD patients, the RFI has shown a reduced blood flow velocity in wet AMD eyes compared with fellow dry AMD eyes.[12] Also, the average blood flow velocity in arteries and veins was significantly lower in AMD patients compared to controls. Anti-VEGF treatment effects on the retina have also been studied with the RFI and showed an increase in blood flow velocity around 7-10 days after the injection, which gradually decreases as the injection effect wanes off.[12] Following intravitreal bevacizumab injections, there was a difference in the retinal blood-flow velocity between responders and non-responders.[12] The RFI can also be used to evaluate the peripapillary blood flow in capillaries perfusing the optic disc to elucidate the vascular pathogenesis in different etiologies of glaucoma [Fig. 8].[13]

Multispectral imaging for retinal oximetry

The absorption spectrum of oxy-haemoglobin and deoxy-hemoglobin is different. This unique property can be

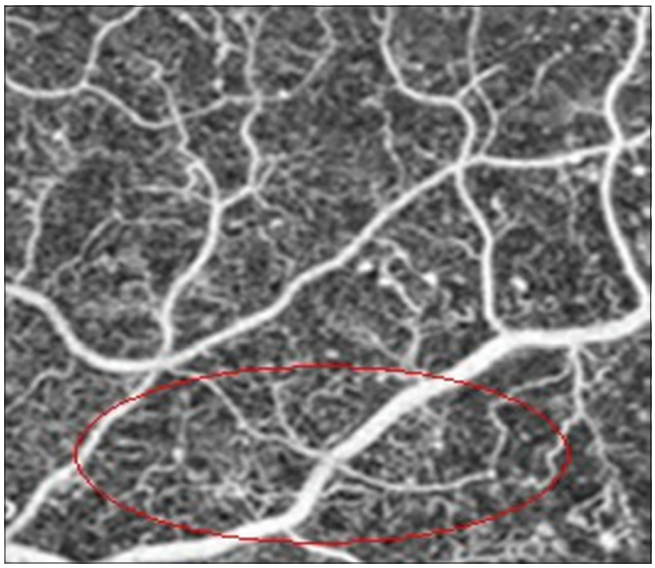

Figure 3: Shunt and anastomotic vessels well delineated in diabetic retinopathy using the RFI

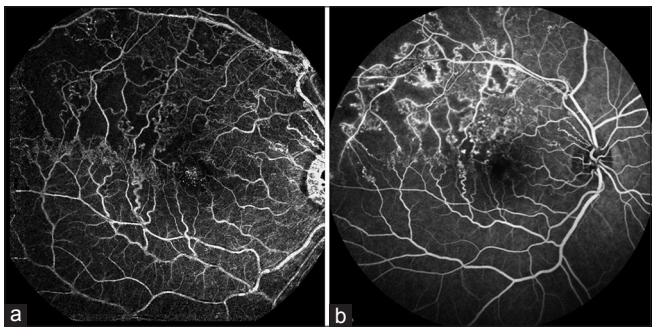

Figure 5: Good correlation of the capillary non-perfusion area between RFI (a) and FFA (b) in a patient with ST BRVO

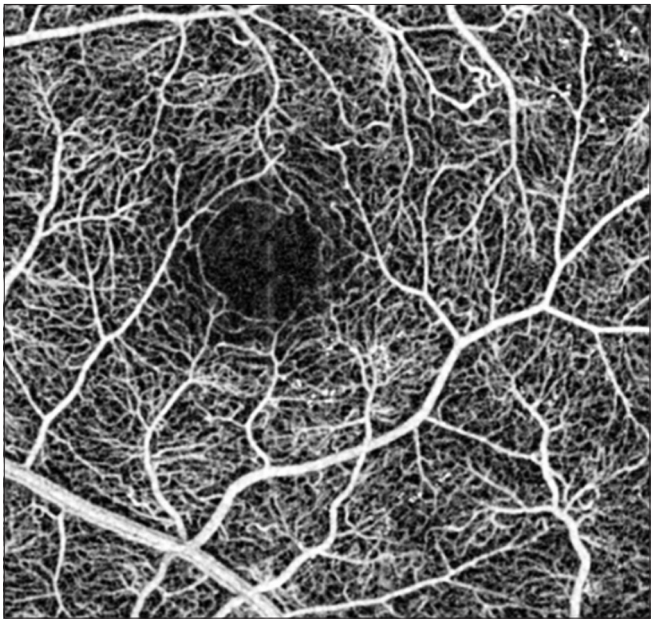

Figure 4: Capillary perfusion maps obtained without any contrast agent. The retinal microvasculature is well delineated

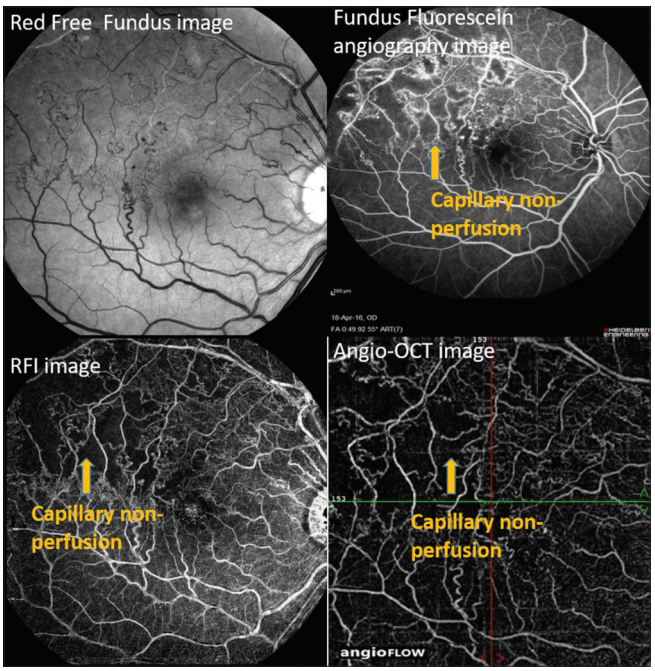

Figure 6: Comparison between the red free fundus image, FFA, RFI and OCTA of the same patient in Figure 5 with ST BRVO

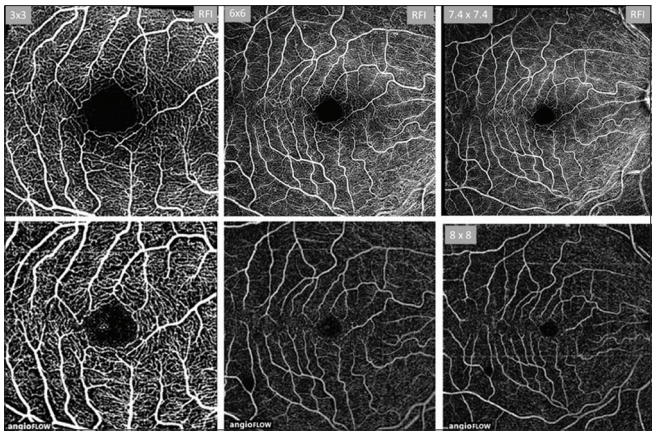

Figure 7: RFI (upper panel) and OCTA images of a normal patient demonstrating a higher resolution in RFI as the scan area increases

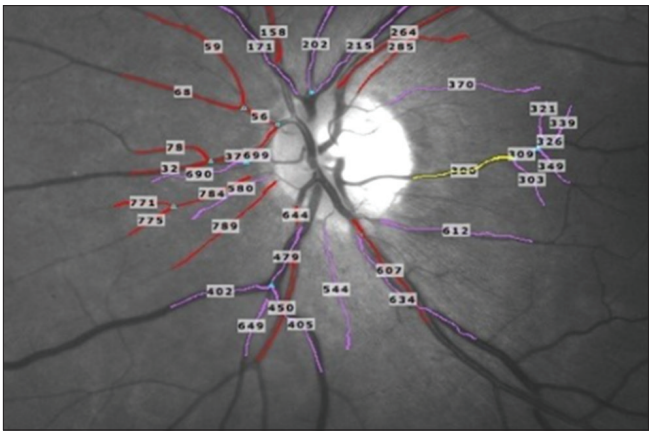

Figure 8: Para papillary blood flow measurements in a patient with glaucoma

used to study and analyse the oxygen saturation of blood with the help of inbuilt spectroscopic methods,[13,14] which otherwise gets altered in diseases affecting the retinal blood vessels. Quantitative and qualitative assessment of oxygen saturation of retinal arteries and veins is possible.[15,16] However, the optical complexity of the retina can hamper the accurate quantitative evaluation of oximetric maps.[17] Perfusion deficits and abnormalities appear as a region of colour distinct from their surroundings. Poor perfusion areas appear blue, whereas highly perfused area appears red. In patients with diabetic retinopathy, these regions appearing grossly normal on conventional FFA, appear patchy and darker in the oximetric maps of RFI suggesting ischemia.[18] This finding underscores the significance of qualitative oximetric imaging as a supplement to angiography to detect anoxia directly. Similar to the CPMs, oximetry maps can also provide high-resolution details of microaneurysms in patients with diabetic retinopathy, as they are not obscured by the leakage that occurs in conventional FFA.[18]

Retinal metabolic function

The RFI can be used to study the retinal functional signal by analysing the reflectance changes in the retina after stimulating it with a visual stimulus. Such functional reflectance signals are small, originating from activity-dependent metabolic, hemodynamic, and fast and slow light-scattering changes.[19] The RFI can image outside the absorption range of photoreceptors with near infrared light (750-840 nm), and hence be used to optically monitor the metabolic demand or the retinal activity in response to a well-defined visual stimulus (562 + 20 nm). The difference between the post-stimulated and pre-stimulated images reflects the metabolic state or demand and the functional state of the activated axons of ganglion cells.[20,21]

Limitations

The RFI is firstly a fundus camera based system versus the OCT A which is an SLO based system and/or uses a light of a much higher wavelength. This implies lesser light scattering and hence clearer images. Hence the nCPM images of the RFI do come with a lot of artefacts which need to be kept in mind while interpreting them. Secondly since the imaging takes eight images it involves the discharge of a high intensity light eight times within a short period into the patients eye. This can cause significant patient discomfort and also render the patient unco-operative. In our experience we also noted that a significantly higher technician skill was required as compared to what was needed for the OCT-A. All the above factors put together makes image aquisition on the OCT-A more reliable as compared to the RFI which can be more of a hit or miss. Future RFI development is thus focussed on addressing these limitations.

Disadvantages of most non-invasive modalities include a smaller field of view with a maximum of 50 degrees compared to 200 degrees in ultra-wide field FFA. This limits the utility to macular diseases currently. In addition, RFI requires that the patient to fixate for several seconds, whereas a single FFA frame can be obtained in seconds. Interpretation and image processing is a time consuming and, at times, a difficult aspect of several newer modalities. Future advancements of the technology and software enhancements are required to overcome these limitations. Vascular leakage, which is of diagnostic importance in several retinal diseases, cannot be appreciated on the RFI. Cost of instrumentation and further upgrades is also a deterrent when compared to the FFA.

Conclusion

In conclusion, the Retinal Functional Imager is a non-invasive technique for estimation of the retinal blood flow velocity, perfusion, oxygen saturation and metabolic demand of tissue. Its ability to detect subtle changes in circulation—both in normal subjects and those with ocular disorders—helps in early diagnosis of retinal diseases. Early detection of abnormalities in functional parameters before structural abnormalities become evident allows intervention before permanent retinal damage occurs. It also widens the opportunities for further research and drug development addressing a wide range of retinal diseases, beyond the capabilities of structural imaging. Besides characterizing the retinal microvasculature under various conditions, it has potential in some central nerve system (CNS) and systemic diseases. Applying the RFI in research and clinical settings should help earlier diagnosis, support disease prevention, and improve management.[22,23]

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Conflicts of interest

There are no conflicts of interest.

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