Chapter 1

Introduction
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1. Corneal vascularization

Corneal vascularization is a common and potentially sight-threatening condition. It can develop from almost any insult to the cornea – from chronic ocular surface inflammation, chemical injury, and contact lens overuse,\(^1\) to the most common infections such as herpes and trachoma – Figure 1.\(^{2,4}\) The healthy human cornea is avascular and transparent; corneal vascularisation is a pathological condition whereby the avascular cornea loses its transparency due to ingrowth of blood vessels. It is believed that the balance of angiogenic and anti-angiogenic factors is important to ensure the avascularity, and this is maintained by the inhibition of immune and inflammatory response.\(^3\) Visual impairment may occur secondary to persistent inflammation, due to corneal edema, lipid deposition, and scarring.\(^5\) Further to this, the vascularization causes a disruption to the immune privilege of the cornea, increasing the risk of rejection should corneal transplantation be required in these eyes.\(^6\)

![Image](Figure 1. Left: Extensive corneal vascularization leading to visual impairment from chronic inflammation and scarring. Right: Corneal vascularization after corneal transplantation, leading to a higher risk of rejection and graft failure.)

2. Current diagnostic techniques for corneal vascularisation are limited

The main modes of assessment for cornea and anterior segment vasculature are slit-lamp photography (SLP) and dye-based angiography. Currently, SLP is the most commonly used method for delineating corneal vasculature in clinical and experimental applications. In SLP, photos of the eye are taken with a specially mounted camera on a microscope that has a primary illumination that can be adjusted from a broad to a narrow slit of light. However, SLP has limited visualisation of vessels in the presence of corneal edema, deposits, or scars; while image analysis often results in underestimation due to poor sensitivity to smaller vessels and interference from background iris vessels. Also, only two-dimension information of the vasculature can be derived.

Fluorescein angiography (FA) and indocyanine green angiography (ICGA) are more reliable methods for evaluating normal and diseased vessels clinically – Figure 2.\(^3\) In FA and ICGA, intravenous fluorescein or indocyanine green dye is injected into the patient and the eye is photographed using specialized video angiography or digital imaging techniques to capture the dye traversing the blood vessels. These methods are traditionally used for imaging the normal circulation and abnormal vessels in the fundus of the eye (retina and choroid). In the cornea, FA and ICGA are infrequently used but it has been demonstrated that these techniques show better vessel delineation than SLP, especially for vessels beneath corneal
In addition, leakage observed in FA and ICGA can give information on vessel maturity (new vessels tend to have leaking walls), and, unlike SLP, FA and ICGA allow for differentiating afferent and efferent vessels. Furthermore, since ICG is a large molecule that remains in vessels for long periods, ICG leakage is likely indicative of a pathological condition. However, these invasive techniques are rarely performed due to infrequent but severe adverse reactions associated with the dyes, including gastrointestinal side effects and anaphylactic shock, even for patients with no risk factors or history of allergies. Patients who have impaired kidney function, or are pregnant, are also not compatible with such techniques. In addition, leakage may prevent visualization of deeper vessels, causing underestimation of the extent of vascularisation. While current angiography methods allow qualitative assessment of the anterior segment vasculature, objective and quantitative evaluation is still lacking.

Figure 2. ICGA angiography of the cornea vessels is rarely performed due to invasiveness, potential side effects, and difficulty in interpretation.

3. Imaging the vessels in the cornea and anterior segment of the eye is a clinical unmet need

A recent round-table expert discussion suggested that objective evaluation of corneal vessels is becoming increasingly important, as new treatment modalities for treating corneal vascularization emerge. As outlined above (Section 1), detecting and treating corneal vascularisation is critical for preventing significant loss in visual acuity or even blindness. Qualitative and quantitative assessment are needed for optimal disease monitoring, treatment planning, and prognostic evaluation. A rapid, objective imaging system to evaluate corneal vascularization would be a welcome addition to our clinical practice. This diagnostic tool would have a direct clinical impact of being able to monitor corneal vessels in patients in common clinical scenarios such as in ocular surface diseases, infections, and corneal transplantation. Furthermore, as neovascularization is not limited to the cornea, non-invasive imaging techniques that can measure vascular changes in other parts of the anterior segment (anterior part of the eye consisting of the cornea, anterior chamber, trabecular meshwork, iris, and lens) as well, are needed.

4. Optical Coherence Tomography is an important imaging tool for the anterior segment of the eye

Optical coherence tomography (OCT) imaging is a well-established technique that enables non-invasive and rapid in vivo imaging of the eye. Since it was first introduced, OCT imaging has become an integral part of clinical assessment, first for the posterior segment (especially the retina) but now also for the anterior segment – Figure 3. By applying low-coherence light and measuring the “echo” of light backscattered from tissue structures, OCT can provide high-resolution three-dimensional structural images. These images are very useful for the management of chronic eye disease like macular degeneration, diabetic retinopathy, and glaucoma, as well as for preoperative diagnosis, intraoperative real-time imaging, and post-operative evaluation of diseases. Regular OCT systems produce poor delineation of

scars.
blood vessels due to scattering of light - blood vessels produce artefacts rather than useful information. Hence, to further the field, a modification that would enable visualization of vascular flow is urgently needed.

Figure 3. OCT imaging of the cornea provides detailed cross-sectional imaging of the cornea and associated pathologies such as Descemet membrane detachment (top left), infection (top right), corneal edema (bottom left) and hydrops (bottom right).

5. OCT Angiography is an emerging imaging technique for vessels in the posterior segment

A recent advancement in OCT technology has led to OCT angiography (OCTA), an emerging technique for imaging ocular vasculature. Basically, it works based on signal decorrelation between consecutive OCT scans. Essentially, changes in intensity between consecutive scans denote flow. OCTA is currently used clinically for the delineation of vessels within the retina, choroid, and optic nerve. The commercially available systems are designed for angiography of retina vessels, and have been useful in the assessment of pathologies in the posterior segment of the eye, including retinal neovascularisation, retinal artery and vein occlusion, and glaucoma - Appendix 1.

6. OCT Angiography is potentially a useful technique for the anterior segment as well

Currently, OCT is widely used to image the cornea and the anterior segment in our daily clinical practice. Thus, adapting OCTA for the cornea would be a logical next step; it would provide not only information on structures in the front of the eye, but also image its vasculature. As mentioned, clinical applications include non-invasive imaging of corneal vascularisation to evaluate inflammation, ocular surface damage, and prognostication of corneal transplantation. Thus OCT angiography would be a welcome addition to the diagnostic armamentarium for the cornea and anterior segment clinician. However, as current OCTA systems are designed for retinal imaging, modifications are needed to image the anterior segment, both of the hardware (an adaptor lens) and of the software.

There are currently four different OCTA systems available in the market. They use different algorithms to compare OCT images to produce angiograms, including full- or split-spectrum amplitude decorrelation angiography (FSADA or SSADA respectively), optical microangiography, and ratio analysis. These systems also differ in scanning speed, scan area, resolution, and internal software that allows for motion correction, projection artefacts removal, or automated segmentation, to name a few. A good image generally requires a good balance between sampling density, field of view, and scan duration. Table 1 summarizes the currently available OCTA systems that may be used specifically for imaging the cornea and anterior segment of the eye.
Table 1. There are currently four main types of OCTA systems that may be adapted for anterior segment imaging available: (1) the AngioVue (Optovue, Inc., Fremont, California, USA), (2) the Angioscan (Nidek Co Ltd, Gamagori, Aichi, Japan), (3) the Triton Prototype DRI-OCT (Topcon Corporation, Tokyo, Japan), and (4) the Plex Elite 9000 (Carl Zeiss Meditec, Dublin, California, USA). AngioVue and Angioscan are spectral-domain OCTA systems, which uses SSADA and optical microangiography algorithm, respectively. Triton and Plex Elite are swept-source OCTA systems, which uses ratio analysis and complex microangiography, respectively.

7. Aim and Outline of Thesis

OCT Angiography would potentially provide a rapid, non-invasive imaging modality for the anterior segment, and especially the cornea, as it produces three-dimensional image constructs of structures and its associated vascular supply simultaneously. However, current systems are not optimized and designed for angiography in the cornea. The aim of this thesis is to:

1. Establish that OCTA systems currently designed for the retina are useful for imaging the cornea
2. Optimize the OCTA images and segmentation for the cornea
3. Compare OCTA to the current gold standard, i.e., ICG angiography

I first begin by confirming the validity and repeatability of OCTA images of corneal limbal vasculature that were acquired using the OCTA designed for the posterior segment and adapted for the cornea, in normal healthy eyes (Chapter 2).

Next, the OCTA images of the cornea require image analysis, thus I describe the technique of en face analysis and segmentation of OCTA images in eyes with abnormal corneal vessels (Chapter 3).
Once the OCTA imaging technique and image analysis is established, in **Chapter 4**, I describe the comparison of OCTA imaging of the cornea compared to the current gold standard (ICGA). The next step is to describe how the OCTA imaging of the cornea is useful for serial scanning and monitoring in abnormal corneal vessels in human eyes for detecting progression as well as treatment response (**Chapter 5**).

In **Chapter 6**, I then evaluate OCTA imaging of the cornea in an animal model by inducing abnormal corneal vascularisation and comparing the assessment of this vascularisation between OCTA, ICGA, histology, and confocal microscopy. Finally, I compare two different OCTA systems adapted for cornea, to highlight that while both systems provide useful images of corneal vascularisation, they cannot be compared to one another in terms of vessel measurements (**Chapter 7**).

**Appendix 1** is a review article that provides an overview and detailed technical aspects of OCT and OCTA for the anterior and posterior segment.

**References**

5. Menzel-Severing J. Emerging techniques to treat corneal neovascularisation. Eye (Lond) 2012;26:2-12.