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Imaging in Diabetic Retinopathy: A Review of Current and Future Techniques

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Imaging in diabetic retinopathy: A review of current and future techniques

Sonul Gajree[*1], Shyamanga Borooah[2] and Baljean Dhillon[2]

[1]*Royal Infirmary of Edinburgh, University of Edinburgh, Edinburgh, Scotland;* [2]*Princess Alexandra Eye Pavilion, University of Edinburgh, Edinburgh, Scotland*

Abstract:

Background
Diabetic eye disease is the most common cause of blindness worldwide in the population under 65 years of age. The prevalence of sight-threatening diabetic eye disease continues to rise rapidly, resulting in an increasing burden on health systems worldwide. This highlights the need to develop new tools to help in the screening, diagnosis and management of diabetic eye disease.

Purpose
This review aims to provide a brief overview of the current standard in care for diabetic eye disease, before providing an up to date overview of newer imaging modalities, with potential application in the management of diabetic eye care.

Methods
A literature search for the terms "enhanced depth imaging OCT", "swept source OCT", "retinal oximetry", "OCT angiography", "fundus autofluorescence" with the term "diabetes" was performed using the pubmed and google scholar databases. Only articles published within the last two years were selected for use in this article.

Discussion
There has been a rapid increase in the available imaging techniques used to manage diabetic eye disease. To date there has been variable use of these next generation imaging techniques. A greater understanding of how phenotypic findings link to the risk of sight loss is required before there is more widespread adoption by mainstream diabetic eye services.

Introduction

Diabetes mellitus is a source of major morbidity and mortality around the world and is rapidly increasing in prevalence. The International Diabetes Federation (IDF) estimates that 387 million people worldwide suffer from diabetes, a figure expected to increase by over 200 million in the next 20 years [1]. The increase in incidence of type 2 diabetes is particularly dramatic, with an increasing life expectancy combined with sedentary lifestyles and an obesity epidemic driving this growth [2].

The increasing number of diabetics has led to a corresponding increase in the number of patients affected by complications secondary to diabetes which include diabetic retinopathy. The increasing prevalence of diabetes will place increasing strain on an already stretched health service, both in the United Kingdom (UK) and worldwide. This highlights the need to use new and more efficient ways to both investigate and manage diabetic patients as outlined in this review.

Diabetic retinopathy (DR) is a chronic and progressive disease affecting the microvasculature of the retina, and is the most common microvascular complication of diabetes. Several studies have demonstrated that nearly all type 1 diabetics, and a large proportion (more than 60%) of type 2 diabetics, will have some degree of retinopathy twenty years following diagnosis [3,4]. Although not all of these patients will suffer significant visual disturbance, diabetic retinopathy remains the leading cause of blindness in the working population of developed countries [3].

Diabetic retinopathy is asymptomatic in its early stages. By the time visual disturbance is detected, substantial and irreversible pathology may have already developed. Many classification systems exist, but broadly speaking DR can be classified using two main features – the presence or absence of new vessel formation (**proliferative** or **non-proliferative diabetic retinopathy** respectively), and the presence or absence of sub foveal macular oedema (**diabetic macular oedema**). The presence of these clinical features allows the ophthalmologist to ascertain the risk of imminent visual impairment, as well as that of future visual loss [5].

There are a growing number of imaging modalities which can be used in the screening, evaluation, diagnosis and treatment of diabetic retinopathy. This review article aims to provide a brief overview of the current standard in care for DR and diabetic macular oedema (DMO), before providing an up to date overview of newer imaging techniques and how they can best be used in the management of this important condition.

Fundus Photography and Diabetic Retinopathy Screening

With the rapidly increasing prevalence of diabetes and the potential sight threatening consequences of untreated diabetic retinopathy, health boards across the United Kingdom recommend a thorough screening programme in order to detect asymptomatic individuals with retinopathy at an early stage of the disease.

The UK National Screening committee recommends that everybody with diabetes, aged 12 or over, should be invited to diabetic eye screening once a year [5,6]. There are some regional differences in the screening programme, however they are all based upon **digital fundus photography**. Digital photography allows a clear and accurate image of the interior surface of the eye, and is relatively easy and cost effective to perform with little discomfort to the patient. With the advent of digital fundus photography, high quality digital colour images of the retina, retinal vessels, optic disc and macula can be obtained quickly, even without dilatation of the pupils [7]. The images are graded for severity of disease by qualified trained graders. Based on the grading of the retinal images, patients are either asked to return annually or are referred on to the local ophthalmology service for further management [8].

Fundus photography is an important imaging technique in the hospital or optometric setting to help monitor disease progression. Digital images also enable immediate review of the images, and give the user the ability to easily adjust or enhance the images to aid diagnosis and ongoing management.

There have been continuing developments in the technology behind fundus photography, and there are now several types which exist.

Currently the most commonly used form of **single field fundus photography** in screening programmes in the UK produces an image of 30º of the posterior pole of the eye, including the macula and the optic nerve [9]. Colour photography is useful for identifying lesions such as hard exudates and cotton wool spots (see **figure 1**), whilst red free imaging can be used to detect other abnormalities [5]. It can be performed, with or without medical dilatation of the pupils, using a fundus camera such as the Canon Cr6-45NM (Canon USA, Lake Success, NY) and is used in screening programmes in the UK because it is widely available and relatively simple and rapid to perform. There is comprehensive evidence that single field photography is an effective initial screening tool for diabetic retinopathy [10], however it has a number of limitations. Single field images do not allow accurate evaluation of retinal thickening or macular oedema, and the periphery of the retina is not visualised in the photograph.

Stereoscopic fundus photography is a method of retinal imaging which involves creating a stereo image of the retina by sequentially taking two images. This allows for examination of the image in three dimensional form, and the added depth perception can help aid the diagnosis of DMO. Its role in clinical practice is controversial however, with *Li et al* showing no difference in reliability between monoscopic and stereoscopic photography in the grading of diabetic retinopathy [13].

The Early Treatment Diabetic Retinopathy Study (ETDRS) group defined the gold standard imaging technique for the detection and classification of diabetic retinopathy as stereoscopic colour fundus photography in 7 standard fields [10,11]. This method of photography allows imaging of the peripheral retina in addition to the central retina and is known as **wide field fundus photography**. It is performed using a traditional fundus camera, which takes multiple images of the posterior fundus from different angles. These can then be viewed separately or stitched together to create a montage view of the fundus, which represents close to a 75° field of view. This allows good visualisation of the mid peripheral retina. Whilst this technique offers a greater field of vision and a high level of accuracy, it is a time consuming process requiring a higher level of operator skill, as well as more complex film processing and thereby a higher cost to run. As it requires a larger number of photographs, it is also less tolerable for the patient. As such, despite its increased image quality, it is not routinely used in the screening of diabetic retinopathy [10].

Newer cameras have been developed recently which can create photographs with an even greater field of vision, described as **ultra-wide field fundus photography.** These include devices such as the Optos (Optos, Dunfermline, UK). This device uses scanning laser ophthalmoscopy and can create ultra-wide field views of up to 200° or more than 80% of the total retinal surface [12]. The greater field of vision is obviously advantageous in the diagnosis and monitoring of pathology in the periphery of the retina. However these ultra-wide field photographs are still limited by availability of the technology and the extra training and resources needed for an operator to be proficient in using such a machine [12].

With the continuing advancement of techniques and equipment, the role of fundus photography in the screening, diagnosis and management of diabetic retinopathy will only increase. It is likely we will see further development in wide field imaging techniques. As costs for devices fall it is also likely that adoption of the technology will become more mainstream.
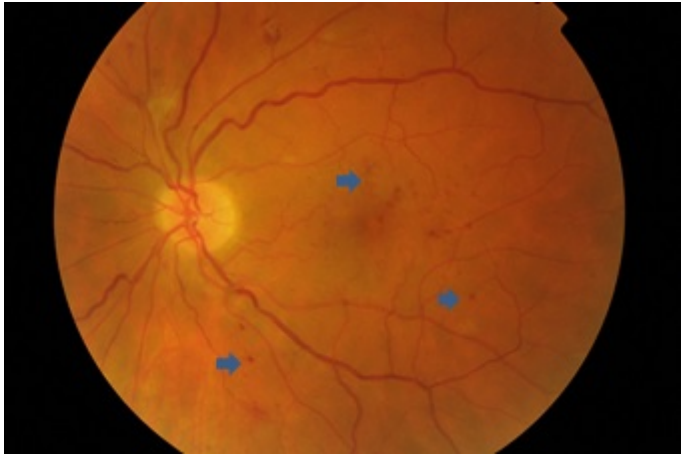
Fig. (1) Fundus photography of the left fundus from a patient with diabetic retinopathy and maculopathy, demonstrating microaneurysms and haemorrhages (blue arrows). Such a patient would be picked up in the screening programme, allowing earlier diagnosis and treatment

Fundus Fluorescein Angiography

Fundus fluorescein angiography (FFA) is an important imaging technique used in the diagnosis and staging of diabetic retinopathy. It remains the gold standard imaging technique for assessing the retinal vasculature and circulation, despite first being introduced by *Novotny and Alvis* as long ago as 1961 [14,15]. Its use is declining, however, with the development of newer imaging modalities which will be described later.

FFA involves the injection of sodium fluorescein into the systemic circulation as a bolus through a peripheral vein. This is a water soluble dye which fluoresces when excited by blue light of wavelength 465 to 490nm [16]. The fluorescein travels quickly in the body's circulation and reaches the arteries of the retina approximately 12 seconds after injection. Over a further short period, the dye passes through the retinal arteries, capillaries and then veins. 10 to 15 minutes following injection, the dye has mostly evacuated from the eye. A series of rapid sequence photographs taken using a fundus camera with appropriate barrier and excitation filters allows the retinal and choroidal circulations to be visualised both as moving images and as stills [17].

The normal progression of the dye is interrupted by diseases affecting the retina, choroid and retinal vasculature. The two main abnormalities in a fluorescein angiogram are **hypofluorescence**, a reduction from the expected level of fluorescence, and **hyperfluorescence**, an increase in the expected level of fluorescence. Hence the fluorescein angiogram of a patient with diabetic retinopathy reveals many irregularities which can be useful in the diagnosis, grading and management of the disease.

Microaneurysms are the most characteristic, although not pathognomonic, lesion of diabetic retinopathy. They are easily detectable on fluorescein angiography (see **figure 2**), appearing as distinct and intense areas of hyperfluorescence [18]. Areas of ischaemia, seen in diabetic retinopathy due to decreased perfusion to retinal capillaries, are identified by patchy areas of hypofluorescence, as these areas cannot fill with fluorescein containing blood [9,18]. FFA is also useful for the identification of neovascularisation, a hallmark of proliferative diabetic retinopathy. The FFA can reveal leakage of new vessels at the optic disc (NVD) or of new vessels elsewhere in the retina (NVE). Additionally in DMO the FFA can highlight areas where there has been a breakdown in the blood retinal barrier, an important identifying feature in DMO.

The identification of such features using FFA is vital in assessing the indications for, and outcomes of, treatment methods such as laser photocoagulation, surgical intervention, and intraocular pharmacological methods (e.g. anti VEGF therapy). Various studies have shown that laser photocoagulation can reduce severe visual loss in patients with neovascularisation or severe non-proliferative retinopathy [6, 19], and FFA plays a crucial role in identifying these patients and monitoring their response to treatment.

With the recent advent of ultra-wide field fundus cameras, the technique of **ultra-wide field fluorescein angiography** is increasing in its use. Ultra-wide field FFA provides greater imaging of the peripheral retina, allowing easier identification of peripheral neovascularisation and retinal ischaemia which would otherwise be difficult to visualise on standard field angiography [20]. This technology allows greater targeting of treatment,

using targeted pattern retinal photocoagulation rather than the traditional pan retinal photocoagulation. The benefit of this is that it targets specific areas of ischaemia whilst sparing better perfused areas from laser scarring [21], thereby reducing some of the complications of pan retinal photocoagulation such as visual field loss.

A pilot study has demonstrated that this technology is effective and has a positive safety profile [21], but the results of full scale clinical trials are still awaited.

FFA is usually well tolerated, however a wide range of complications are possible and should be mentioned. The commonest adverse reactions include nausea and vomiting which occur in less than three percent of patients [22]. More serious adverse events are very rare (<1%), including skin necrosis if there is extravasation of dye into local tissue, anaphylaxis, and myocardial infarction.

As such, despite the advent of newer imaging modalities, fluorescein angiography remains an important tool in the imaging of diabetic retinopathy, and ultra-wide fluorescein angiography will likely have an increasing role in the treatment of the disease in the coming years.
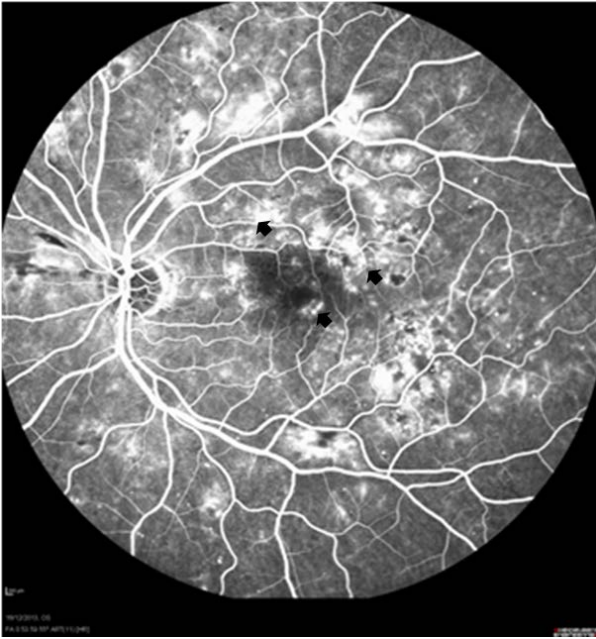
Fig. (2) Venous phase fluorescein angiography of the left eye of a patient with severe non-proliferative diabetic retinopathy and diabetic macular oedema. Areas of ischaemia (patchy areas of hypofluorescence) and leak from capillaries can easily be identified (black arrows). Numerous microaneurysms can also be seen as distinct and intense areas of hyperfluorescence. The macula has an enlarged foveal avascular zone and shows areas of leakage.

Optical Coherence Tomography

The development of **Optical Coherence Tomography (OCT)** has been the most significant advance in DR imaging to have been developed in the last few decades. It was first introduced by *Huang et al* in 1991 [23]. Successive improvements in image acquisition and resolution have revolutionised ophthalmic clinical practice. OCT is a non-invasive imaging modality which can produce a high resolution, cross sectional image of the retina with a fast acquisition time. OCT is particularly useful in identifying and quantifying macular oedema, and is an important tool in guiding the treatment of patients with DMO.

OCT generates a cross sectional image by measuring the time delay and magnitude change of a beam of light entering the eye and being reflected back from the retina, in a way analogous to ultrasonography, using light rather than sound waves to create the image. It is based on a technique known as low-coherence interferometry. Light is emitted from a light source and split into a reference and a sample beam, to obtain a reflectivity versus depth profile of the retina [24]. The light waves which are backscattered from the retina interfere with the reference beam this interference pattern is used to create an image [24]. By combining a series of these axial scans (A-scans) a cross-sectional reconstruction of the retina (B-Scan) can be produced. These scans can be analysed in a variety of methods and using various software tools to allow empirical measurements to be made (e.g. retinal thickness) and qualitative morphological information to be obtained [25].

The technology behind OCT has developed rapidly since its introduction in 1991. The first generation of OCT was known as **Time Domain Optical Coherence Tomography (TD-OCT)**, and was conducted using commercially available OCT systems such as the Stratus OCT (Carl Zeiss Meditec, Inc., Dublin, CA)**.** In this method of OCT, a moving reference mirror is used to generate axial scans. These machines are limited by the speed of the reference mirror, and therefore were limited to scan rates of around 400 axial scans per second, with an axial resolution of around 10 µm [26].

There have been several advances in OCT technology since the first TD-OCT systems became available. The use of broadband light sources in newer OCT systems has increased the axial resolution to 2-3 µm [27], allowing far higher resolution images to be obtained and decreasing the risk of missing pathology. The introduction of a newer generation of OCT, known as **Spectral Domain Optical Coherence Tomography (SD-OCT)**, has allowed a dramatic increase in the possible scanning rates. SD-OCT does not require a movable reference mirror, but instead uses a specially designed high speed spectrometer to simultaneously collect all the frequency signals. A mathematical relationship known as the Fournier transform is then used to create an image [26]. SD-OCT can be conducted using a commercially available machine including the Bioptigen SD-OCT (Bioptigen, Research Triangle Park, NC) or the Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA) which can achieve scan rates of over 20,000 axial scans per second, or the Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany) which can achieve 40,000 axial scans per second, with increased resolution, resulting in a significant advancement from TD-OCT. This allows high quality images to be obtained in a relatively short amount of time.

The images produced by OCT can show a wide variety of pathology in diabetic retinopathy (see **figure 3**). Areas of reduced reflectivity are most often due to intra-retinal or subretinal fluid accumulation. This could be secondary to macular oedema or retinal detachment. Other lesions seen in diabetic retinopathy such as hard exudates, intraretinal haemorrhages and neovascular membranes, all appear as areas of hyper reflectivity [28].

Using OCT images, it is possible to accurately and reliably measure retinal thickness [29], which enables the detection of macular oedema, the main feature of diabetic maculopathy. OCT is particularly useful in determining whether the oedema is centre involving or not, which is vital in assessing which patients may benefit from anti-VEGF therapy (those with centre involving oedema), and which patients would benefit from laser treatment (extrafoveal oedema) [5]. Other findings on OCT in a patient with diabetic maculopathy include intraretinal cystic changes, subretinal fluid, flattening of the foveal depression, and tractional changes [30].

As such, OCT is playing an ever increasing role in the diagnosis of diabetic maculopathy, and in the assessment of treatment outcomes. The technological advances in scanning speeds and image resolutions have made OCT one of the most important imaging techniques in ophthalmology, and it is expected that, with further innovations, the field will continue to grow in the coming years. Some of these innovations will are described below.
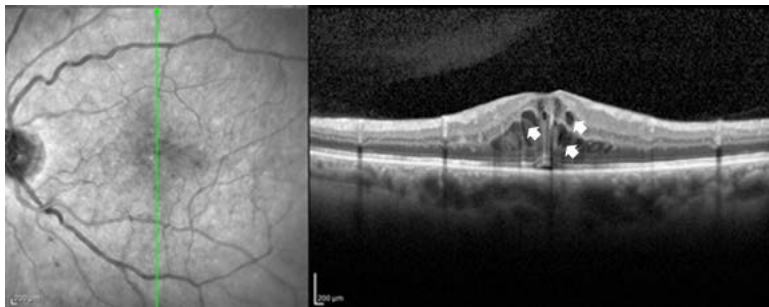

Fig. (3) OCT of the same patient as the fundus photograph in Figure 1. The cross sectional imaging demonstrates the different layers of the retina clearly. Significant macular oedema is observed here as the darker, cystoid spaces (white arrows) where normally the foveal depression would be found. OCT allows accurate quantification of the macular oedema, and can be used to measure response to treatment.

Enhanced Depth Imaging OCT

Current methods of OCT are limited by the difficulty in imaging the choroid, the vascular layer beneath the retina. Several histopathological studies have demonstrated choroidal abnormalities in diabetic eyes [32,33], and choroidal thickness may have future potential as a marker of the severity of the disease [24].

Enhanced Depth Imaging OCT (EDI-OCT) is a relatively recently developed adaptation of spectral domain OCT which allows improved imaging of the choroid. This technique was first described in 2008 by *Spaide et al* [34], and since then numerous studies have demonstrated the utilisation of this imaging technique in various clinical conditions, including diabetic retinopathy [35].

EDI-OCT can be performed with conventional SD-OCT equipment, and many of these devices, such as the Spectralis OCT system (Heidelberg Engineering, Germany), now come with an EDI mode option. Images of the choroid are obtained by positioning the device closer to the eye than in normal practice. This results in an inverted image with the most tightly focused illumination at the level of the choroid [36]. To improve the image and improve the signal to noise ratio, an average of multiple images is taken. This is best accomplished using a device which has eye tracking, to allow the same location to be imaged in multiple scans. An area of 5° by 30° is commonly used, with seven sections each composed from one hundred averaged scans [37].

The resulting image can then be re-inverted to match the orientation of traditional OCT images. EDI-OCT performed in this manner allows good visualisation of the choroid, and using software available with the OCT devices it is possible to accurately and reproducibly measure choroidal thickness [36].

As described, this ability to image the choroid is set to become increasingly important in the management of diabetic retinopathy. EDI-OCT offers a relatively easy way to visualise the choroid and can be performed using existing devices. Using this technique, images of the choroid are now readily available in clinical practice, and if future studies can demonstrate an association between diabetic retinopathy and certain choroidal abnormalities or characteristics, EDI –OCT will play a significant role in the management of these patients.

Swept Source Optical Coherence Tomography

Swept source optical coherence tomography (SS-OCT) is one of the most recent developments in OCT technology, and offers several advantages over the more widely available spectral domain OCT. SS-OCT uses a narrow bandwidth laser light source which sweeps through a broad optical spectrum, and uses a photodetector to detect backscattered light [26]. This is in contrast to SD-OCT which, as described above, uses a broad bandwidth light source, together with a spectrometer and charge coupled device camera to create an image.

Swept source OCT devices, such as the Triton DRI-OCT (Topcon, Japan), offer speed and sensitivity advantages over SD-OCT devices. The scan speed in such an instrument can be well over 100,000 axial scans / second, enabling significantly faster acquisition of images and allowing wide-field imaging, making it possible to view both the optic nerve and the macula on the same single scan [31].

A disadvantage of spectral domain OCT is the difficulty in visualising structures deep to the retinal pigment epithelium due to signal drop off and the scattering of light. Swept source OCT overcomes this by using a photodetector instead of the charge coupled device camera, as well as using a longer wavelength of light (1050 nm vs 840 nm). This allows a greater resolution of images (around 1µm) as well as excellent visualisation of the choroidal layers which is difficult with SD-OCT (see **figure 4**). The longer wavelength used in SS-OCT also has the advantage of overcoming media opacities, such as cataract, when imaging the retina. This is another downfall of older OCT techniques.

Swept Source OCT is not commonly available at present; however, with the increasing understanding of the role of the choroid in retinal disease, it is likely that it will become an important imaging modality in the management of diabetic retinopathy in the coming years. The high resolution wide field images and the fast acquisition rates, make this an exciting prospect for the future.
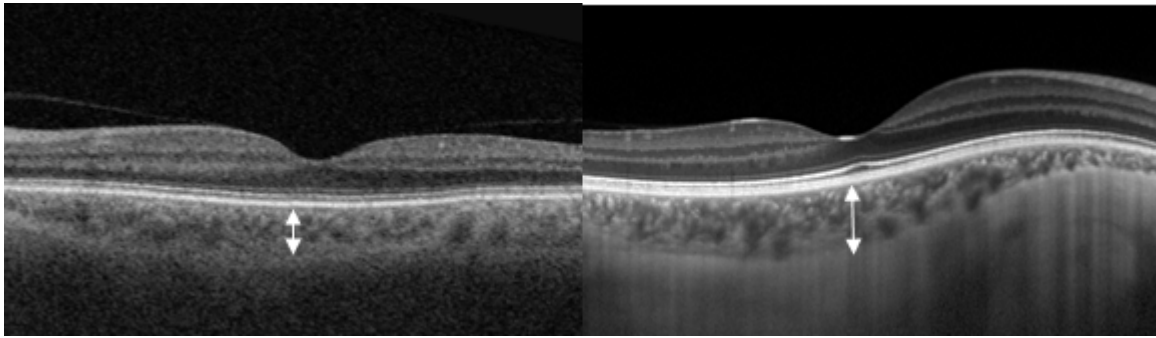
Fig. (4) An example of a swept source OCT image (left) and enhanced depth imaging OCT (EDI-OCT) (right). The swept source images allow greater depth and enable the visualisation of the choroid vessels below the retina (white arrows highlight the choroidal depth).

OCT Angiography

It is clear that optical coherence tomography is a vital tool in the imaging of various retinal pathologies, and as such it has become one of the most commonly used imaging modalities in the ophthalmology clinic. Whilst the latest devices are useful in providing high resolution 3-D images of the structure of the retina, they are limited by the inability to visualise the vascular changes associated with diabetic retinopathy, such as capillary drop out and new vessel formation. Fluorescein angiography is still required to visualise such vascular abnormalities. Whilst fluorescein angiography has played an important role in the imaging of diabetic retinopathy for many years, it is limited by its side effect profile together with it being relatively expensive and time consuming to perform. The images from FA are also only two-dimensional, adding difficulty to interpretation.

OCT angiography is a novel, non-invasive technique which allows the high-resolution, three dimensional visualisation of retinal and choroidal vasculature. Several methods of using OCT to detect retinal blood flow have been described, but the most effective method of OCT angiography utilises a technique known as *split spectrum amplitude decorrelation angiography (SSADA)* [38]. This method detects the movement of erythrocytes through retinal blood vessels by measuring the variation in reflected OCT signal amplitude between consecutive cross-sectional scans [39]. SSADA works to eliminate axial bulk motion from patient movement to improve the signal to noise ratio, so that sites of movement seen between repeated cross sectional scans represent erythrocyte flow through the vessel lumen [40]. By comparing these signal differences an accurate map of blood flow can be produced.

OCT angiography can be performed in this manner using a device such as the recently commercially available AngioVue OCTA system (Optovue, Inc., Fremont, CA). The software can also be installed on some current SD-OCT or SS-OCT devices without any particular hardware modification [39].
It is currently approved for clinical use in Europe and Asia, but awaits full approval in the USA.

Fluorescein angiography is the current gold standard imaging technique for detecting retinal vascular abnormalities, and *Matsunga et al* demonstrated that OCT angiograms were at least equivalent in showing vascular detail as fluorescein angiograms [40,44].

Fluorescein angiography has known limitations in imaging the vasculature of all the retinal layers. A study by *Spaide et al,* showed that while fluorescein angiography could not image radial peripapillary or the deep capillary networks well, OCT angiography was able to image all layers of the vasculature non-invasively [41]. At present, OCT angiography is able to capture up to an 8mm by 8mm view of the retina with adequate resolution to detect retinal and choroidal vascular abnormalities [39]. Each scan takes only approximately six seconds to take.

A recent prospective pilot study by *Ishibazawa et al,* investigated the use of OCT angiography in diabetic retinopathy [42]. Their results demonstrated that OCT angiography was effective at detecting microaneurysms

and retinal non-perfused areas, as well as providing quantitative information about neovascularisation (see **figure 5**). Other studies have also shown OCT angiography to be useful in identifying neovascularisation [43].

Some limitations of OCT angiography are important to note. These include the inability to adequately visualise leakage from vessels, its current limited field of view (although this is likely to increase in the future), and the difficulty in imaging in the presence of lens opacity such as cataract, which is a common finding in diabetic patients.

OCT angiography remains an emerging imaging technique with a huge potential for use in both a clinical and research setting. It offers many advantages over fluorescein angiography as have been described, but further studies are required to prove its clinical utility in the context of diabetic retinopathy. It is likely that OCT angiography will become one of the most important imaging techniques in the management of diabetic retinopathy in the years to come. The fact that it is non-invasive and quick to perform, means that it could also serve as a useful monitoring method in future clinical trials into new treatments for diabetic retinopathy.
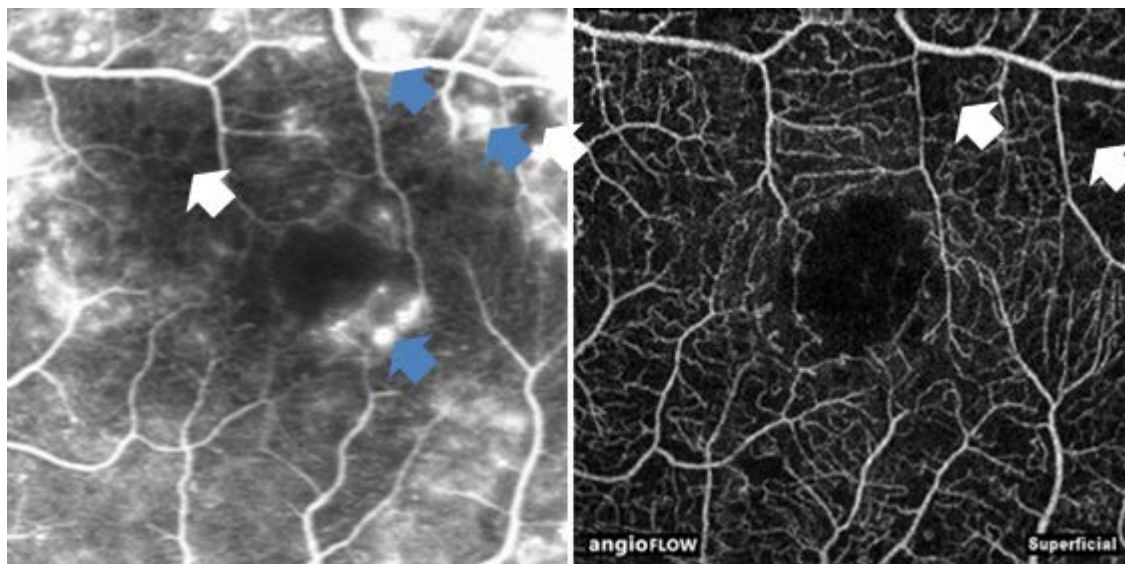

Fig. (5) Comparison of FFA (left) and OCT angiogram (right) imaging in diabetic macular oedema (right). Venous phase FFA shows capillary drop out (white arrows). The Foveal avascular zone is not clearly visible in later phase FFA and is obscured by leakage in the perifoveal region and generalised leakage in the macula (blue arrows). The OCT angiogram clearly shows the vasculature and only shows mild foveal avascular zone enlargement. There are small focal areas of capillary drop out (white arrows). However, the OCT angiogram does not clearly highlight areas of leakage.

Fundus Autofluorescence

Fundus autofluorescence (FAF) is a non-invasive retinal imaging modality which has been developed over the last ten years. It uses the autofluorescent properties of pigments in the retina to generate images which can be used in the diagnosis and management of various retinal disorders.

The predominant source of autofluorescence in the ocular fundus is **lipofuscin**, a pigment which accumulates in the retinal pigment epithelium as a by-product of metabolism and cell function [45]. When excited by short to medium wavelength light, lipofuscin granules autoflouresce, emitting broad spectrum of light with a peak wavelength of 630nm which can be imaged in several ways.

There are two main methods of creating images using FAF; using a confocal scanning laser ophthalmoscope (cSLO) such as the Heidelberg Retinal Angiograph (HRA2, Heidelberg Engineering, Germany), or by modifying a fundus camera such as the Topcon fundus camera (Topcon, Japan), as described by *Spaide et al* in 2003 [48]. Both of these techniques are frequently used in practice, however they use different wavelengths to obtain the images. The cSLO uses a 488 nm laser for excitation and a barrier filter at 500nm or 520nm [49], the same wavelengths used in fluorescein angiography. The modified fundus cameras use an excitation filter at 580 and a barrier filter at 695nm [48]. Despite the differences in wavelength used, both methods of FAF imaging produce similar images demonstrating fundus autofluorescence, although there are some small differences [49]. The longer wavelengths used in the fundus camera for example, mean the images produced show a much lesser extent of macular pigment absorption, and the signal is less decreased over blood vessels and the optic nerve head [54].

FAF images appear similar in appearance to fluorescein angiograms in their grey scale representation. Retinal vessels appear dark on the images due to blood flow blocking the autofluorescence signal from the RPE. The optic nerve and foveal area also both appear dark, or hypofluorescent, on a normal FAF image. Areas of hyperfluorescence are caused by an increase in autofluorescence signal and can be caused by reduced clearance by the RPE, increased turnover of photoreceptors, increase in other cells such as macrophages which contain lipofuscin, and window defects [16]. Areas of hypofluorescence are conversely caused by a decrease in AF signal and are caused by RPE loss or inactivity amongst others [16].

Lipofuscin deposits increase with age [46], but excessive accumulation is associated with various retinal diseases, most notably age related macular degeneration [47]. Its role and potential in diabetic retinopathy is being increasingly studied.

DMO is a common complication of diabetic retinopathy and a leading cause of visual disturbance. Studies have shown that fundus autofluorescence imaging is effective at detecting cystoid macular oedema with high sensitivity and specificity [50,51], offering a useful non-invasive method of studying the disease. Macular oedema is visualised by areas of hyperfluorescence at the macula, thought to be due to the disruption of macular pigment by the intraretinal fluid resulting in reduction in the masking effect of macular pigment and increased

penetration of the natural autofluorescence of the lipofuscin in that area. The amount of hyperfluorescence would appear to correlate with the severity of the macular oedema [52].

The detection of macular oedema by fundus autofluorescence has been shown to correlate well with the findings of optical coherence tomography [51] and fluorescein angiography [50]. As such, fundus autofluorescence is a useful imaging modality in both clinical care and in research in diabetic maculopathy. It can plays a role in understanding the pathophysiological disease, aiding in diagnosis, monitoring the efficacy of therapeutic interventions and identifying markers for of disease progression [53]. New developments such as widefield imaging, and quantitative measurement of autofluorescence which has been shown possible in mice studies so far [54], will serve to increase the clinical utility of fundus autofluorescence imaging in the years to come.

Fundus autofluorescence is already commonly used in the management of retinal disorders such as age related macular degeneration, and it is likely that, with further research, it will become an increasingly useful imaging modality in diabetic retinopathy in the next decade.

Retinal Oximetry

Retinal oximetry is an imaging technique which allows the non-invasive measurement of the relative oxygen saturations of retinal blood vessels. It was first described by *Hickam et al* in 1959, and has advanced significantly since then [62]. Currently, the most popular device for measuring retinal oxygen saturations is the Oxymap Retinal Oximeter (Oxymap, Reykjavik, Iceland). This device is attached to a conventional fundus camera (Topcon, Japan) which is operated in the same manner as for digital fundus photography. The device simultaneously captures to images of the same area of the retina at two different wavelengths of light, 570nm, which is insensitive to oxygen saturation, and 600nm, which is sensitive to oxygen saturation [63]. The two images are then processed using specialised software (Oxymap Analyser Software, Oxymap, Reykjavik, Iceland). The software automatically detects blood vessels and calculates the light absorbance (optical density) along the vessels at the two different wavelengths. The ratio of the two optical densities has been shown to have an inverse and approximately linear relationship to the oxygen saturation of the vessels [64]. In this way the oxygen saturation of the blood flowing through retinal vessels can be calculated.

Diabetic retinopathy has been shown in preliminary studies to be associated with changes in the oxygen saturations of retinal vessels [56,57,58]. *Hardarson et al* demonstrated that retinal vessel oxygen saturation is higher in patients with DR than normal controls. Possible explanations for this include the shunting of blood to bypass non-perfused capillaries, resulting in hypoxia in some areas of the retina whilst larger vessels demonstrate higher oxygen saturations [56]. Another possible explanation is the decreased oxygen consumption by retinal tissue damaged by diabetic retinopathy [59]. A separate study by *Hammer et al* showed a trend of increasing arterial and venous oxygen saturations correlating to the severity of retinopathy in diabetic patients [57], a trend also supported by *Khoobehi et al* [58].

Hypoxia is a key factor in the pathogenesis of DR, and is particularly important in the development of retinal neovascularisation, the hallmark of proliferative diabetic retinopathy [60]. By monitoring retinal vessel saturations in patients with early or no retinopathy, there is the potential to recognise early signs of vascular compromise before the clinical signs of retinopathy are visible on examination [61]. Measuring saturations in patients with known retinopathy may play an increasing role in the monitoring of the disease and measuring the outcomes following treatment.

As research continues to improve the reliability and sensitivity of retinal oximetry, it will play an increasing role in the management of various retinal vascular disorders. Further studies are needed to confirm the findings of preliminary studies in diabetic retinopathy. Once these have been validated, retinal oximetry will become a useful imaging technique in the early diagnosis, and continued management of this condition.

Conclusion

Effective imaging is vital to assist screening, diagnosis, and management of diabetic eye disease. Commonly used techniques such as digital fundus photography, optical coherence tomography, and fundus fluorescence angiography, will continue to play a central role in the management of disease. However, ophthalmic imaging is rapidly evolving in diabetic eye care. Several techniques have already shown promise in specialist situations. These include OCT angiography which provides a rapid, non-invasive route to identify ischaemia and proliferative retinopathy. Meanwhile other techniques such as retinal oximetry, swept source OCT and enhanced depth imaging OCT of the choroid still require further study to understand how imaging findings correlate with prognosis of sight loss. However, adoption of these techniques may ultimately depend on convergence of methods using multimodal imaging devices. In the near future machines will be available which combine many of the techniques described above. These developments together with updated on-device imaging analysis software are likely to bring a step change in understanding and managing diabetic eye disease

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