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Fine structure in diabetic retinopathy lesions as observed by adaptive optics imaging. A qualitative study

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ABSTRACT.

Purpose: **Diabetic retinopathy is diagnosed by fundus photography and optical coherence tomography (OCT) scanning. However, adaptive optics (AO) imaging can be expected to add new aspects to the knowledge of diabetic retinopathy because photographic resolution is improved by reducing the influence of optical aberrations on retinal imaging.**

Methods: **Nineteen patients with diabetes mellitus were subjected to fundus photography, OCT scanning and AO imaging. The fundus photographs were scaled to the same magnification as that of the AO image, and qualitative aspects of AO images of each retinopathy lesion observed on fundus photographs and OCT scans were assessed.**

Results: **All red lesions on fundus photographs appeared on AO images as dark hyporeflective elements, but it could not be verified whether lesions represented haemorrhages or microaneurysms. The smallest of these lesions were circular with a size corresponding to that of blood cells. Hard exudates had irregular surfaces with buddings of various sizes protruding from the lesions. Areas of retinal oedema observed by fundus imaging and OCT scanning resulted in blurring of AO images, but cystoid spaces observed by OCT could be seen on AO images to have a sharp delimitation with a darker hyporeflective rim at the internal lining of the cyst wall.**

Conclusion: **AO imaging may potentially assist in detecting diabetic retinopathy at an earlier stage, may help elucidating the pathophysiology of the diseases and may be used for evaluating the effects of clinical interventions on diabetic retinopathy and other retinal vascular diseases.**

Key words: adaptive optics imaging – diabetic retinopathy – haemorrhages – hard exudates – leucocytes

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Introduction

Diabetic retinopathy is characterized by morphological lesions in the retina secondary to impairment of retinal vascular supply (Curtis et al. 2008). The severity and type of retinopathy depends on the type, the number and the location of retinal lesions, which is in daily clinical practise evaluated by direct inspection of the retina through the optics of the eye (Klein et al. 1986; Aldington et al. 1995; Bek 2013) and by a quantification of retinal oedema by optical coherence tomography (OCT) scanning (Buabud et al. 2010; Vujosevic & Midena 2013). However, these imaging modalities are limited by imperfections in the optical components of the eye (Bek 1998; Bernardes et al. 2011), which may hinder the detection of retinal lesions of small size and lesions with low contrast to the surrounding retina. In diabetic retinopathy, the detection of such lesions might potentially be important for discovering diabetic retinopathy in its earliest stages, to study progression of retinal lesions in more detail and to shed new light on the pathophysiology of the disease.

Adaptive optics (AO) imaging is a newer technique that allows the detection of retinal structures at the cellular level beyond the resolution of normal fundus inspection and OCT scanning. The high resolution can be obtained because the image of the ocular fundus is corrected for the influence of aberrations generated during passage through the optics of the eye (Zhang et al. 2005; Ramaswamy & Devaney 2013). Recently, AO imaging has been used to study retinal capillaries in diabetic retinopathy (Tam et al. 2012; Lombardo et al. 2013), but there is lack of knowledge about the appearance of other diabetic retinopathy changes as observed by AO imaging and their correlation with existing imaging modalities.

The purpose of this study was to describe qualitative aspects of changes observed by AO scanning in patients with diabetic retinopathy and to correlate these changes with the corresponding retinal morphology observed by fundus imaging and OCT scanning.

Materials and Methods

Patients

Nineteen patients with diabetes mellitus (14 males and five females) successively referred to the Department of Ophthalmology, Aarhus University Hospital for the evaluation of possible diabetic macular oedema were studied.

Examination procedure

The persons underwent a routine examination in the department's clinic for diabetic retinopathy. The name, birth date, personal identification number, and previous treatment for diabetic retinopathy or other eye diseases were noted, and the patients were asked about the time of diagnosis, age of onset and treatment of diabetes mellitus. The weight and height were measured using a digital weight with a telescopic height measure (SECA, Hamburg, Germany). The diagnosis of diabetes mellitus had been made by a diabetologist, and the patients were classified as having type 1 diabetes mellitus (T1D) if the age of onset was below 30 years, or if the age of onset was between 30 and 40 years, insulin treatment had been commenced within 1 year after the onset of diabetes mellitus and the body mass index was below 25. The remaining patients were classified as having type 2 diabetes mellitus (T2D). Information about the most recent measurement of HbA1c (within the preceding 3 months) and plasma glucose (within the preceding week) was obtained from the electronic database generated by the central laboratory at the University Hospital. The clinical background data of the patients are shown in Table 1.

The ophthalmological examination included the measurement of best corrected visual acuity on ETDRS charts, slit lamp examination and pneumotonometry (Nidek Tonoref *II*, Gamagori, Japan), and dilatation of the pupil was induced with phenylephrine 10% (SAD, Copenhagen, Denmark) and tropicamide 1% (Alcon, Rødovre, Denmark). After 15 min of rest, the blood pressure was measured using an electronic sphygmomanometer (Omron M4, HEM-722c1-E), and two 60 degrees images were taken using a Canon CF 60Z fundus camera (Canon, Amstelveen, Holland), one centred on the fovea and another nasally displaced image centred on the optic disc. Mean and range of best corrected visual acuity transferred to decimal values were (0.8, 0.2–1.7) on the right eye and (1.0, 0.3–1.7) on the left eye.

Optical coherence tomography (OCT) scanning was performed using the Heidelberg Spectralis version 5.4.7 (Heidelberg Engineering, Heidelberg, Germany) using the IR & OCT 30 degrees ART examination procedure, which includes a 30 degrees infrared fundus image centred on the fovea and an array of 19 horizontal OCT scans, each with a length of 20 degrees and spaced with a vertical interval of 0.8 degrees.

Diabetic retinopathy lesions were identified by the author on the basis of fundus photographs and biomicroscopy followed by evaluation of optical coherence tomography scans in the macular area. All patients had mild or moderate non-proliferative diabetic retinopathy on both eyes.

Adaptive optics imaging

Adaptive optics (AO) imaging was performed and the images processed using the rtx1 Adaptive Optics Camera (Imagine Eyes, Orsay, France). This camera consists of a Hartmann-Shack aberrometer that records small aberrations during imaging of the ocular fundus through the optics of the eye, and an adjustable mirror that corrects the disturbances in the retinal image caused by these aberrations (Lombardo et al. 2012).

Examination

The examinations were carried out in both eyes of all patients, resulting in the study of 38 eyes. The patients were seated in front of the camera and were asked to look at a fixation cross in the

Table 1. The background data of the studied patients at the time of examination. Non-available data are indicated with a hyphen.

Patient	Diabetes type	Treatment insulin (i) or peroral (o)	Age of onset (years)	Known diabetes duration (years)	Diastolic blood pressure (mmHg)	Systolic blood pressure (mmHg)	HbA1c (%)	Plasma glucose (mM)	Body mass index
1	2	i	52	17	82	140	8.2	12.9	32
2	2	i	46	17	83	151	9.5	12.5	36
3	2	o	53	2	75	136	6.7	8.1	27
4	2	i	44	11	86	157	9.5	12.5	32
5	1	i	7	48	74	127	9.2	12.0	28
6	2	i	42	11	76	149	8.3	10.6	33
7	2	i	41	12	87	140	–	15.3	21
8	2	o	37	13	74	102	7.3	12.9	24
9	2	i	40	10	70	136	8.3	–	22
10	2	i	37	11	81	139	9.6	–	27
11	1	i	28	19	100	157	8.5	8.9	33
12	2	i	46	0	96	141	9.5	20.4	23
13	1	i	12	30	91	137	9.0	15.1	33
14	2	i	38	2	90	135	6.6	–	53
15	2	i	31	10	74	129	11.4	19.7	22
16	1	i	16	12	95	145	8.2	–	28
17	1	i	28	11	106	154	–	–	36
18	1	i	4	33	91	151	8.9	33.0	59
19	1	i	13	23	82	121	8.8	–	25

eye piece of the apparatus. The fixation cross was moved so that the 4 × 4 degrees imaging field included red dots (haemorrhages and/or microaneurysms), and if present, hard exudates and/or macular oedema. Additionally, a recording was always performed with the field centred vertically and displaced 2 degrees temporally with the fixation point at the nasal border of the imaging field. The retina was studied from a plane where the photoreceptors was in focus and with the image plane successively moved with ten microns intervals through the retina to reach the inner retinal surface. The photoreceptors and the inner retinal surface as well as discernible structures located in between these limits were recorded and stored for the subsequent analysis. During a recording, the apparatus discarded images obtained during blinking and eye movements, and sampling of images lasted until a sufficient number of images for the adaptive optics correction had been obtained.

All examinations were part of the routine procedures for the evaluation of diabetic retinopathy in the clinic at the time and therefore did not require approval by the local committee for scientific ethics. However, otherwise the study adhered to the tenets of the declaration of Helsinki, and the patients had given their consent to participate according to the Danish legislation.

Data analysis

The fundus photographs obtained from the Heidelberg Spectralis were cropped (Microsoft Image Office Picture Manager; Microsoft, Palo Alto, CA, USA) and scaled (Microsoft Office Power Point 2007; Microsoft) to the same magnification as that of the AO image, and on the basis of the vascular tree, the infrared fundus image with the marking of an OCT scanning line was superimposed onto the AO image, and the distance in microns on the OCT scan was transferred to the other imaging modalities. The colour fundus photographs were used to verify that dark lesions on the infrared images were red to represent haemorrhages and/or microaneurysms. Subsequently, the appearance on AO images of each retinopathy lesion observed on fundus photographs and OCT scans was analysed, and features in common for all observed lesions of a given type were noted.

Results

In addition to the dot haemorrhages and/or microaneurysms that were present in all fundus images, fundus imaging and OCT scanning showed hard exudates in eleven patients and retinal oedema in fifteen patients of which three had cystic elements. Cotton wool spots were not detected in any of the patients, and in three of the patients, the presence of hard exudates or oedema could not be confirmed (ETDRSG 1985). There were no differences in the appearance of lesions from patients with type 1 and type 2 diabetes mellitus.

The AO images showed all the red dots or blots observed by fundus imaging and elements blocking OCT scans in the deeper retinal layers as dark hyporeflective lesions, but it was not possible to verify whether lesions represented haemorrhages or microaneurysms. Additionally, in all patients, the AO image showed dark elements that were smaller than what could be resolved by fundus imaging and OCT scanning. The smallest of these lesions were circular with a size corresponding to both leucocytes (diameter approximately 20 microns) and erythrocytes (diameter approximately 7 microns). These elements were most frequently observed at the retinal surface, but were also arranged in arrays to suggest plugging in a small retinal vessel (Fig. 1).

All hard exudates observed by fundus imaging and OCT scanning were also observed by AO imaging, but the lesions had a heterogenous reflection pattern consisting of interchanging dark and white areas. In high resolution, the hard exudates had an irregular surface with buddings of various sizes protruding from the lesions (Fig. 2).

Areas of retinal oedema observed by fundus imaging and OCT scanning appeared to produce blurring of the

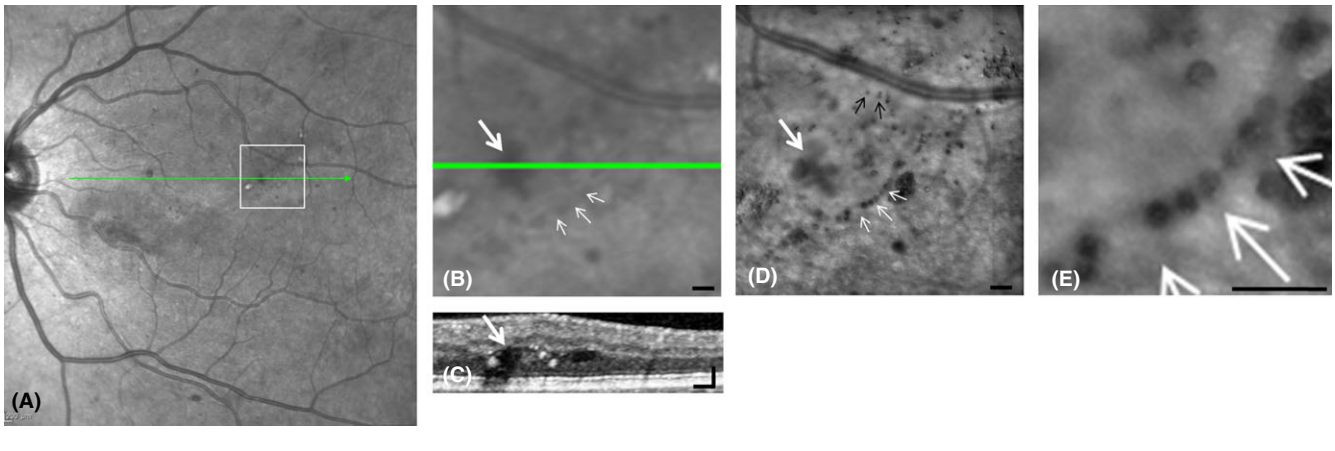

Fig. 1. (A) Fundus image from the left eye of a patient with diabetic maculopathy characterized by hard exudates. (B) Higher magnification of the area delimited in (A). Large white arrow points to a retinal haemorrhage, whereas smaller white arrows point to an unsharply delimited dark line. (C) Optical coherence tomography (OCT) scan corresponding to the green line in (A) and (B) showing that the haemorrhage is located deeply in the retina. (D) Adaptive optics image corresponding to B resolves retinal lesions of much smaller size than the fundus image. (E) Magnification ×4 of (D) showing that the dark line marked by the three white arrows represent an array of cells with a diameter of approximately 20 microns. Smaller cell-like elements with the diameter of approximately 7 microns (black arrows) are observed near the arteriole traversing the image horizontally. Vertical bar on OCT scans and all horizontal bars correspond to 100 microns.

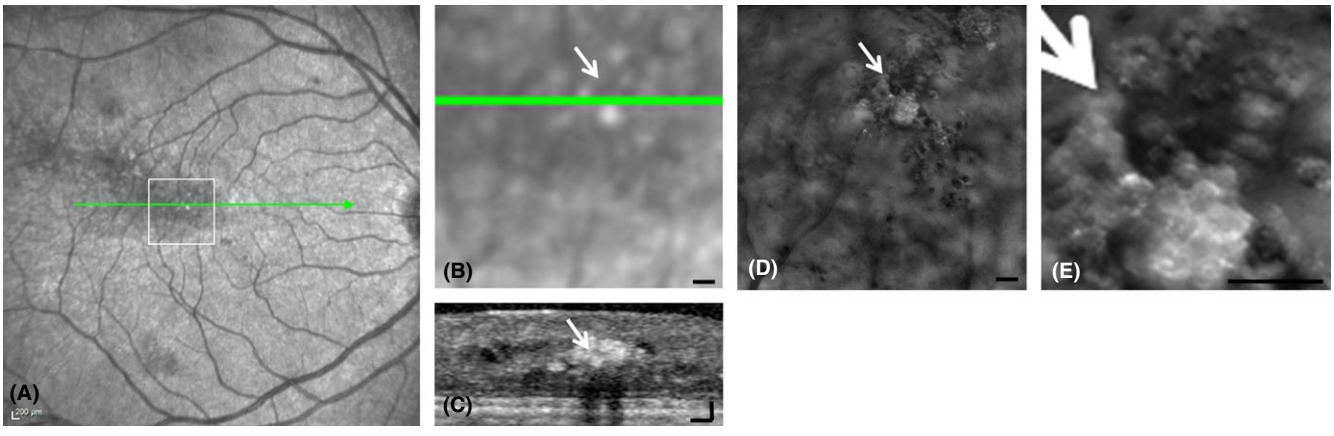

Fig. 2. (A) Fundus image from the right eye of a patient with diabetic maculopathy characterized by hard exudates. (B) Higher magnification of the area delimited in (A). Large arrow points to a hard exudate. (C) Optical coherence tomography (OCT) scan corresponding to the green line in (A) and (B) showing that the exudate is located in the middle retinal layers. (D) Adaptive optics image corresponding to B resolves the surface of the exudate in more detail than the fundus image. (E) Magnification ×4 of (D) showing that the surface of the exudate consists of numerous buddings of different size protruding from the surface of the lesions. Vertical bar on OCT scans and all horizontal bars correspond to 100 microns.

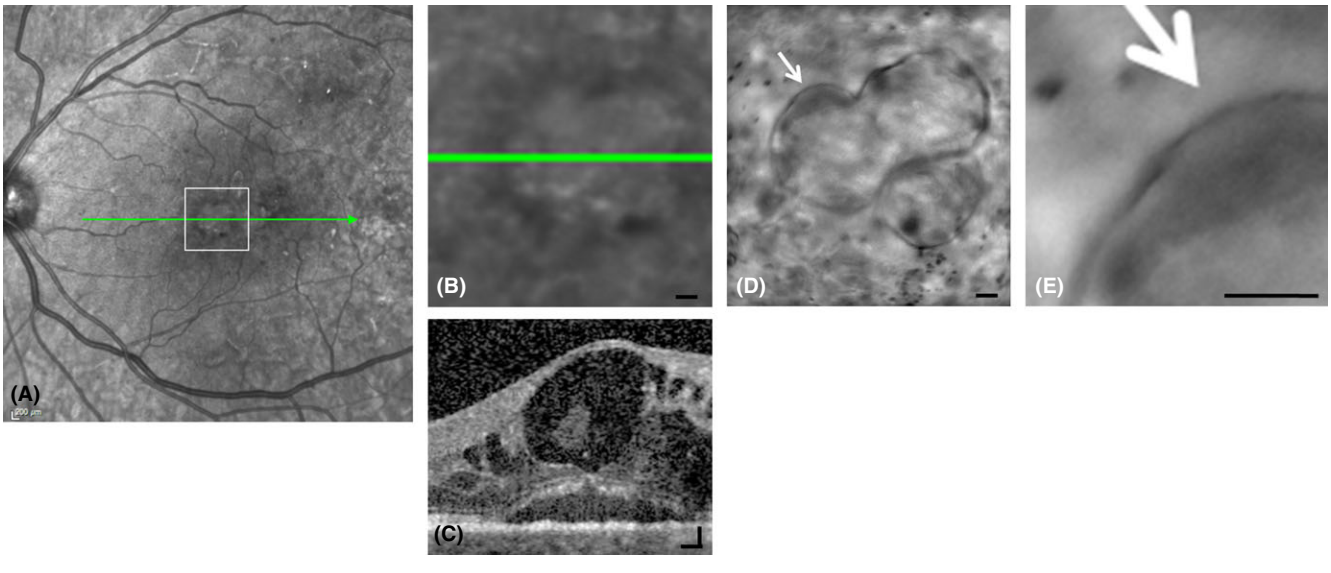

Fig. 3. (A) Fundus image from the left eye of a patient with diabetic maculopathy characterized by hard exudates and retinal oedema. (B) Higher magnification of the area delimited in (A). (C) Optical coherence tomography (OCT) scan corresponding to the green line in (A) and (B) showing cystic retinal oedema. (D) Adaptive optics image showing a sharp delimitation of the walls of the cysts with a different representation than in the fundus image and the OCT scan. (E) Magnification ×4 of (D) showing that the oedematous areas on each side of the cyst wall appear blurred, but with a dark rim at the inner side of the wall. Vertical bar on OCT scans and all horizontal bars correspond to 100 microns.

retinal image, but cystoid spaces observed by OCT could be seen on AO images to have a sharp delimitation with a darker hyporeflective rim at the internal aspect of the cyst wall (Fig. 3).

Discussion

The present study is the first to report qualitative aspects of the microstructure of extravascular diabetic retinopathy lesions *in vivo* using adaptive optics (AO) imaging, which allows a better resolution of retinal structures than what can be obtained by ordinary fundus photography and by optical coherence tomography (OCT) scanning. The reported features could not be subjected to a quantitative analysis, but were common for all lesions of a given type. Using a binomial distribution, it can be calculated that at least six identical observations are needed for the observation of a given qualitative feature to be significant, which was fulfilled for the features characterizing retinal haemorrhages and hard exudates.

The high quality of AO images is achieved by eliminating the influence of aberrations in the optics of the eye to result in a much higher resolution of details at the retinal plane (Gocho et al. 2013; Ramaswamy & Devaney 2013). The studied patients had been referred successively and therefore also included cases with other risk factors for the development of diabetic retinopathy, such as arterial hypertension. However, it is unlikely that this had invalidated the conclusions as AO imaging was performed temporal from the fovea from where diabetic retinopathy lesions tend to develop and become most pronounced (Taylor &

Dobree 1970; Bek & Helgesen 2001; Hove et al. 2006). The identification of haemorrhages as dark hyporeflective elements on AO images confirmed that the constituents of these lesions absorb light and the lesions can be located in all retinal layers. The fact that AO imaging was able to demonstrate haemorrhage-like lesions that were smaller than what could be resolved by fundus imaging indicates that this imaging modality might be more sensitive in detecting the initial lesions developing in diabetic retinopathy. In addition, the observation of elements resembling individual blood cells with the size of both erytrocytes and leucocytes indicates that the mechanisms that are active when these cells escape from retinal vessels in diabetic retinopathy may involve features that can only be studied *in vivo* at the high resolution that can be achieved by AO imaging. The lack of identification of microaneurysms might be coincidental, but might also be due to the fact that blood cells inside these lesions had been packed to the same density as that of retinal haemorrhages, or had been in motion as part of the blood flow. The sampling of images from all retinal layers ensured that all studied lesions were in focus; therefore, it is less likely that the lack of detection of microaneurysms is due to inaccurate focussing. A more detailed evaluation of whether AO imaging allows the detection of microaneurysms requires the inclusion of fluorescein angiography that enables the differentiation of these lesions from haemorrhages (Baudoin et al. 1983). The sampling of a sufficient number of AO images to allow the correction for aberrations takes several seconds, which precludes the resolution of formed elements in the flowing blood (Uji et al. 2012). Therefore, the observation of rows of cell-like structures most likely represents retinal vessels packed with non-flowing blood cells. Whether this implies that the discontinuation of the blood flow in these vessels has been intermittent (Bek 1999) or permanent (Bek 1994) remains to be elucidated. The size of the cellular elements in these vessels of approximately 20 microns suggests that the structures represent leucocytes and supports the notion that leucostasis may be involved in the pathophysiology of diabetic retinopathy (Patel 2009). If the observation reflects occlusion that has become permanent, it may represent the stage that precedes the invasion of Müller cells that can be observed in areas of retinal vascular occlusion observed in diabetic retinopathy post-mortem (Bek 1997a,b). The findings indicate that AO imaging might be a suitable technique for differentiating vessels with flowing blood from vessels in which the blood flow has arrested, and thereby a technique for studying intermittent properties of blood flow in the retinal microcirculation (Bek et al. 2013). Additionally, the finding of cellular elements with the size of leucocytes dispersed at the surface of the retina supports the notion that the development of diabetic retinopathy can involve an inflammatory response (Tang & Kern 2011) and may explain why corticosteroids can reduce diabetic macular oedema transiently (Stewart 2012).

The hard exudates observed on fundus images corresponded to whitish areas containing both hyper-reflective whitish and hyporeflective dark elements probably representing heterogeneity of the composition of the lesions. In high resolution, the surface of the hard exudates appeared irregular with buddings of various sizes, which might represent the sites where plasma proteins are added and resorbed during the dynamic changes of these lesions, and may be related to the hyper-reflective foci that have been suggested to represent early stages of hard exudate formation (Bolz et al. 2009). Therefore, it is possible that a more detailed study of the dynamics of these surface structures might contribute to elucidating the mechanisms involved in the formation and resolution of hard exudates and the accompanying retinal oedema in diabetic retinopathy (Chew 1997; Bek 2011).

All cases of retinal oedema seen by OCT scanning induced blurring of the AO image in all layers below the retinal surface, including the photoreceptor layer. This implies that the adaptive optics had been unable to adjust for the imperfections in the imaging imposed by the passage of light through the oedematous retina and points to limitations for the study of retinal diseases characterized by tissue oedema, such as diabetic maculopathy. This is opposed to adequate adaptive optics imaging in retinoschisis where the retina is split but is less swollen (Duncan et al. 2011). In the present study, the sharp delimitation of retinal cysts and the dark rim inside the cyst walls probably reflected optical properties in the internal lining of these cysts. It is likely that a further exploration of the nature of these optical properties of cystic lesions may help explaining disturbances in central vision in diabetic maculopathy other than a reduction of visual acuity (Klein & Klein 1990; Rodgers et al. 2009).

Altogether, the study suggests that AO imaging may become a tool to open new gates to the understanding diabetic retinopathy. The method may potentially contribute to the detection of diabetic retinopathy at an earlier stage than what is possible with current techniques and may help explaining the pathophysiology of the disease. This may be achieved in prospective observational studies of the development and resolution of retinal haemorrhages, vascular occlusion and hard exudates. Finally, the technique might potentially contribute to an evaluation of the therapeutic effects of clinical intervention on pathological cellular responses in the retina in diabetic retinopathy and other retinal vascular diseases.

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