## Structural and spectroscopic in vivo imaging of the human retina with scanning light ophthalmoscopy

Damodaran, M.

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# 7

# Discussion and outlook

## 7.1 Background

Assessment of retinal health for diagnosis and monitoring of retinal pathologies is predominantly based on its appearance and is visualised by various imaging techniques such as scanning laser ophthalmoscopy (SLO). To this end, a compact, low-cost experimental SLO based on a digital micromirror device (DMD) as described in **Chapter 3** was designed and implemented. Almost all retinal diseases affect the optical properties of the retina. Quantitatively determining these optical properties or changes thereof is of primary interest to our understanding of these diseases and for effective clinical evaluation, follow-up and treatment. Spectral signatures in the retina are related to the chemical composition of the retina and can be used to assess important physiological parameters such as the oxygenation of retinal blood. This was the main motivation in constructing the multispectral SLO. A multi-spectral SLO based on a supercontinuum source was constructed as described in **Chapters 4-6**. Both these *en face* techniques mentioned above show the result of the interaction of a light source with the retinal tissue and were developed to translate them to clinical use for diagnostic and research purposes. **Chapters 3-6** were introduced with a detailed discussion focusing on the research question addressed in those chapters. In this chapter, a short description of key technical aspects is presented, followed by a discussion of its advantages and limitations. Additionally, technologies that can aid in retinal disease diagnostics in the future is presented. Finally, the conclusion of the thesis is given.

## 7.2 Digital micromirror based SLO



In this thesis, an extension to the line of work on DMD based ophthalmic scanners is presented to demonstrate the value of using the compact ophthalmoscope for imaging at higher speeds while not compromising the signal to noise ratio. In this work, a stand-alone DMD was used to, in contrast to the DLP projector used by others [1, 2]. Concentric circle illumination was used to provide fixation for the subject during measurement. As shown in **Chapter 3**, the ophthalmoscope can produce high contrast images at a 7 Hz imaging speed. The DMD based SLO potentially reduces cost and increases flexibility. Shifting the centre of the concentric circles to visualise different regions is a novel way to provide fixation while imaging different peri- and para-foveal regions. The system is modular with respect to the light source and enables changing the wavelength for imaging. This is advantageous, especially in achieving multispectral imaging.

The main limitation of the DMD based SLO is that the illumination light effi-

ciency is reduced. The DMD is operated at low fill-factor resulting in the majority of power is unused. Hence a high power and low divergence LED is needed to provide sufficient power for imaging the retina. Although a low divergence LED was used in our system, this might not be available for all wavelengths, particularly for the wavelengths of interest for multispectral applications. This is expected to improve in the future with the progress in LED source development. As we currently are a factor of 4 below the maximum permissible exposure according to the IEC standard 60825-1 [3], there is still room for further improvement. The near-infrared (NIR) illumination of the SLO provides a significant gain in the detected signal to noise ratio for structural imaging compared to visible light illuminations. The laser safety standards typically limit the visible illumination power. This, combined with the lack of low étendue sources, results in a lower signal to noise ratios compared with the NIR imaging. The polarization optics to reduce the stray reflections in the system lead to polarisation artefacts in the images due to birefringence of the cornea [4] and retina [5], influencing the images being recorded. Further, for extending the system to a multispectral retinal oximeter faced challenges due to the lack of high power, low divergence LED's in the suitable wavelengths for accurate estimation of retinal oxygenation. It was possible to extract motion information and use this to track the eyes movement using the DMD based SLO. When done fast enough, it will also enable doing eye motion correction in real-time [6–8] to improve the quality of the images by averaging multiple images at the same location and correct for the minor motion artefacts present in these images. Image registration could potentially be better with the parallel illumination as all the illumination points of a single frame are undistorted.

Multispectral retinal imaging has gained interest in recent years as it helps to gather quantitative information on retinal health. Multispectral imaging is more sensitive to subtle variations in the retina, compared to structural imaging. Using the DMD based technique, multispectral imaging can be extended to spatial frequency domain imaging (SFDI) [9] of the retina by projecting spatially confined sinusoidal patterns of different spatial frequencies to extract the absorption and scattering properties of the retina. SFDI can spatially resolve optical absorption and scattering properties and thus allows wide-field quantitative mapping of tissue optical properties. By separating and quantifying the multispectral absorption and scattering optical properties. SFDI provides a more direct assessment of tissue state and aids in measuring physiologically relevant parameters. SFDI can spatially resolve local properties in the retina such as oxygen saturation, total blood volume, and water abundance. It involves projecting sinusoidal illumination patterns of multiple spatial frequencies over a large area of the retina. The reflected image is

modified from the illumination pattern due to the specific retinal optical properties. These spatially-modulated waves are demodulated to calculate the retinal modulation transfer function. Using model-based analysis, the diffuse reflectance, and subsequently the tissue optical properties, specifically, the quantitative absorption ($\mu_a$) and reduced scattering ($\mu_s^{'}$) parameters can be extracted by this technique.

## 7.3 Quantitative retinal imaging

The potential range of applications for multispectral retinal imaging includes diseases that show abnormal colouring on routine ophthalmoscopy. Besides, spectral signatures are related to the chemical composition of the retina can be used to assess the oxygenation of retinal blood. This was the main motivation in constructing the multispectral SLO based on a supercontinuum source.

### 7.3.1 Retinal oximetry

**Chapter 4** presents the *in silico* analysis of the error propagation of measurement noise in retinal oximetry, to identify optimal wavelengths which will yield the lowest uncertainty in saturation estimation for a given measurement noise level. In these analyses, the effect of haemoglobin packing in discrete blood vessels, the so-called 'pigment packing effect' was also incorporated. This effect may result in a non-negligible bias in saturation estimation if unaccounted for under specific geometrical conditions such as sub-diffuse sampling of smaller blood vessels located deeper within the retina. The Optimal wavelength combination for retinal oximetry was identified based on these analyses. To validate the simulations, an SLO was constructed. Confocal reflectance measurements were then conducted on a tissue-mimicking scattering phantom with optical properties similar to retinal tissue. Narrow channels filled with absorbing dyes to mimic blood vessels were fabricated. By imaging at three optimal wavelengths, the 'saturation' of the dye combination was calculated. The experimental values showed good agreement with our theoretical derivations. As a follow-up, *in vivo* oxygenation measurements were performed in two healthy volunteers as presented in **Chapter 5**.

From a technical standpoint, **Chapter 5** describes an SLO system based on a supercontinuum source and high-quality passive double-clad fibres (DCFs) and of DCF couplers which improved the collection efficiency by approximately 2.5 times. The larger inner cladding for DCF was used for quasi-confocal detection to increase throughput and signal to noise. A balanced detection scheme was implemented to suppress the relative intensity noise of the supercontinuum source. Finally, by per-

forming a wavelength sweep between 485 and 608 nm, we determined an approximately linear relationship between the effective path length of photons through the blood vessels and the vessel diameter for our SLO geometry.

A demanding factor for clinically relevant retinal oximetry is to be able to determine blood oxygen saturation in small retinal vessels — capillaries, venules and arterioles. SLO based retinal oximetry has benefits over traditional fundus camera-based oximetry methods especially in terms of : resolution and contrast. It is in these microvessels that the oxygen saturation is expected to vary the most in response to increased metabolic demand or decreased oxygen delivery capacity. The larger retinal vessels ($>100\,\mu m$) are suspected to be less sensitive to variations in tissue metabolic requirement or microvascular dysfunction and are hence not ideal as early hypoxia markers. The clinical value of fundus camera-based oximeters is most likely limited due to the low spatial resolution. Even if the oxygen saturation estimates would be robust with such systems and more accurate taking into account our optimised wavelengths and our proposed algorithms that include pigment packaging (**Chapters 4 and 5**).

SLO based oximeters are not new to ophthalmic imaging, but their performance was limited due to sub-optimal choices for the wavelengths used due to lack of lasers at optimum wavelengths. Further pigment packaging, which is likely a non-negligible factor for smaller blood vessels located deeper in the retina, was not taken into account. The effective path length that photons travel through a tissue volume before being collected depends on the illumination and collection geometry and is also a function of the optical properties of the tissue and is, therefore, wavelength-dependent. We have assumed that the effective path length for the two optimum wavelengths in **Chapters 4 and 5** to calculate the ODs is almost the same. In the 450-600 nm wavelength range absorption remains of the same order and scattering varies slowly with wavelength (roughly as $1/\lambda$). However, a slight mismatch in the path lengths results in an offset in the saturation estimation. Additionally, in a diffuse sub approach, pigments which are present in the retina, especially in the retinal pigment epithelium (RPE), and absorption due to choroid blood can affect the recorded intensity. The effect of melanin on backscattered light varies with concentration [10], and a *a priori* knowledge of the pigment concentration in RPE melanin and its contribution to the backscattered light can aid in removing the influence of RPE in the backscattered light.

Saturation error and reproducibility are the major factors which contribute to the 'total cost of quality' of an oximetry system. To achieve acceptable levels of saturation error, a large number of points along the blood vessel and the tissue location had to be averaged (as explained in **Chapter 4**). This is a challenge for all

the three techniques mentioned in section 2.3.3, namely the fundus photography, SLO and visible-light optical coherence tomography (OCT). A *in vivo* image contains structural information and choosing many points might lead to an incorrect estimation of the intensity in the absence of a blood vessel. A smaller number of points around the blood vessel should be averaged to overcome this problem (as was demonstrated in **Chapter 5**), and this requires multiple images to be produced within a short amount of time, as is done with an SLO. Fundus cameras are based on snapshot imaging, and it might not always be possible to acquire multiple images continuously without causing discomfort to the subject or exceeding the safety limit for radiant exposure. Visible light OCT offers the possibility of functional retinal imaging, as most retinal chromophores possess clear absorption signatures in the visible spectrum. In OCT-based oximetry methods, layers of the retina free from the influence of RPE can be used for extracting the intensities. Nevertheless, OCT being a coherent detection method suffers from speckles, and a significant amount of averaging has to be performed to improve the image quality and reduce the error on the mean intensity down to 1%. The SLO can be easily integrated with the 1050 nm ophthalmic OCT to get the overall metabolism of the retina (oxygenation and flow) combined with the blood flow in the choroid. By performing a wavelength-sweep hyperspectral imaging (**Chapter 5**), spectral unmixing can be achieved by decomposing the mixed spectral signature of different chromophores into a set of chromophores and their corresponding abundances [11].

As a side note, the system described in **Chapter 5** can be combined with endoscopic scanners to extract blood oxygenation of the pulmonary and gastrointestinal tracts in a minimally invasive manner. In combination with the flow and immuno-NIRF [12], this would give useful information, for instance, on tumour development.



### 7.3.2 Retinal haemoglobin concentration

In **Chapter 6**, the first results are shown from the same experimental SLO system to measure the haemoglobin content in the retina. Although the demonstration of the method has been done with the sophisticated setup using a supercontinuum source, the method can be translated to clinics, and for field applications using a simpler and compact prototype. For example, a fundus camera employing low-cost hardware, and filters with transmission windows corresponding to the optimum wavelengths could be implemented for clinical applications.

## 7.4 Future directions

**Systemic diseases and retinal imaging**

Various systemic diseases affect the retina. There are multiple reports of retinal imaging findings in systemic diseases ranging from neurological [13] to blood infections [14]. The retinal vessel network is optimised for efficient flow [15], and malfunctions that are revealed in abnormal geometric parameters measured on fundus images can give indications of pathologies. These parameters include, but are not limited to vessel diameters fluctuations within a vessel, changes in vessel bifurcation geometry, blood vessel tortuosity and global complexity of the visible network. Such abnormal vessel patterning increases metabolic energy costs and reduces the efficiency of nutrient transport. Retinal microvascular health is indicated by both adequate vessel diameters and optimal branching architecture [16]. A few examples of the potential of fundus imaging in diagnosing and monitoring systemic diseases are described as follows:

**Diabetes** is a disease due to inadequate production of insulin or owing to cells becoming resistant to insulin. Diabetes has a profound effect on the body's microvasculature. Consequently, retinal vascular changes are also evident on fundus images with a widening of venular diameters found to be associated with subsequent incidence and progression of diabetes. Diabetes can have serious long-term complications, such as diabetic retinopathy (DR). Using retinal imaging to identify the early signs of DR in the eye can help in preventing vision loss and blindness from this disease, and for early intervention. Also, identification of worsening DR signals unstable systemic control of diabetes, prompting a re-evaluation of treatment and other risk factors.

Fundus imaging has been proposed as a screening and monitoring tool for **Alzheimer's disease** (AD) [17]. Frost *et al.* [18] demonstrated altered vessel geometry such as reduced vessel diameters, and less tortuous venules etc. in patients with AD compared to healthy controls. Furthermore, evidence from the *Rotterdam study* on retinal vascular abnormalities in 655 dementia patients [19] underscore the potential role of fundus imaging in distinguishing different forms of dementia. Einarsdottir *et al.* [20] showed that oxygen saturation in moderate Alzheimer's disease was statistically significantly increased compared to healthy individuals.

Retinal oxygenation measurements in subjects with chronic **cardiac or pulmonary disease** [21] has shown reliable results in identifying hypoxia. *Oxymap* and *Imedos* retinal oximetry findings show a good statistical correlation with peripheral finger oximetry. Blood from radial artery of pulmonary disease patients corresponds to

retinal oximetry measurements [22], there by showing a potential direction in exploring retinal imaging and retinal oximetry for cardiac and pulmonary ailments.

**Artificial intelligence in ophthalmology:**

Artificial intelligence (AI) has been used in recent years in ophthalmology for the automatic classification of eye diseases. There has been a paradigm shift in image interpretation and analysis due to the use of AI and specifically, deep learning. Deep learning is a collection of AI techniques that permit an algorithm to determine the relevant predictive features by models from a large set of examples rather than requiring features to be specifically predefined. Deep convolutional neural networks (CNV) has been employed for highly accurate classification and diagnosis of retinal pathologies [23] with accuracy comparable to that of experts.

Ophthalmic imaging is well suited for the implementation of AI-assisted automatic screening and investigation because of the wide-spread use of ophthalmic images which provide large amounts of data. The limited availability of retina specialists and trained human graders to diagnose these diseases is a major challenge in many countries. Hence, unmanned automatic applications and AI can be employed as a potential alternative to ophthalmologists and retina experts for detection and follow up of retinal pathologies.

For example, compact fundus cameras or phone add-on applications [24] can be used to collect retinal data. These devices not only make retinal imaging less expensive and accessible to various populations but also facilitate collection of large amounts of data which can be used to develop algorithms for classification, diagnosis and providing treatment options and advice. The major criticism of these portable fundus camera devices [25] is the inferior image quality. However, identifying good quality images in field conditions using automated image quality assessment is also gathering interest [26]. There can be two main approaches to achieve clinical diagnosis using such a portable or mobile device. In isolated areas where connectivity and access are poor, all the analysis needs to be performed on the device itself. On the other hand, in a setting with data connectivity, the images can be collected and analysed in a central cloud-based architecture for more advanced image processing and diagnostic analysis.

Combining retinal image classifications and diagnostic algorithms at the camera level can be beneficial for solving the need for high computation power. This will provide a paradigm shift in the way retinal imaging is viewed as a diagnostic tool and perhaps will provide a push towards 'point-of-care' and personal care retinal imaging devices.

'smart camera architectures' [27] which uses AI, for example, the deep-lens camera by Amazon web services which embeds advanced deep learning and image processing algorithms at camera level can be adapted for fundus imaging [28].

## 7.5 Thesis conclusion

This thesis presents imaging systems whose purpose is visualising the structure and function of the retina *in vivo*. The systems have been designed and implemented to demonstrate the technical capabilities and usefulness of the techniques. First, an *in vivo* ophthalmoscope based on a digital micromirror device was implemented. Annulus illumination was implemented to block most of the corneal reflections using a circular aperture in the detection arm.

Secondly, we have demonstrated *in vivo* human retinal imaging with a novel SLO based on a supercontinuum source. The RIN noise of the illumination source has been addressed by implementing balanced detection. The balanced detection increased the SNR, and therefore improved the image quality and accuracy of oximetry calculations. The implementation of an achromatizing lens reduced the effect of chromatic aberrations introduced by the human eye, although this did not have a large effect on saturation estimation. The use of a double-clad fibre with large inner cladding provided the best trade-off between image contrast and SNR. This allowed for high-resolution *in vivo* visualization of the oxygenation of retinal blood vessels in healthy volunteers. The oxygen saturation of the larger vessels of the retinas of two human volunteers was in agreement with the *Oxymap* derived saturation; besides, our SLO system was able to provide oxygen saturation in much smaller vessels. The saturation measured in 3 different imaging sessions of the same volunteer (8 minutes apart) demonstrated the reproducibility of our measurements. Several innovative methods to extract information from the retina are described. A novel vessel segmentation algorithm that delineates the blood vessels and tissue was described to facilitate the segmentation of small blood vessels for oximetry estimation. It is expected that the SLO will be a valuable tool to study the effect of degenerative retinal diseases on the oxygenation in the retinal microvasculature. Finally, by performing a wavelength sweep between 485 and 608 nm we determined an approximately linear relationship between the effective path length of photons through the blood vessels and vessel diameter for our SLO geometry that employs a DCF with a large inner cladding. Using this relationship, first results on the haemoglobin content in the retinal blood vessels was calculated using two isosbestic wavelengths.

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