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REVIEW ARTICLE

Retinal imaging as a source of biomarkers for diagnosis, characterization and prognosis of chronic illness or long-term conditions

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ABSTRACT

The black void behind the pupil was optically impenetrable before the invention of the ophthalmoscope by von Helmholtz over 150 years ago. Advances in retinal imaging and image processing, especially over the past decade, have opened a route to another unexplored landscape, the retinal neurovascular architecture and the retinal ganglion pathways linking to the central nervous system beyond. Exploiting these research opportunities requires multidisciplinary teams to explore the interface sitting at the border between ophthalmology, neurology and computing science. It is from the detail and depth of retinal phenotyping that novel metrics and candidate biomarkers are likely to emerge. Confirmation that *in vivo* retinal neurovascular measures are predictive of microvascular change in the brain and other organs is likely to be a major area of research activity over the next decade. Unlocking this hidden potential within the retina requires integration of structural and functional data sets, that is, multimodal mapping and longitudinal studies spanning the natural history of the disease process. And with further advances in imaging, it is likely that this area of retinal research will remain active and clinically relevant for many years to come. Accordingly, this review looks at state-of-the-art retinal imaging and its application to diagnosis, characterization and prognosis of chronic illness or long-term conditions.

Currently about 20 million people in the UK suffer from at least 1 long-term condition (a health problem that cannot be cured but can be controlled by medication or other therapies), and this is set to increase 3-fold over the next decade placing an enormous economic and human-resource strain on the National Health Service.^{1–3} The retina is unique in the body in allowing easy observation of blood vessels with simple, non-invasive instruments. It is also the only accessible site for studying the central nervous system (CNS) *in vivo*.⁴ This makes it a ripe environment for the study of systemic and neurological disease, in a patient-acceptable way. Changes to the retina can occur in some chronic diseases years before other signs become apparent.^{5–9} Thus, studying the retina may provide an additional means for clinicians to stratify risk and help identify people who would benefit from early lifestyle changes and preventative therapies, as well as assessing the efficacy of new treatments. In addition, there is some

early evidence that certain retinal measures may correlate with disease characterization and also prognosis, which not only provides clinicians with a useful assessment tool but also offers research scientists new avenues to explore in the understanding of the pathophysiology of these diseases.

Detailed clinical observations of characteristic fundus features have led to the identification of early indicators of a diverse range of long-term conditions such as diabetes mellitus,⁵ stroke,⁶ hypertension⁷ and cardiovascular disease.⁸ Furthermore, blood vessels of the retina are thought to predict neurovascular disease in particular because they are part of the brain's vascular system and so share anatomical features and respond similarly to stress and disease.⁹ Additionally, the inner layer of the retina, known as the retinal ganglion cell (RGC) layer, projects axons through the optic nerve to the brain and consequently neurodegenerative diseases may have retinal manifestations.⁴

In this article, we review state-of-the-art retinal imaging and discuss its application to the diagnosis, characterization and prognosis of chronic illness or long-term conditions. A recap of eye anatomy is followed by an overview of imaging technology for retinal examinations. Retinal manifestations of (non-ocular) chronic disease are presented. The rationale and methods for computerized image processing and analysis are reviewed. A separate section is devoted to large studies featuring endeavours at retinal imaging biomarker identification. Finally, the future of retinal imaging as a clinically useful tool is considered.

EYE ANATOMY

The inner surface of the human eye, the retina (Figure 1), comprises layers of tissue consisting of neurons and supporting cells, which are interconnected by synapses. The innermost cellular layer of the retina, known as the RGC layer, projects its axons across the inner retina—the retinal nerve fibre layer (RNFL)—through the optic nerve to the brain. It is therefore an extension

of the CNS, and consequently neurodegenerative diseases may not only have retinal manifestations but also measurements of the nerve integrity within the retina may be a biomarker of global CNS status.

In an adult human, the retina is around 22 mm in diameter and approximately spherical in shape. The optic nerve fibres leave the eye at the *optic disc* (OD), while the *fovea* is responsible for our central and sharpest vision and is located in the middle of the *macula*. The inner layer of the retina is supplied by the central retinal artery, which is a branch of the ophthalmic artery and runs alongside the optic nerve. The central arteriole and venule bifurcate several times with the arteriolar and venular branches running mostly in parallel with some crossovers.

The retina is the only part of the CNS and the brain (both the retina and the optic nerve originate as outgrowths of the developing human brain) that can be visualized non-invasively—the pupil

Figure 1. Anatomy of the adult human eye and retinal layers.¹⁰ (a) Sagittal view showing the main structures. (b) Diagram of the organization of the retinal layers and cells.

acts as both an entrance to and exit for the imaging light rays—making direct observation of the body's circulation, specifically the retinal microvasculature, possible and reflecting the state of both the systemic and brain microcirculations. The transparent neuroretina has been more difficult to study in conventional photography, however, the development of new technologies, particularly optical coherence tomography (OCT) has revolutionized our ability to visualize this tissue. The newest generation of OCT known as spectral-domain OCT (SD-OCT) provides sufficiently high resolution to enable visual identification of the individual retinal layers, including the RNFL. Thus, the visible retina permits the study of the structure and pathology of the CNS and of the circulation with the possibility of detecting changes relating to the development of disease. Serial, long-term monitoring is entirely feasible without any negative side effect issue to the patient.

RETINAL IMAGING

In the ophthalmology clinic, retinal imaging devices are primarily used in the diagnosis of retinal disease as well as serial monitoring in retinal conditions such as age-related macular degeneration to monitor response to treatment. However, the detail with which the eye can be visualized non-invasively opens up investigative possibilities for a variety of long-term conditions. We outline the three principal imaging technologies for the retina, namely *fundus camera imaging*, *scanning laser ophthalmoscopy* and OCT. Table 1 summarizes key detail relating to these modalities.

Fundus camera imaging

Fundus imaging generates a two-dimensional (2D) image of the interior three-dimensional (3D) surface of the eye (Figure 2) and

is performed with a system that consists of a specialized low-power microscope and an attached camera.¹¹ The patient sits with his/her chin in a rest and forehead placed against a bar, while the operator focuses and aligns the camera before pressing the shutter release to fire a flash and create the image. This image is an upright, magnified picture of the fundus with typical angles of view of 30°, 45° or 60° and with a magnification of $\times 2.5$, depending on the system optics. Some modifications to these parameters are achievable through zoom or auxiliary lenses. For example, a 15° lens can provide $\times 5$ magnification, whereas a 140° wide angle lens captures a larger area of the fundus. A larger field of view (FOV) can be achieved by composing multiple images acquired at different fixation points. Also, images of higher quality can often be achieved by dilating the pupils beforehand with mydriatic eye drops to enlarge the FOV of the fundus and improve image quality. Current image resolutions are around 3000×3000 pixels.

Most commonly, the retina is illuminated by white light and examined in full colour. However, the imaging light can be filtered to remove red components, creating a *red-free* image with improved contrast of retinal and choroidal blood vessels and other structures. Alternatively, with *fluorescein angiography* (FA) or *indocyanine green angiography*, the subject receives an intravenous injection of a fluorescent dye while the retina is illuminated at an excitation wavelength (465–490 nm) that fluoresces light of another colour where the dye is present. This enables a high-contrast image of the blood vessels and also highlights areas of damage where the dye escapes into the surrounding tissue. Flow dynamics and related pathologies are revealed by capturing a timed sequence of images of the progression of the dye into the vessels.

Table 1. A summary of the principal retinal imaging modalities, including the method of image formation, typical resolution (in micrometers) and some of the key advantages and limitations

Modality	Image formation	Resolution (μm)	Advantages	Limitations
Fundus camera	Colour photograph of retinal surface	7–20	Blood vessels—marker of microvascular health	Dilation of pupils often needed
			Lesions, exudates, haemorrhages—common signs of diabetic retinopathy	
Scanning laser ophthalmoscope	Focused laser beam scans retinal surface	10–15	Larger field of view in one image than fundus camera	Typically more expensive and complex
			Examine fundus features in the peripheral retina	
Angiography	Retina illuminated at wavelength to excite fluorescent dye	7–20	Improved contrast and detail of vasculature	Invasive—intravenous injection
			Flow velocity, leakages, blockages—markers of microcirculatory health	
Optical coherence tomography	Near-infrared light penetrates retina; interferometry resolves tissue layers	4	Cross-sectional view of internal retinal structures	Susceptible to media opacities
			Changes in the retinal nerve fibre layer—possible markers of neurodegeneration	

Figure 2. Imaging the retina. (a) Fundus camera image acquired with a Canon CR-DGi (Canon, Tokyo, Japan) non-mydratric camera at 45° field of view (FOV) and (b) scanning laser ophthalmoscope image acquired with an Optos® P200C (Optos, Dunfermline, Scotland) at approximately 200° FOV. Although not of the same person, the images demonstrate the difference in area viewable with the two devices, which is revealed by comparing the optic nerve head (*i.e.* the bright circular region) as it appears in each image.

Fundus camera imaging is extensively used in diabetic retinopathy (DR) screening where it has been well validated as a screening tool,¹² identifying pre-symptomatic stages of retinopathy, where treatment interventions can protect sight. It is also used routinely in a variety of other ophthalmic conditions such as glaucoma where serial photographs can reveal subtle changes in the optic nerve, or pigmented retina lesions such as choroidal naevi, where again serial monitoring is used to pick up growth or change.

Scanning laser ophthalmoscope

A scanning laser ophthalmoscope (SLO), like the fundus camera, creates a 2D image (Figure 2) but scans a laser beam across the 3D retinal surface instead of using a bright flash of white light.¹³ It is a *confocal imaging* technique, that is, the retina is scanned point by point in a raster fashion by a focused laser beam and the reflected light is captured through a small aperture, the *confocal pinhole*. The confocal pinhole suppresses light reflected or scattered from outside of the focal plane, which would otherwise blur the image. Scanning mirrors are utilized to direct the laser beam to regions of the retina inaccessible to a fundus camera. Obtained images are typically rectangular owing to the raster scanning pattern, while the angle of view varies by instrument from 15–45° (*i.e.* comparable to fundus cameras) up to 200°, the so called *ultra-widefield*.

Unlike the fundus camera, the SLO does not capture full colour images of the retina as typically only one or two wavelengths of laser light are used. However, laser illumination combined with the confocal optical system produces high-contrast, finely detailed images. Current image resolutions are in the region of 3000 × 2800 pixels. Additionally, the narrow wavelength band from the laser light delivers a more efficient excitation of fluorescence than does the filtered flash illumination of the fundus camera. Using SLO to perform FA allows for the measurement of capillary flow velocity. Such measurements provide important information on the health of the microcirculation.

Although various SLO devices image a larger FOV and show the peripheral fundus, resolution can sometimes be lower than that of a fundus camera image. The cost and complexity of devices often see the SLO confined to research and ophthalmology

clinics. However, instrument manufacturers recognize that ease of use and accessibility for non-ophthalmology specialists are key to competing with fundus cameras, which have limited capabilities in terms of the amount of retina that can be imaged. The SLO is more acceptable for the patient, as there is no visible flash of light with some devices, and multiple images can be captured quickly and without patient fatigue.

Optical coherence tomography

OCT is a non-invasive and non-contact method for *in vivo* cross-sectional imaging of the internal retinal structures.¹⁴ It is an optical scanning technique employing near-infrared light and can be thought of as “optical ultrasound” in use and in image interpretation; albeit with a limited tissue depth of just 2 mm. An optical beam is directed at the target tissue and interferometry resolves the back-scattered light signals. The scanning beam is split with a beam splitter sending some to the target tissue (target arm) and the remaining portion to a reference mirror (reference arm). Both beams are reflected back to the beam splitter from the target and the reference mirror, respectively, and then directed together to a detector.

When the distance to the reference mirror in the reference arm is equal to the distance to the reflecting target within the tissue, interference occurs that is used to infer the depth of the reflecting structure in the target. In conventional time-domain OCT, the reference arm is moved to different distances from the beam source allowing sampling of the target at different depths. This relatively slow, mechanical movement of the reference arm limits both the amount of data that can be captured as well as the quality of the image. In state-of-the-art SD-OCT, the depth image is computed by analysing the interference signal based on the wavelength of light. The need for a moving reference mirror is removed, which improves imaging speed, resolution and data quality.

Increasingly, SD-OCT is combined with SLO to create multi-modal imaging devices (Figure 3). The SLO, in addition to showing the retinal surface and features, allows the operator to localize OCT acquisition. Incorporating eye tracking technology further minimizes motion artefact and reduces noise while also

Figure 3. Multimodal retinal imaging. (a) Scanning laser ophthalmoscope image focused on OD, showing peripapillary optical coherence tomography (OCT) scan protocol (white circle), and (b) OCT image showing retinal layers, including retinal nerve fibre layer (RNFL) and others below. Acquired with Heidelberg SPECTRALIS® OCT (Heidelberg Engineering, Heidelberg, Germany). Segmentation of RNFL quantifies temporal thinning: a described finding in multiple sclerosis.

providing precise location registration between patient visits, ensuring the same area of retina is scanned. This is essential for accurate longitudinal change measurement.

RETINAL MANIFESTATIONS OF DISEASE

Many diseases can involve the eye, both primary retinal conditions and systemic disorders. For the purpose of this article, we focus on diseases of the circulation, the CNS and the brain.

The retinal microvascular network develops in a way that is optimized for efficient flow.^{15,16} Deviations away from this optimal state, which are revealed in abnormal or suboptimal geometric parameters measured on fundus images (e.g. arteriolar and venular diameters, vessel bifurcation geometry, vascular tortuosity and global complexity of the visible network; Figure 4), occur in disease processes and may be related to microvascular damage. Such suboptimal patterning increases energy costs and reduces efficiency of metabolic transport; microvascular health is maintained by both adequate vessel diameters and optimal branching architecture.¹⁷ The key question becomes whether changes detected in the microvasculature are early markers of disease secondary to the disease process or representative of the primary cause to the development of disease.

Within the RNFL, there are unmyelinated axons that directly synapse into the CNS, thus making the eye a significant site for investigating axonal degradation in neurologic disease and neurodegeneration. Several neurologic and neurodegenerative conditions have pathological changes in the RNFL, which creates a potential surrogate marker for disease derivable from OCT.^{4,18}

Diabetes mellitus

Diabetes is a disease process manifesting as hyperglycaemia, either owing to inadequate production of insulin by the pancreas or owing to cells becoming resistant to insulin. Diabetes can have serious long-term complications such as DR which is the most common cause of blindness in the developed world.⁵ In the UK, annual screening for DR routinely takes place in all patients with diabetes who are 12 years or older. Monitoring of patients is further aided by software that links primary and secondary services (i.e. general practice, hospital and laboratory) making it possible to access both electronic patient records and retinal images. DR is not curable, but using retinal imaging to identify

the signs early means that vision loss and blindness from this disease is preventable and early intervention salvages eye sight. In addition, identification of worsening DR often signifies unstable systemic control of diabetes, prompting re-evaluation of treatment and other risk factors.

The early stages of DR are termed *non-proliferative* DR (mild, moderate and severe). Whilst asymptomatic, the retinal signs of microaneurysms, exudates, cotton wool spots and haemorrhages are easily detectable with fundus imaging, and also with FA, which reveals areas of retinal ischaemia (i.e. narrowed or blocked retinal blood vessels leading to non-perfusion of retinal tissue). *Proliferative* DR is the sight-threatening stage (Figure 5) and

Figure 4. Features of interest in a fundus image. The optic nerve head boundary provides a reference point for locating and quantifying other features of interest. Measurement of parameters such as the vessel diameter, bifurcation geometry and vascular tortuosity (i.e. how much it twists and turns) can reveal a suboptimal microvascular network in the retina, which may be related to microvascular damage and an indicator of disease. Different diseases may affect arterioles and venules differently, so differentiating between the two types of vessels is important.

Figure 5. Scanning laser ophthalmoscope (SLO) image showing signs of proliferative diabetic retinopathy, with diabetic maculopathy. With a wider field of view than a fundus camera, diabetic retinopathy signs in the periphery as well as near to the macula are identifiable in a single image acquisition. Acquired with Optos® P200C SLO (Optos, Dunfermline, Scotland).

indicates the presence of new abnormal blood vessels growing on the surface of the retina (neovascularization). These fragile vessels are prone to bleeding, which ranges from small transient events to larger bleeds and subsequent permanent damage.

Diabetes has a profound effect on the body's microvasculature and consequently retinal vascular changes are also evident on fundus images with widening of venular diameters found to be associated with subsequent incidence and progression of DR.^{19,20} The retinal vascular network is more tortuous in patients with diabetes while increased arteriolar tortuosity is associated with non-proliferative DR and early stage kidney dysfunction, which suggests potential for fundus imaging in aiding risk-stratification for diabetic complications.^{21,22}

Cardiovascular disease

Cardiovascular disease affects multiple organs and most often manifests itself as hypertension and atherosclerosis, while ageing is considered a physiological cause of cardiovascular disorder.

Systemic hypertension and hypertensive retinopathy (*i.e.* damage to the retina and retinal circulation owing to high blood pressure) are recognizable in the fundus.²³ A key feature of hypertension is increased peripheral vascular resistance, which is determined by arteriolar narrowing, although it remains uncertain whether this precedes high blood pressure or occurs as a secondary adaption. Generalized retinal arteriolar narrowing, observed with fundus imaging, is an important marker of systemic hypertension as well as a sign of damage to the retina, and, in normotensives, it is an early indicator of increased risk of developing hypertension.²⁴ Another sign of hypertension in the retina is *arteriovenous nicking* (Figure 6), which is when the more pliant venule is compressed by the stiffer arteriole at their crossing point in response to a rise in blood pressure.⁷ Arteriovenous nicking is associated with both current blood pressure and also past blood pressure, suggesting that it is a persistent and long-term marker of hypertension. Further markers of hypertension include reduced arteriolar bifurcation angles and increased arteriolar tortuosity, which reflect a sub-optimal or diseased retinal vascular network.^{25,26}

Atherosclerosis causes coronary heart disease (CHD), which leads to over 80,000 deaths in the UK every year and is the country's biggest killer.²⁷ It is estimated that 2.7 million people in the UK are living with the condition and 2 million people are affected by angina, the most common symptom of CHD. Traditional risk factors such as hypertension, hyperlipidaemia and diabetes permit clinicians to identify and treat high-risk patients. However, a substantial amount of cardiovascular disease is not explained by traditional risk factors alone. There is evidence that retinal vascular changes, identifiable on fundus imaging parallel pathology in the coronary micro- and macrocirculations and so may provide markers of CHD.^{28–31}

Narrowing of retinal arterioles is strongly associated with reduced myocardial perfusion and a decline in function (measured from cardiac MRI).^{28,31} Retinopathy signs are associated with coronary artery calcification on CT, a subclinical marker of coronary artery disease and predictor of future coronary events, with more severe lesions associated with worse coronary artery disease.^{29,30} Thus, retinopathy signs may be markers for large artery atherosclerosis, whereas retinal arteriolar narrowing may be a marker for coronary microvascular disease.

Stroke

Stroke occurs when there is the rapid loss of brain function owing to disturbance in the blood supply to the brain, and may be ischaemia or haemorrhagic. Some underlying mechanisms of stroke are poorly understood. For example, lacunar stroke, which accounts for a quarter of ischaemic stroke cases (and a fifth of all strokes), is known to be a small-vessel disease, but exact underlying pathologies are still uncertain.³² Retinal vessels share ontogeny, size and physiological characteristics with cerebral small vessels, suggesting potential for using retinal imaging in studying small-vessel disease.^{9,33}

Retinal venules are wider in patients with lacunar strokes than those in patients with cortical strokes (which are commonly caused by embolism from the heart or large arteries),³⁴ while analysis of the microvascular network visible in fundus images showed lacunar stroke and increasing age are associated with

Figure 6. Fundus camera image showing signs of hypertensive retinopathy, *i.e.* arteriovenous nicking indicated by arrows. Image acquired with a Canon CR-DGi (Canon, Tokyo, Japan) non-mydratic camera at 45° field of view.

a loss of branching complexity.³⁵ Thus, parameters demonstrating a suboptimal retinal vascular network act as a surrogate marker for diseased cerebral vessels. This suggests that a distinct vasculopathy may cause lacunar stroke, which has implications for improving prevention and treatment. Moreover, retinal venular dilation rather than arteriolar narrowing has been revealed as a strong predictor of clinical stroke,⁷ which further intimates that venules are a dynamic component of the vascular network and respond to changes in the microcirculation.

The presence of both white matter lesions on MRI, thought to have a microvascular aetiology and to precede the development of clinical stroke, and retinopathy (*e.g.* microaneurysm, haemorrhage and soft exudate) on fundus imaging is associated with a larger stroke risk than the absence of either finding.³⁶ This suggests people with both cerebral and retinal markers of microvascular disease may have more severe or extensive cerebral microvascular pathology. In addition, these retinal lesions that are strongly related to stroke are most commonly seen when there is a breakdown of the blood–retinal barrier,³⁷ which further supports the notion that disruption of the cerebral microcirculation’s blood–brain barrier may be an important pathophysiological feature in the occurrence of cerebrovascular disorders.³⁸

Neurodegenerative disease

Major neurodegenerative diseases such as multiple sclerosis (MS), Alzheimer’s disease (AD), Parkinson’s disease (PD) and motor neurone disease are currently incurable, and even disease-modifying treatments are variable in efficacy as well as being expensive.³⁹ Given the complexity of these disorders and our limited understanding of their causes, there is a strong need to both identify the early pre-clinical stages of disease and also to develop tools to closely monitor their progression, particularly the impact of any interventions or treatments.

OCT has been suggested as a potentially useful biomarker in the assessment of CNS neurodegeneration in MS.⁴⁰ It has been used to measure RNFL thickness in MS eyes with and without a history of optic neuritis (ON), as well as in disease-free control eyes. Reduced RNFL thickness was found in both MS groups along with a correlation of RNFL thickness with visual acuity and

contrast sensitivity. Correlation of RNFL thickness with brain atrophy in MS has also been reported.^{41–43} OCT measures of RNFL thickness could also have prognostic value.⁴⁴ Baseline temporal quadrant RNFL atrophy was found to be associated with new relapses and changes in the Expanded Disability Status Scale. Other studies have since also shown temporal quadrant atrophy associated with physical disability.⁴⁵

To determine the natural history of the RNFL changes, a collaborative study appeared in 2010, following 299 patients with MS with OCT and low-contrast visual acuity.⁴⁶ They found that eyes with visual loss showed more RNFL thinning than that of eyes with stable vision, and that in general, RNFL thinning increased over time, with or without a history of ON. A Spanish group followed 34 patients with MS after an episode of ON, and compared their affected and unaffected eyes.⁴⁷ Whilst they unsurprisingly found RNFL thinning in the eye affected by ON, the background progressive loss was similar between the two eyes, suggesting that whilst ON gives a single dramatic kick, the chronic degeneration continues in the background, secondary to the disease. The same group published further results on a larger study population, this time showing that patients with MS relapses showed a greater reduction of RNFL thickness than those with non-relapsing cases.⁴⁸

Although much of the research in this field involved measuring the RNFL—the axons of the ganglion cells, that run across the inner retina, to the optic nerve, and onwards—recent advances in OCT technology provide the opportunity to look at the immediate adjacent layer, the ganglion cell layer (GCL), which consists of the main body of the ganglion cell or neurone. GCL thinning in MS eyes (versus controls), particularly those with a history of ON, was discovered⁴⁹ and was consistent with post-mortem histological evidence of GCL thinning in all MS subtypes.⁵⁰

OCT measurements are starting to be used as part of the data collection in trials of treatments. Patients with MS and healthy controls were followed up for 3 years, measuring multiple ophthalmic items including OCT.⁵¹ About half of the patients with MS were receiving treatment—with β -interferon 1b, β -interferon 1a or glatiramer. Not only did the MS group

show RNFL thinning, but untreated patients with MS also showed more degeneration in the mean and superior RNFL thicknesses than those treated. There was no difference between the treatments.

Defined as a first neurological episode (monofocal in which symptoms present at a single site in the CNS or multifocal in which multiple sites exhibit symptoms, and lasting longer than 24 h), clinically isolated syndrome progresses to MS in around 45% of individuals.⁵² Risk can be stratified on MRI findings but is otherwise difficult to predict, while clinical trials of treatments to delay a second clinical event would benefit from additional markers of risk of progression. A cross-sectional study prospectively followed such individuals.⁵³ 13 out of 24 patients had decreased RNFL thickness in at least 1 quadrant at presentation; this finding had a sensitivity of 75% and specificity of 56% for predicting MS. Given the small numbers and low predictive probability, more evidence is needed.

In 2010, a pilot study emerged from New York, NY, using OCT to look for any difference between individuals with PD and controls.⁵⁴ Peripapillary RNFL thickness was measured along with inner retinal layer thickness and macula thickness, finding only differences in macula thickness (outer superior subfield 2.8% thinner and outer nasal and inferior inner subfields 2.8% thicker) compared with published normal values.

The natural history of RNFL thickness is incompletely understood, both in health and in disease. Firstly, it must be remembered that physiological variations, pharmacology and biochemistry, as well as pathological processes can increase or decrease retinal layer thicknesses. In addition, the published studies generally used regression lines to demonstrate correlations. This means that the conclusions apply to the sample population and are not necessarily directly applicable to the individual patient. In fact, the pattern of RNFL thinning in an individual eye may not be linear over time. Indeed the RNFL in some eyes studied increased in thickness over time. It seems therefore that with only limited evidence thus far, caution will be needed in interpreting the rate of change of an individual's RNFL thickness in terms of their neurological status. And given the physiological variations in RNFL thickness, single time-point measurements in individual patients is likely to have limited value. The future of OCT in longitudinal monitoring and estimating prognosis for neurodegeneration will almost certainly lie in its integration with other retinal imaging modalities such as fundus imaging, and the formation of composite scoring.

Fundus imaging has been proposed as a source of biomarkers for AD,⁵⁵ but application to other neurodegenerative conditions is sparse. AD is known to have a vascular component with small-vessel disease, micro-infarction and cerebral amyloid angiopathy.⁵⁶ So the homology between cerebral and retinal microvasculatures would suggest that changes in the retina might also occur in AD.⁹ A recent study demonstrated reduced vessel diameters (venular and arteriolar), reduced complexity of the branching pattern, reduced optimality of the branching geometry and less tortuous venules in patients with AD than in healthy controls.⁵⁷ Furthermore, retinal abnormalities in AD that oppose those previously reported in vascular dementia were also found (*i.e.* wider

retinal venules are associated with an increased risk of vascular dementia),⁵⁸ suggesting a potential role for fundus imaging in distinguishing between these most common forms of dementia.

While fundus imaging of longitudinal retinal changes might facilitate more accurate pre-clinical detection or monitoring of AD and response to intervention, relationships between retinal vascular abnormalities and other neurodegenerative disorders appear under-researched.

IMAGE PROCESSING AND ANALYSIS FOR THE RETINA

Various software tools, exploiting image processing and analysis techniques, enable quantitative and reproducible measurements of the retinal vasculature in both fundus images and also the different retinal layers observed with OCT.^{15,18,59} Such computer-assisted methods measure morphometric properties, and their variations or abnormalities, in the retinal microvasculature and in the retinal layers that might be imperceptible to or missed by a human grader, in an objective and repeatable fashion. Furthermore, software automation of recurrent measurement processes enables high throughput of images. Here, we focus on some of the key tasks of retinal image analysis.

Retinal lesion detection

Software to aid DR screening involves the computer-assisted detection of specific lesions in fundus images (*e.g.* microaneurysms, exudates, cotton wool spots and haemorrhages). Given the high incidence of diabetes, considerable research has been devoted to automatic screening systems analysing the retinal fundus.^{60–68} Automatic detection is challenging as lesions are often faint or small and there can be a great variation in appearance and scale. Generally, image processing transforms based on grayscale intensity, shape, texture and size are utilized to detect candidate lesions.^{60,62} *Supervised classification* algorithms (which require a set of annotated data to produce an inferred function for classifying new examples) are often employed to distinguish valid lesions for spurious responses or false negatives.⁶¹

Retinal vessel detection

Many different methods for automatic detection of the retinal vascular structure in fundus camera images have been reported.^{28,69} This includes vessel tracking where vessel centre lines are followed guided by local information;⁷⁰ matched filters to highlight blood vessels;⁷¹ deformable models;⁷² Hessian measures are used to steer the application of matched filters and confidence measures;⁷³ and gradient vector fields.⁷⁴ Classifying image pixels as either vessel or non-vessel sees another application for supervised classification techniques.⁷⁵ By comparison, vessel detection for SLO is vastly under-researched,⁷⁶ possibly because the devices are less prevalent than fundus cameras.

Retinal vessel segmentation (Figure 7) is the precursor to measurement processes such as vessel diameter,⁷⁷ deviation of vessel bifurcation geometry (branching angle and junction exponent) from optimality⁷⁸ and vascular tortuosity.⁷⁹ Additional, further automation includes distinguishing venules from arterioles, with vessel diameters subsequently summarized as a central retinal

Figure 7. Automatic retinal vessel detection. The output is a vessel map showing vessel locations in white. Examples shown are for the fundus images in Figure 2, with (a) the resulting map for the fundus camera and (b) the map for scanning laser ophthalmoscope. Vessel detection is the precursor to the measurement of parameters such as vessel diameter, bifurcation geometry and vascular tortuosity. The complexity of the map can also be quantified using fractal analysis to yield a fractal dimension that combines contributions of individual vessel parameters into a single global value.

arteriolar equivalent and central retinal venular equivalent to give an *arteriole-to-venule ratio* (AVR).^{80,81}

Fractal analysis quantifies the complexity or density of the vessel branching pattern visible in a fundus image in terms of a fractal dimension.^{17,35} Such a measurement combines contributions of individual vessel parameters within the branching pattern into a single global value that summarizes the global complexity. Variations in the fractal dimension are an indicator of deviation away from the normal or optimized network, and so a potential marker of disease.

Optic disc detection

While detecting and quantifying the optic nerve head region in fundus imaging is valuable for recording gradual damage due to disease such as glaucoma, it also provides an important reference point for the location and quantification of other features of interest in the retina. The OD is normally the brightest component on a fundus image and so can be identified via a grouping of pixels with high intensity, although further refinement is usually required, utilizing shape and vessel locations, to improve success and yield reliable boundaries.^{82,83}

Building on methods locating the OD automatically, various authors have attempted an automatic assessment of glaucoma. Recently reported systems for automatic glaucoma assessment include ARGALI (Automatic cup-to-disc Ratio measurement system for Glaucoma Analysis using Level-set Image processing)^{84–86} and AGLAIA (Automatic Glaucoma Diagnosis and Its Genetic Association Study through Medical Image Informatics).^{87,88} The latter aims to a holistic glaucoma analysis integrating clinical and genomic data. Stereo vision techniques, estimating the 3D cupping of the OD using conventional fundus cameras, have also been tried.^{89–92}

Registration

Registration (aligning in a mosaic or montage) of fundus images^{93,94} is useful to expand the FOV, using images of different regions, and to analyse longitudinal changes, using images of the same region taken at different times. This involves identification and extraction of features or landmarks derived from

the retinal vasculature that are segmented separately from the individual images.⁹⁵ After vascular segmentation, vascular branching points, for example, yield stable landmarks for determining image-to-image correspondence.

Increasingly, OCT is combined with SLO by manufacturers into one device, giving co-registered data as an output (Figure 8). Fundus camera image to SLO/OCT registration then becomes a task similar to the one described above, whereby aligning with the SLO image via common vascular features will achieve the desired result. However, fundus to OCT registration can also be achieved for separate devices through identification of prominent landmarks identifiable in both modalities (e.g. blood vessels, the optic nerve head and macula) and 3D alignment of the data.⁹⁶

Computational fluid dynamics

Computational fluid dynamics (CFD) provides additional insight into how structural changes to the retinal vascular network that are associated with disease can result in potentially disadvantageous

Figure 8. Optical coherence tomography (OCT) volume scan co-registered with fundus image from scanning laser ophthalmoscope. Acquired with Heidelberg SPECTRALIS® OCT (Heidelberg Engineering, Heidelberg, Germany).

blood flow and oxygen distribution.⁹⁷ The 2D vascular segmentations from fundus imaging are used as the basis for constructing 3D computer models of the networks. CFD simulations are applied to these in order to investigate functional characteristics of the retinal microcirculation, and then whether haemodynamic parameters could act as potentially sensitive markers of disease.

Optical coherence tomography analysis

One of the major tasks in analysing OCT is the accurate segmentation of retinal layers, especially in the presence of lesions, as thickness changes can be an important indicator of disease status. OCT images are inherently noisy making the delineation of the different layers challenging. Pre-processing such as median filtering is employed to reduce the influence of noise prior to one-dimensional peak detection on every column in the image to find points on each border of interest.⁹⁸

Texture analysis refers to the characterization of regions in an image by their texture content, and it is applicable to OCT for assessing changes in structure or tissue that cannot be ascertained from changes in thickness alone.⁹⁹ It is also useful for detecting features such as retinal lesions and the retinal vasculature in 3D SD-OCT volumes,¹⁰⁰ which is also useful for OCT-to-fundus and OCT-to-OCT image registration.

Validation

A comprehensive review of validation in retinal image analysis is given in the study by Trucco *et al*,¹⁰¹ from which we summarize some key considerations. Validation means showing that a computer algorithm for measuring a retinal parameter performs correctly by comparing its output with a reference standard, defined by expert performance. Hence validation involves selecting an image set representative for the task at hand, collecting reference standard annotations, running the software to validate on the sample images, and assessing the statistical agreement of the output with the reference standard, for example, sensitivity, specificity, positive- and negative-predictive value and area under the receiver operating characteristic curve.

BIOMARKER IDENTIFICATION

A number of large scale longitudinal studies have been undertaken internationally with the shared aim of improving the prevention, diagnosis and treatment of a wide range of long-term conditions and chronic illness.^{24,102–113} The target has been the identification of biomarkers that will enable systematic patient surveillance and the identification of high-risk patients and also surrogate end points that will accelerate the discovery of new interventions. These studies featured the retina predominantly as a non-invasive proxy of microvascular health through the analysis of blood vessels in fundus images; for a summary, see [Table 2](#).

The Wisconsin Epidemiologic Study of Diabetic Retinopathy began in the USA in 1979 with the purpose of communicating the frequency and incidence of complications associated with diabetes (e.g. DR and visual loss, kidney complications such as diabetic nephropathy and amputations) and to identify contributing risk factors such as poor glycaemic control, smoking and high blood pressure.¹⁰² Recruitment and initial examination featured

younger-onset Type 1 diabetes ($n = 996$) and older-onset persons mostly with Type 2 diabetes ($n = 1370$) with a further five follow-up examinations from 1984 through 2012. Good control of blood sugar was associated with less risk of incidence and progression of DR and diabetic kidney disease.^{103,104}

Also in the USA, the Atherosclerosis Risk in Communities (ARIC) study started in 1987 and recruited over 10,000 participants. It was devised to investigate the causes of atherosclerosis and its clinical outcomes and variation in cardiovascular risk factors. ARIC was one of the first studies to quantify arteriolar and venular diameters in fundus images.¹⁰⁵ Lower AVR predicted a 3-year risk of CHD events in females (and not males), although it was not clear if arteriolar narrowing or venular widening were responsible. Retinal microvascular abnormalities were related to current blood pressure, to past blood pressure independently of current blood pressure and to various markers of inflammation and endothelial dysfunction.⁸

Commencing in 1992 in Australia, the Blue Mountains Eye Study (BMES) featured 3654 participants. Researchers examined the association of vessel diameter variations with CHD mortality, looking at arterioles and venules separately, and found that wider venules predicted a 9-year risk of CHD death in both males and females without a history of pre-existing CHD, while retinal arteriolar narrowing additionally predicted CHD death in females.¹⁰⁶ However, the results were not replicated by the Beaver Dam Eye Study (BDES), which included 4926 individuals in the USA.¹⁰⁷ This inconsistency was addressed through a combined data analysis of participants from BMES and BDES who did not have a history of CHD. Both narrower arterioles and wider retinal venules predicted 40–70% higher risk of CHD mortality in persons aged 43–69 years, although this was limited to CHD mortality outcome and not CHD events.¹⁰⁸

The Rotterdam study from Netherlands was set up in 1990 and designed to investigate the prevalence and incidence of and risk factors for chronic diseases in the elderly.¹⁰⁹ This included cardiovascular, neurological, locomotor and ophthalmologic diseases. In total, 7983 people participated in the study. Similar to the results of other studies, arteriolar narrowing was associated with an increased risk of hypertension.²⁴ Also, retinal venular widening was associated with an increased risk of vascular dementia⁵⁸ and larger retinal venular diameters were associated with an increased risk of stroke and cerebral infarction.¹¹⁰ These findings were in line with other observations and add further weight to the use of retinal imaging in investigating cerebral small-vessel disease, which is a major risk factor for both stroke and vascular dementia.

The Multi-Ethnic Study of Atherosclerosis was a prospective cohort study of 6814 participants, aged 45–84 years, without a history of clinical cardiovascular disease, sampled from 6 US communities.¹¹¹ Cardiac MRI was performed at the first examination (July 2000 to July 2002), and fundus imaging was performed at the second examination (August 2002 to January 2004). 4593 participants had both fundus images that were suitable for measurements and cardiac MRI data, enabling investigations into links between the retina and CHD. Retinal

Table 2. A summary of large-scale longitudinal studies featuring quantitative analysis of retina imaging (predominantly fundus camera)

Study name	Aims	Number of participants	Findings
Wisconsin Epidemiologic Study of Diabetic Retinopathy	Establish frequency and incidence of complications associated with diabetes, and to identify contributing risk factors	2366	Good control of blood sugar associated with less risk of incidence and progression of DR and diabetic kidney disease ^{103,104}
Atherosclerosis Risk in Communities	Investigate the causes of atherosclerosis and its clinical outcomes and variation in cardiovascular risk factors	>10,000	Lower arteriole-to-venule ratio predicted 3-year risk of CHD events in females (and not males). ¹⁰⁵ Retinal microvascular abnormalities were related to current blood pressure, to past blood pressure independently of current blood pressure and to various markers of inflammation and endothelial dysfunction ⁸
Beaver Dam Eye Study	Inform on the prevalence and incidence of common eye diseases causing loss of vision in an ageing population. Examine other ageing problems such as development of kidney and heart disease	4926	Narrower arterioles and wider retinal venules predicted 40–70% higher risk of CHD mortality in persons aged 43–69 years (CHD mortality outcome and not CHD events) ¹⁰⁸
Blue Mountains Eye Study	Assess visual impairment and common eye diseases of a representative older sample, as well as other ageing problems	3654	
Rotterdam Study	Investigate the prevalence and incidence of and risk factors for chronic diseases in the elderly	7983	Arteriolar narrowing associated with an increased risk of hypertension. ²⁴ Retinal venular widening associated with an increased risk of vascular dementia. ⁵⁸ Larger retinal venular diameters associated with an increased risk of stroke and cerebral infarction ¹¹⁰
Multi-Ethnic Study of Atherosclerosis	Investigate the prevalence, correlates and progression of subclinical CVD and risk factors that predict progression to clinically overt CVD, and that predict progression of subclinical disease itself	6814	Retinal arteriolar and venular diameter associated with cardiovascular risk factors—hypertension, diabetes, measures of obesity and dyslipidemia. Venular diameter associated with systemic inflammation. ¹¹² Narrower retinal arteriolar diameters and retinopathy in non-diabetic participants associated with increased risk of stroke independent of traditional risk factors and measures of atherosclerosis ¹¹³
UK Biobank	Creation of a databank, accessible to international researchers, for improving the prevention, diagnosis and treatment of a wide range of long-term conditions and chronic illness	67,716	No findings yet reported (to date)

CHD, coronary heart disease; CVD, cardiovascular disease; DR, diabetic retinopathy.

arteriolar and venular diameters are associated with a range of cardiovascular risk factors, including hypertension, diabetes, measures of obesity and dyslipidemia, while venular diameter is also associated with systemic inflammation.¹¹² Furthermore, in

this relatively healthy multi-ethnic cohort, narrower retinal arteriolar diameters and retinopathy in non-diabetic participants were associated with increased risk of stroke independent of traditional risk factors and measures of atherosclerosis.¹¹³

Important, new resources that feature retinal data exist within the UK. The largest is the UK Biobank which recruited 502,656 people aged between 40 and 69 years in 2006–10.¹¹⁴ With informed consent, participants completed a detailed touchscreen questionnaire about their lifestyle, environment, medical and family history, underwent a range of physical measures and provided blood, urine and saliva samples for future analysis. They also agreed to long-term follow-up of their health. A subset of 68,554 underwent fundus camera imaging and OCT. Genetics of Diabetes Audit and Research in Tayside (GoDARTS) recruited over 9000 participants (to date) in the Tayside region with a particular focus on Type 2 diabetes and collecting clinical measures, samples and medical history along with electronic linking to retinal images from screening.¹¹⁵ Both these resources represent examples of valuable data sets to mine for future investigations into retinal markers of chronic illness or long-term conditions. Further exciting opportunities will be created with the new Farr Institute, in which charities, Research Councils, Government and other bodies are investing at total of £39 million to support the safe use of patient and research data for medical research across all diseases, linking research in 19 universities around the UK.¹¹⁶

THE FUTURE OF RETINAL IMAGING AS A SOURCE OF BIOMARKERS

Early identification of chronic illness or long-term conditions through biomarkers derived from a simple and economical technique such as retinal imaging would allow patients to be treated more effectively, increasing patient benefit and decreasing care costs. To be clinically useful, retinal imaging biomarkers must demonstrate additional prognostic information for disease risk prediction over contributions from traditional risk factors. Traditional risk factors already have a moderately high predictive ability, but retinal image analysis has the potential to offer significant, additional insights. Furthermore, as larger and larger collections of cross-linked patient data become available, computational methods, image analysis and data science ("big data") will play an increasingly important role, following

the trend already well-established in life sciences, e.g. for genome analysis. This will in turn require an ever greater integration of imaging scientists and clinicians, and the increasing number of relevant, interdisciplinary funding calls issued by UK Research Council and the European Union (Framework VII and Horizon 2020) is a distinct push in this direction.

Interdisciplinary teams will be able to explore the interface sitting at the border between ophthalmology, neurology and computing science. It is from the detail and depth of retinal phenotyping from which novel metrics and candidate biomarkers are likely to emerge. Confirmation that *in vivo* retinal neurovascular measures are predictive of microvascular change in the brain and other organs is likely to be a major area of research activity over the next decade. Unlocking this hidden potential within the retina requires integration of structural and functional data sets, that is, multimodal mapping and longitudinal studies spanning the natural history of the disease process. And with further advances in imaging, for example, swept-source OCT, it is likely that this area of retinal research will remain active and clinically relevant for many years to come.

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