High-resolution in vivo fundus angiography without the need for an adaptive optics imaging system

Mali Okada, MMed[1,2]; Tjebo FC Heeren, MD[2,3]; Pádraig J Mulholland, PhD[2,3,4]; Peter M Maloca, MD[2,5-7]; Marketa Cilkova, MSc[3]; Vincent Rocco[2]; Marcus Fruttiger PhD[3]; Catherine A Egan, FRANZCO[2,3]; Roger S Anderson, DSc[2,3,4]; Adnan Tufail, MD FRCOphth[2,3]

1. Royal Victorian Eye and Ear Hospital, Melbourne Australia
2. Moorfields Eye Hospital NHS Foundation Trust, London, United Kingdom
3. Institute of Ophthalmology, University College London, London, United Kingdom
4. Optometry and Vision Sciences Research Group, School of Biomedical Science, Ulster University, Coleraine, Northern Ireland
5. OCTlab, Department of Ophthalmology, University Hospital Basel, Basel, Switzerland
6. Institute of Molecular and Clinical Ophthalmology Basel (IOB), Basel, Switzerland
7. Department of Ophthalmology, University of Basel, Basel, Switzerland

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Address for correspondence:
Adnan Tufail, MD, FRCOphth
Moorfields Eye Hospital NHS Trust
162 City Road, London, United Kingdom
E-mail: Adnan.Tufail@moorfields.nhs.uk

Abstract

Purpose:

To provide a proof of concept for the detailed characterization of retinal capillary features and surrounding photoreceptor mosaic using a customized non-adaptive optics angiography imaging system.

Methods:

High-resolution fluorescein angiography (FFA) and/or indocyanine green angiography (ICGA) images were obtained using a modified Heidelberg Retina Angiograph (HRA2) device with a reduced scan angle enabling 3° field of view. Co-localized images of the photoreceptor mosaic were also captured *in vivo* using the same instrument. Visibility of vascular sub-branches were compared between high-resolution images and conventional fundus angiography (FA) with a 30° field of view.

Results:

High-resolution angiographic and infrared images (3° x 3° field of view, a 10-fold magnification) were obtained in ten participants. These included seven patients with various retinal diseases, including myopic degeneration, diabetic retinopathy, macular telangiectasia and central serous chorioretinopathy, as well as three healthy controls. Images of the retinal vasculature down to the capillary level were obtained on angiography with the ability to visualize a mean 1.2 levels more sub-branches as compared to conventional FA. In addition, imaging of the photoreceptor cone mosaic, to a sufficient resolution to calculate cone density, was possible. Movement of blood cells within the vasculature was also discernible on infrared videography.

Conclusion:

This exploratory study demonstrates that fast high-resolution angiography and cone visualization is feasible using a commercially available imaging system.

Translational Relevance:

This offers potential to better understand the relationship between the retinal neuro-vascular system in health and disease and the timing of therapeutic interventions in disease states.

Word Count: 247

Introduction

Fundus fluorescein angiography (FFA) was first described by Novotny and Alvis in 1961 and has since become the gold standard imaging technique for assessing macular and retinovascular disorders.[1] Along with indocyanine green angiography (ICGA) for examining the choroidal circulation, FFA has been instrumental in developing our understanding of the pathogenesis of many retinal and choroidal diseases. However, fundus angiography (FA) in its current form has several limitations. Although recent advances have been made with ultra-wide field angiography, the resolution of FA remains a limiting factor for studying microscopic features of the retinal vasculature and the associated neuroretina.

In the search for accessible higher resolution retinal imaging, optical coherence tomography angiography (OCTA) is an emerging imaging modality that has been proposed as an alternative to FA for mapping retinal vessels. It is non-invasive and provides detailed delineation of the retinal capillary beds. However, it is subject to projection and motion artefacts and the current technology is unable to demonstrate flow or leakage.[2] In addition, although OCTA provides some structural information about the neuroretina, this is not at the cellular level (e.g. photoreceptor mosaics).

Adaptive optics (AO) based imaging system is another approach that has demonstrated great potential for imaging the retinal vasculature down to the capillary level.[3,4] This method has been widely used to investigate the neuroretinal structure, principally cone photoreceptor mosaics[3–5]. Two methods of adaptive optics scanning light ophthalmoscopy (AOSLO) system have been employed to image retinal vessels, including a confocal system combined with oral fluorescein, as well as a non-confocal AOSLO system, which is coupled with motion contrast to remove the need for contrast agents.[6–8]

Although these AO based imaging systems provide unparalleled resolution of the retinal microstructure, they are complex, expensive, require significant patient cooperation and image processing. As such, it is currently impractical to use in routine clinical practice or in large-scale clinical trials. A simpler, non-AO based system that can resolve images *in vivo* down to the level of photoreceptors and small retinal capillaries, has the potential to provide novel insights into retinal disease and

may provide endpoints for future clinical studies. The ability to image larger numbers of patients with various levels of disease than is currently feasible using current AO systems would also help to generate new biomarkers in our understanding of retinovascular diseases. Although we have previously demonstrated that the paramacular cone mosaic may be imaged in vivo using a modified scanning laser ophthalmoscope,[9,10] the capabilities of the device to provide high resolution angiography has not been examined. Therefore, the aim of this exploratory study is to provide a proof-of-concept demonstration of high-resolution angiography alongside photoreceptor imaging using a modified commercially available non-adaptive optics imaging device.

Methods

Participants

Participants for this exploratory study were recruited from the medical retina clinics at Moorfields Eye Hospital. They were invited to join the study if they had retinal pathology requiring FFA and/or ICGA diagnostic workup as part of their clinical care. Additional healthy control participants were also recruited for comparative purposes. Participants were excluded if they had an allergy to intravenous dye, were aged less than 18 years, or had significant media pathology that would preclude good quality imaging on conventional FA.

Ethics approval was obtained approved by the local institutional review board and conducted according to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

High-resolution Fundus Angiography

High-resolution imaging was performed using a modified Heidelberg Retina Angiograph 2 (HRA2; Heidelberg Engineering GmbH, Heidelberg, Germany). This modification has been previously described for use in cone imaging by our team.[9] In summary, the standard scan angle of a conventional HRA2 was reduced by a factor of x10, from 30° field of view down to 3°, with the narrow angle enabling a magnification of the image whilst retaining the same 768 x 768 density of pixels (Figure 1). The field of imaging was 3° x 3°, which corresponds to an area on the retina of 0.825 x 0.825 mm, when calculated using standardized conversion rates at

equivalent retinal loci.[11] The original commercial filters for FFA and ICG in the HRA2 were left in situ. The incident beam power however of the blue laser (488 nm) used for FFA and for blue reflectance imaging was reduced to 100µW to meet the requirements for Class 1 emission limit according to the International Electrotechnical Commission 60825-1 guidelines. The high-resolution ICGA was acquired with the standard diode laser emitting 785 nm, with no change to laser output needed to meet safety guidelines. Cone imaging of the same location was also obtained using the infrared laser but with the image acquisition set to reflectance mode. Near infrared autofluorescence (IRAF) was also performed in some patients to attempt to image the retinal pigment epithelium and choriocapillaris layer.

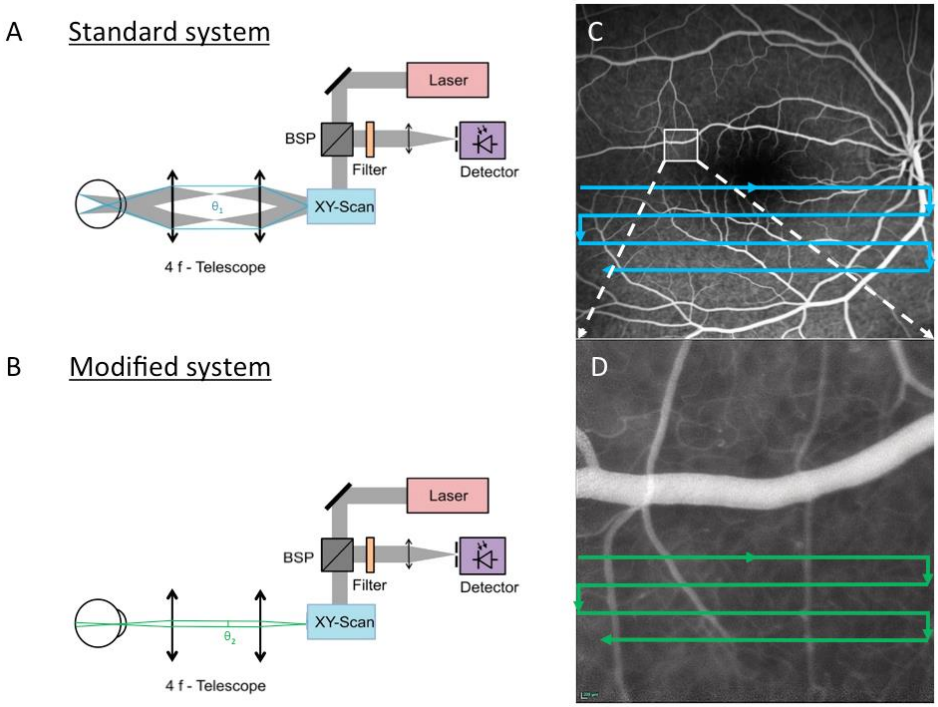

Figure 1. Schematic of (A) conventional Heidelberg HRA2 device with standard scanning angle (θ_1) and (B) modified high-resolution device with reduced scan angle (θ_2). (C) High resolution image with a scan angle of 3° superimposed onto conventional image of 30°

Imaging Protocol

In vivo imaging was performed through dilated pupils with different areas of the fundus imaged by adjustment of the internal fixation lights. Standard doses of either 2-3 mls of 20% sodium fluorescein dye or 25mg/5ml of indocyanine green dye were injected for FFA and ICGA respectively. Based on the clinical indication for the conventional FA, the majority of participants had early phase angiography images

conducted on a conventional non-modified HRA2 device with late phase images (> 5 minutes) on the high-resolution device. This was done in order to have both conventional and high resolution FA performed in the same sitting. In one participant, imaging was performed on three FA devices to enable comparisons across different imaging platforms: conventional HRA2, high-resolution HRA2 and Topcon TRC-NW8 retinal camera (Topcon Medical Systems Inc., USA). In each participant, the device was then switched to infrared reflectance mode to capture the cone mosaic pattern at the same location as the FA imaging. Single, non-averaged images as well as averaged real time (ART) images of 10-40 frames were acquired for both conventional and high-resolution images. Videography was also obtained to record blood flow.

Image Analysis

Raw images were exported from Heidelberg Eye Explorer with the image borders cropped to remove other features (eg. date information, patient-identifying data). The high-resolution images, comprising a 3° x 3° field (768 x 768 pixels), were exported into Adobe Photoshop CS 5.1 (Adobe Systems Inc, California, USA) and scaled to size to enable manual overlay onto the corresponding conventional image for localization and comparison (Figure 2).

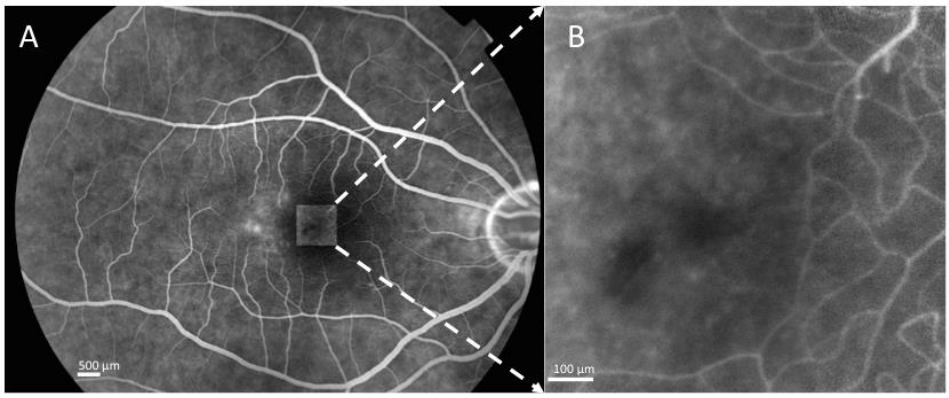

Figure 2. Localization and scaling of conventional image (A) and high-resolution image (B)

Comparison between conventional and high-resolution images was made for each patient on a qualitative basis on the clarity of large vessels and quantitatively by the visibility of sub-branches off the main retinal vascular arcade at the same location with hierarchical numbering from branch 1 indicating main retinal arcade and 4 indicating capillary network (Figure 3). The difference between the two was

calculated with a higher number indicating a step further in the hierarchy of vascular branching. Analysis of cone density was performed using Matlab software (R2014b, Mathworks Inc.), according to the automated method described by Li and Roorda for use in AO images.[12] In brief, image labels were cropped from the raw image and a low-pass filter then applied. The image was then converted back to the spatial domain and maximum local luminance was detected and plotted as cone centers, ensuring that identified cones were not closer than physiologically possible. Manual calculation was also performed to account for cones erroneously detected over any large blood vessels. Voronoi analysis was used to examine the packing arrangements of the cone photoreceptors.

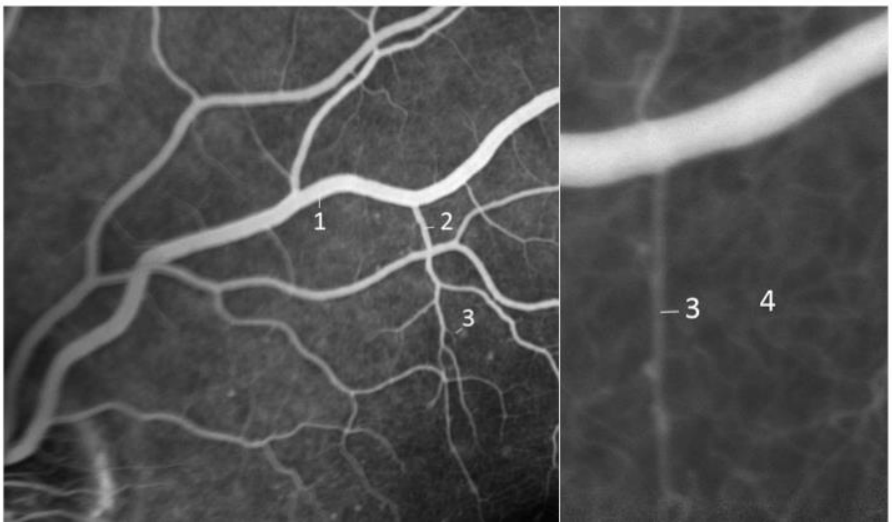

Figure 3. Grading scheme of retinal vasculature into sub-branches of progressively smaller caliber

Results

A total of 10 participants were enrolled in the study, with a mean age of 47.5 years (range 27 – 77) with half being male (n=5, 50%). The demographic features and diagnosis for each participant are listed in Table 1. All patients tolerated the imaging well.

High-resolution Fluorescein and Indocyanine Green Angiography

In contrast to the standard images, the high-resolution images demonstrated greater visualization of smaller caliber vessels including its sub-branches (mean 1.2 greater sub-branches visible, median of 1 level, range 0-2) (Table 2). In the participant with

FFA performed on three different FA devices, the highest resolution, as determined by visibility of sub-branches, was seen in the modified HRA2 device, followed by the Topcon TRC-NW8 then the conventional HRA2 (Figure 4). Similarly, high resolution ICGA demonstrated visualization of smaller caliber branches with greater appreciation of the spatial relationship between vessels (Figure 5 and 6). Across all participants imaged using the high-resolution device however, there were variations in the clarity and fluorescence strength of the images obtained. In the first patient imaged, the HRA2 image was of poor quality and ungradable, likely due to delayed timing of the imaging. Earlier onset of angiography imaging immediately after dye injection produced better quality images of the capillary network, compared to when imaging was delayed by > 10 minutes.

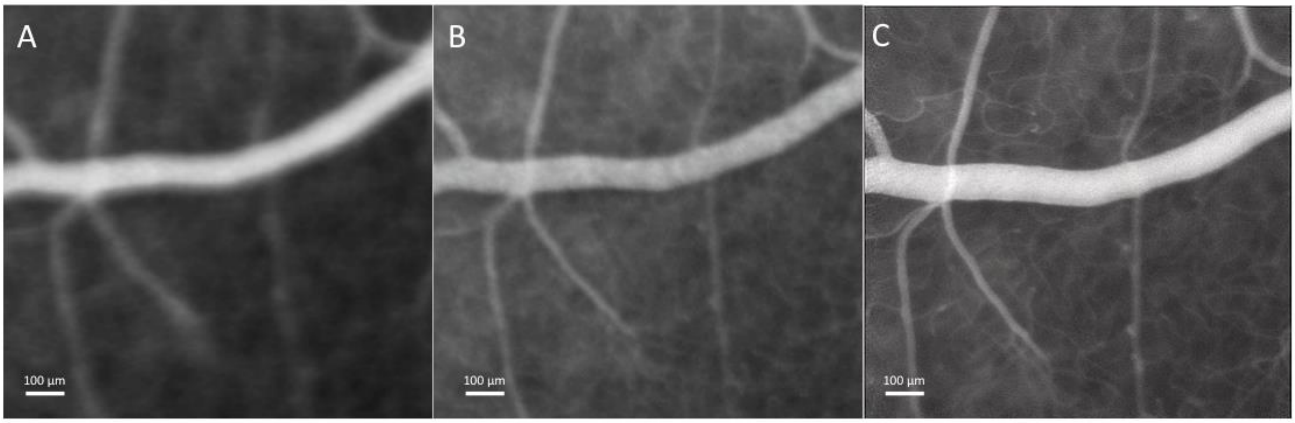

Figure 4. Comparison of enlarged conventional fundus fluorescein angiography (FFA) on Heidelberg retina angiography 2 (HRA) (A), Topcon TRC-NW8 (B) and high-resolution HRA2 (C) in the same location

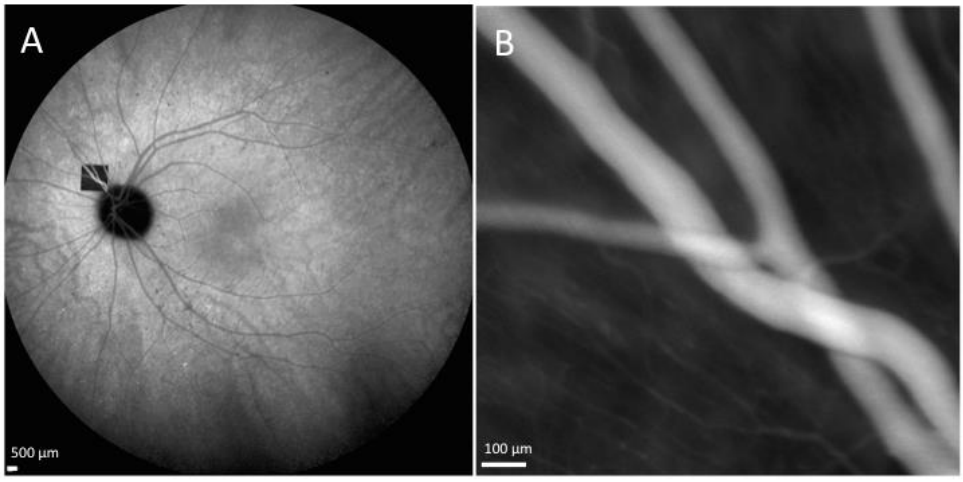

Figure 5. Localization (A) and (B) early phase high-resolution indocyanine green angiography

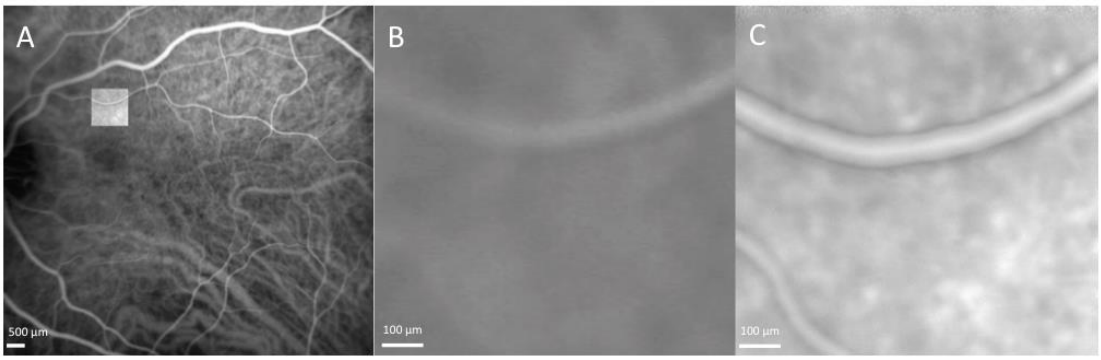

Figure 6. Localization and scaling of conventional indocyanine green angiography image (A) with enlarged conventional image (B) and high-resolution image (C)

Aside from capturing static images, the HRA2 can also be employed to take videos of the retinal vasculature in real time. No differences were seen on the videography setting when performing the high-resolution FFA or ICGA as compared to the static images. However, when the modified device was set to continuous infrared mode, flow of blood cells was discernible, visualized as continuous movement within the retinal circulation (supplementary material video file).

Photoreceptor Mosaic Imaging and Analysis

High-resolution images of the photoreceptor mosaic were also acquired using the infrared and blue reflectance setting. Imaging of the cone photoreceptors was resolved to a level sufficient for manual cone counting in both modalities (Figure 7, cone density 3964 cells/mm^2 including blood vessels, and 4695 cells/mm^2 accounting for blood vessels, hexagonal percentage 6273%, 39.6 µm spacing). Assessment of the photoreceptor density could also be obtained around microvascular structures of interest, such as a large microaneurysm in a diabetic patient (Figure 8, cone density 6397 cells/mm^2 without blood vessels, hexagonal percentage 10164%, 36.8 µm spacing). The device could be easily switched from infrared to angiography mode to provide an estimate of the cone density relative to perfusion in a given location (Figure 9, cone density 4846 cells/mm^2 without blood vessels, hexagonal percentage 6521%, 40.3 µm spacing).

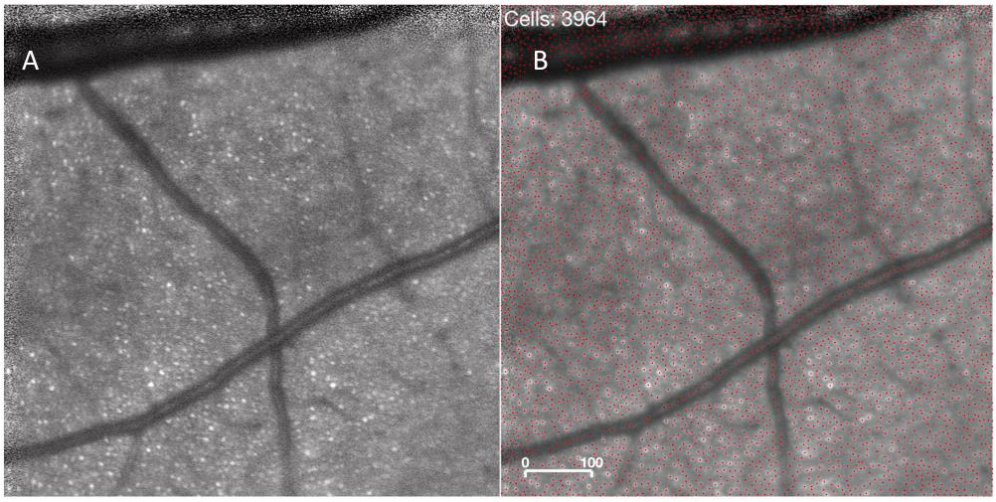

Figure 7. (A) High-resolution infrared image focused at the level of photoreceptor mosaic. (B) Automated assessment of cone density. Note few cones were detected within vessels and were manually excluded from the overall count (manual correction 4695 cells/mm^2).

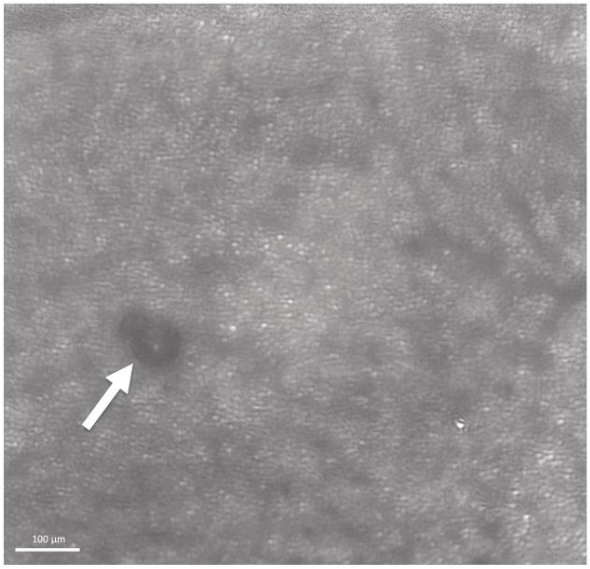

Figure 8. High resolution infrared image of a patient with diabetic retinopathy demonstrating large microaneurysm (arrow) and adjacent photoreceptor mosaic (cone density 6397 cells/mm^2 excluding blood vessels)

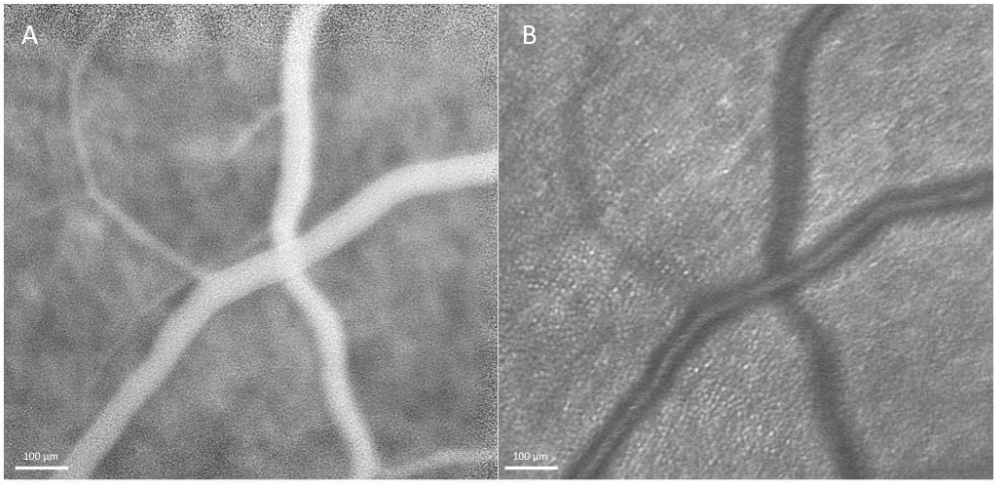

Figure 9. High-resolution fluorescein angiography images (A) and infrared imaging of photoreceptor mosaic (B) at the same location (cone density 4846 cells/mm^2 excluding blood vessels)

Imaging of Choriocapillaris

In addition to standard angiography, the modified device also enabled imaging of the choriocapillaris layer by using the IRAF setting with ICG dye in situ. (Figure 10).

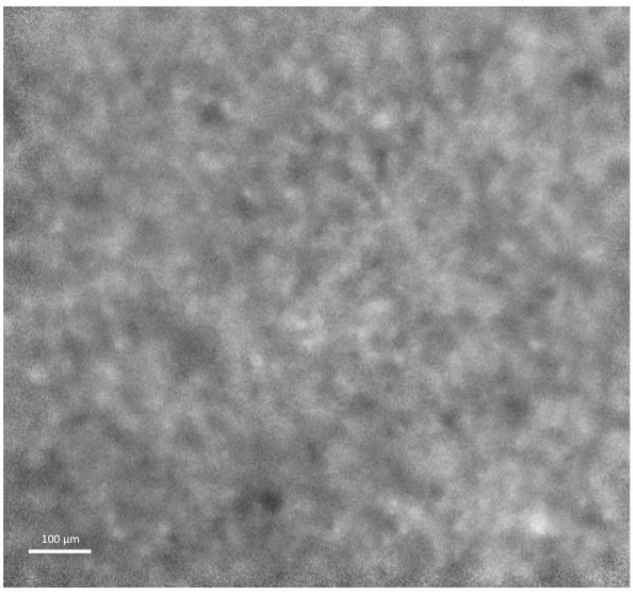

Figure 10. Imaging of choriocapillaris layer with high-resolution indocyanine green angiography

Discussion

This is the first study to demonstrate that high-resolution *in vivo* fundus angiography is possible without the need for complex adaptive optics imaging systems. Fine retinal capillaries and its sub-branches were visualized with the narrow angle modification and there was improved spatial resolution of larger vessels on FFA and ICGA compared to conventional images. The system was also capable of demonstrating the topographic relationship between vessels and the photoreceptor mosaic on a single device, with cone density counts comparable to levels previously reported on AOSLO and on histology.[13,14] In addition, the videographic capabilities of the modified HRA2 system was also able to capture, movement of blood cells through the retinal circulation in real time.

Our results are comparable to those obtained using AOSLO FA. In a study by Pinhas et al., FFA was performed using confocal AOSLO in ten healthy subjects given oral or intravenous fluorescein dye.[7] Compared to conventional FFA, confocal AOLSO

FA demonstrated increased transverse and axial resolution and was able to resolve details of the retinal capillary bed and its sub-branches. Although we did not perform AOSLO FA in our subjects, retinal images acquired using the modified HRA2 device in this study had potential resolution down to the same capillary level as seen in the confocal AOSLO FA report.[7]

The ability to resolve fine retinal capillary details without the use of AO systems represents a significant advantage. Image acquisition using the high-resolution device is fast and can be viewed immediately without the need for complex image processing. In addition, the device is a modification of a conventional HRA2, a platform already familiar to many technicians, thus facilitating ease of use. It can also potentially be used to image a wider spectrum of patients including those with small pupils. However, both AOSLO FA and the modified HRA2 require oral or intravenous dye administration to view the retinal vasculature.

Optical coherence tomography angiography is another imaging modality that can provide high resolution images of the retinal vasculature. It does not require dye injection and as it is non-invasive, can be repeated at same or multiple visits with good reproducibility.[15] Although limited in its field of view, newer models in development are utilizing montaging protocols to allow wider field images of up to 70 degrees.[16,17] Compared to traditional dye based angiography such as this however, its main disadvantage is its inability to demonstrate vascular leakage or areas of slow flow.

Limitations of this study include the small number of patients and the variation in the start time and fluorescence signal of the high-resolution FA due to the need to move patients from one device to another. In order to avoid subjecting patients to repeat angiograms, early phase images were only captured on one device at a time. A greater difference between the two devices may have been obtained if all the high-resolution imaging was performed in the early phase. There were also technical challenges in the reproducibility of the quality of the images. In addition, although the modified device provided improved visualization of smaller capillaries, the reduced field of view led to difficulties localizing where the image was being taken at the exact time of imaging. Montaging the images may help recreate a larger field of view; future modifications to the system to enable switching from the conventional to high-

resolution scanning angle within the same device would also be desirable to allow rapid examination of targeted features. However, as an exploratory study, the results of this study suggest the potential level of detail that can be obtained. Further studies, including early phase high-resolution FFA in all patients as compared with either OCTA or FA AOSLO, and dedicated imaging of pathology such as microaneurysms or neovascularization will help clarify the scope of this technology.

The study of microvascular abnormalities remains vital to understanding the pathobiology of retinal vascular diseases. Advances in imaging technology such as this, which allow detailed in vivo assessment of the vascular network simultaneously with the surrounding neuroretina, has the potential to identify earlier manifestations of a disease and improve our understanding of the relationship between photoreceptors and retinal vasculature. This is important, not only for diagnostic purposes, but may have a role in phenotyping patients for novel future therapies. In addition, the ability to assess photoreceptor density alongside vascular abnormalities can provide insight into the relationship between the two, an important factor in determining the timing of intervention needed for disease prevention. This high-resolution FA technology provides a promising avenue to better understand microvascular changes in both health and disease.

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Tables

Table 1. Clinical characteristics of participants

Participant	Age (years)	Gender	Right BCVA (Snellen)	Left BCVA (Snellen)	Diagnosis
1	68	F	6/6	6/7.5	Macular telangiectasia type 2
2	36	M	6/4	6/4	Central serous chorioretinopathy
3	58	M	6/9.5	6/8	Age related macular degeneration
4	52	F	6/5	6/6	Macular telangiectasia type 2
5	56	F	6/5	6/5	Proliferative diabetic retinopathy
6	38	M	6/5	6/12	Central serous chorioretinopathy
7	77	F	6/7.5	6/9	Macular telangiectasia type 2
8	30	F	6/6	6/6	Healthy control
9	33	M	6/6	6.6	Healthy control
10	27	M	6/6	6/6	Healthy control

F = Female; M = Male; BCVA = best-corrected visual acuity

Table 2. Comparison of retinal vascular tree sub-branches visible between conventional and high-resolution fundus angiography

Participant	Conventional Image	High-resolution Image	Difference
1	2	Poor quality	Not gradable
2	2	2	0
3	2	3	+1
4	3	4	+1
5	1	2	+2
6	2	4	+2
7	2	2	0
8	2	4	+2
9	2	4	+2
10	3	4	+1