Development of cellular resolution Gabor-domain optical coherence microscopy for biomedical applications

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ABSTRACT

We have developed a cellular resolution imaging modality, Gabor-Domain Optical Coherence Microscopy, which combines the high lateral resolution of confocal microscopy with the high sectioning capability of optical coherence tomography to image deep layers in tissues with high-contrast and volumetric resolution of 2 \textmu m. A novelty of the custom microscope is the biomimetics that incorporates a liquid lens, as in whales’s eyes, for robust and rapid acquisition of volumetric imaging of deep layers in tissue down to 2 mm, thus overcoming the tradeoff between lateral resolution and depth of focus. The system incorporates a handheld scanning optical imaging head and fits on a movable cart that offers the flexibility in different biomedical applications and clinical settings, including ophthalmology. In the later, the microscope has successfully revealed micro-structures within the cornea and in particular the endothelial cells micro-environment, an important step in understanding the mechanisms of Fuchs’ dystrophy, a leading cause of the loss of corneal transparency. Also, the system was able to provide high definition of the edge of soft contact lenses, which is important for the fitting of the lens and the comfort of the patient. Overall, the imaging modality provides the opportunity to observe the three-dimensional features of different structures with micrometer resolution, which opens a wide range of future applications.

Keywords: Optical coherence tomography, imaging system, image processing, GPU parallel computing, biomedical optics, soft contact lens metrology, corneal imaging

1. INTRODUCTION

Optical Coherence Tomography (OCT) is an optical imaging technique that has led to impressive developments during the past decades and is still presenting a greatly untapped potential for the future\textsuperscript{1-3}. Focused investigations across various application fields are driving the advancement of the capabilities of OCT to achieve higher resolution and speed. The axial resolution of OCT has reached the sub-micrometer scale along the rapid development of ultra-broadband sources. The lateral resolution of conventional OCT instruments is limited to tens of micrometers and hampers the adoption of OCT in a wide range of applications that require cellular resolution comparable to or approaching histology. Indeed, the numerical aperture (NA) of the optics sets the lateral resolution in the focal plane of the optics and throughout the depth of focus. However, the depth of focus is inversely proportional to NA\textsuperscript{2}. As a result, OCT typically operates at low NA with a corresponding lateral resolution in the order of 10 to 20 \textmu m, which enables a long depth of focus on the millimeter scale at the expense of lateral resolution. The need of cellular-resolution images of deep layers in tissues, up to millimeters deep, is one of the challenges in biomedical imaging, nevertheless crucial in reducing the need of biopsy and allowing in-vivo investigation of disease mechanisms. Different approaches, including hardware implementation\textsuperscript{4-6} and computational technique\textsuperscript{7,8}, have been proposed to achieve high lateral resolution comparable to confocal microscopy, while preserving the long depth of imaging offered by OCT technique. Full field en face OCT was also developed as a solution to provide high lateral resolution imaging at the expense of lower volumetric imaging speed and lower sensitivity\textsuperscript{9}. Optical coherence microscopy (OCM) was introduced to achieve cellular resolution by combining...
the high axial resolution of OCT and high lateral resolution of confocal microscopy gating to achieve high-contrast imaging of deep layers in tissue\textsuperscript{10}. The gain in lateral resolution in OCM is reached at the expense of a limited depth of focus in the order of 100 to 200 µm\textsuperscript{11}. In this paper, we highlight the development of Gabor-domain optical coherence microscopy (GD-OCM) conceived to extend the imaging depth of OCM to the millimeter range while maintaining a constant cellular resolution of 2 µm in biological tissue. Section 2 of this paper will review the development of GD-OCM from the concept to a mature system with clinical settings. In section 3, we will focus on our advancements in two different biomedical applications: corneal imaging and soft contact lens metrology. Section 4 will conclude this study with some perspectives.

2. DEVELOPMENT OF GABOR-DOMAIN OPTICAL COHERENCE MICROSCOPY

2.1 History

The concept of GD-OCM was first introduced in 2008 \textsuperscript{12}. A liquid lens was incorporated in a bio-inspired optical imaging head to dynamically refocus at different depth locations up to 2 mm to acquire multiple images that were then combined in a single volume using automatic Gabor-based fusion technique\textsuperscript{13,14}. The optical imaging head was evaluated to produce images with a constant lateral resolution of 2 µm across a 2 mm x 2 mm field of view\textsuperscript{15}. After the manufacturing and integration of the custom imaging optics, different aspects of the GD-OCM system were completed, including a custom broadband astigmatism-corrected Czerny-Turner spectrometer developed for higher sensitivity detection\textsuperscript{16}. The first demonstrations of the volumetric cellular resolution achieved with GD-OCM in biomedical application were performed in skin imaging\textsuperscript{17,18}. However, the large dataset and the multiple computational tasks associated with GD-OCM compound the need for fast processing. Performing the processing steps on conventional central processing unit (CPU) architectures was taken about two orders of magnitude longer than the acquisition steps. Critical to the adoption of GD-OCM in the clinical workflow is a fast, real-time processing and rendering of the high-resolution images. A parallel computational framework using multiple-graphic processing units (GPUs) to enable real-time imaging capabilities of GD-OCM was developed and reported\textsuperscript{19}. In 2013, the entire system was moved from the bench to a movable cart to allow the flexibility needed in clinical settings.

2.2 System description

The current imaging system (Fig. 1) with micron-class resolution (i.e., 2 µm both laterally and axially) and high-speed processing is described below. The source is a super luminescent diode laser centered at 840 nm and with 100 nm FWHM (BroadLighter D-840-HP-I, Superlum®, Ireland). The reference arm integrates a polarization controller and a custom dispersion compensator that allow maintaining constant resolution across the thickness of up to 2 mm\textsuperscript{20}. The sample arm, equipped with a galvanometer-based scanner (Dual axis, Cambridge Technologies Inc.), is configured to scan a 1 mm x 1 mm field of view with a 1 µm sampling step. The liquid lens focusing range is calibrated to image different depths of the sample while maintaining a lateral resolution of 2 µm within the imaging depth adjustable up to 2 mm. A high-sensitivity custom spectrometer with a high-speed CMOS camera (spl4096 –140km, Basler Inc., Exton, Pennsylvania) is used to capture the spectral information\textsuperscript{16}. The handheld scanning probe is attached to a rotatable arm that allows flexibility in imaging under multiple configurations. The multi-GPU framework was recently updated to four NVIDIA® GTX Titans to allow real time visualization of high-resolution images. During the acquisition of the each zone, which consists of a data size of 1000 x 1000 A-scans, the data is held in a temporary CPU buffer. When the acquisition of this zone is completed, the data is structured and divided into four sections, which are transferred to the four GPUs via the four PCIe-2.0 interfaces. During that time, the focal length of the liquid lens is shifted by 100 µm to acquire the next zone. The acquisition of the next zone is done in parallel with the processing of the previous zone. We reported a visualization of dataset with 1000 x 1000 x 400 voxels 6 seconds after the completion of the acquisition step that takes ~1.5 minutes\textsuperscript{19}.
3. APPLICATIONS IN BIOMEDICAL FIELD

3.1 Assessing the microenvironment of corneal endothelial cells

The cornea is the outermost component of our visual system and plays key roles such as protecting the eye against germs, dust and harmful matter, as well as refracting the incoming light in the eye. The cornea is composed of several layers (the epithelium, the Bowman’s layer, the stroma, and the endothelium and its basement membrane – Descemet’s membrane (DM) –), each playing distinct and important functions. One of the most important characteristics of the cornea is its perfect transparency due to the hyper-regular organization of the collagen fibrils in the stroma and maintained by the deturgescence state of the cornea.

The corneal endothelium is the innermost corneal layer made of a monolayer of cells whose primary function is to maintain the corneal transparency by pumping excess fluid out of the stroma to aqueous humor. Dysfunction of endothelial cells (ECs) leads to greater hydration of the corneal stroma, which can cause irreversible corneal edema, itself causing opacity and blindness. Corneal transplantation is nowadays the only therapy available to treat corneal opacity caused by EC dysfunction, such as Fuchs’ endothelial dystrophy (FED).

A better understanding of in vivo ECs microanatomy through non-invasive imaging is crucial to identify anomalies at the early stage of FED before the disease becomes symptomatic. The capability of GD-OCM to provide the 3D distribution of corneal microstructures was first investigated and validated using fresh pig eyeballs obtained from a local abattoir. The experiments were performed within three hours postmortem. As shown in Fig. 2, the 3D high definition images of the central porcine cornea, which thickness was measured to be ~850 µm, can be achieved with a field of view of 1 mm × 1 mm. The different layers of the cornea can be distinguished in the x–z cross-sectional image (Fig. 2b). En face x–y images at different depths in the cornea also show a corneal nerve, stromal keratocytes, and the posterior layer: endothelium with its basement, DM (Fig. 2d–f). Importantly, a 3D view of ECs with a field of view of 1 mm × 1 mm using OCT-based technology was demonstrated (Fig. 2c).
Such a large field of view is beneficial for increasing the reliability of quantitative analysis of ECs morphology and EC density calculation, which are key factors in the diagnosis of endothelial dystrophies, the evaluation of donor corneas, as well as in the follow up of patients having received a corneal transplantation. Future work will investigate excised human corneas at different stage of the FED in order to provide insights into the progression of the disease.

3.2 Evaluation of the edge of soft contact lenses

Today, more than 125 million people worldwide wear contact lenses, with about 38 million in the United States alone. This number is expected to gradually increase, given the various benefits that contact lenses provide in daily activities compared to eyeglasses. Contact lenses correct refractive error of the eye without disturbing daily activities and sports. However, contact lens wearers might easily experience discomfort primarily caused by the interaction of the lens with the ocular surface, as supported by the reports of the TFOS International Workshop on Contact Lens Discomfort. The interaction between a contact lens and the eye primarily involves the movement of the CL, which allows for the exchange of tears and removal of metabolic waste products when the eye blinks. Shen et al. indicated the possibility that this interaction with the CL, especially near the edge, largely determines the comfort of the wearer and the health of the ocular surface. The significance of the edge in terms of discomfort and quality of vision has also been supported with recent studies. For instance, Shen et al. reported that the thinner edge configuration helps to form a smoother transition between the edge of the lens and the conjunctiva and thus reduces mechanical irritation of the ocular surface. The quality control of the contact lens edge during and after manufacturing is needed in order to maintain the optimum comfort of the patient and the health of the ocular surface. Davidson et al. used OCT to measure the mechanical prism of contact lenses at 0.3 mm from the edge. Their automated algorithm and analysis established the capability of spectral domain OCT as a tool for non-destructive, fast, and reliable contact lens metrology. An extension to the developed methods would include the ability to assess the full edge and to extend the 2D scans imaging to 3D provided that the 3D orientation of the lens is critical to the accuracy of the measurement, especially at the edge. We report on the investigation of the capability of GD-OCM to provide high definition 3D imaging of the contact lens edge (Fig.3). The soft contact lens was imaged in three different configurations (Air, immersed in the water, and fit onto the cornea of the porcine eyeball). As compared to conventional OCT where lateral resolution is limited to tens of micrometers, the 2 µm resolution in three dimensions of GD-OCM is highlighted in this study for contact lens metrology. Future work will develop algorithms to accurately and automatically evaluate the edge-thickness of soft contact lenses.
Figure 3. High definition imaging of the edge of a soft contact lens (CL) using GD-OCM in three different configurations: (a) soft CL in air, (b) CL in plastic bag filled with water, and (c) CL on cornea of a porcine eyeball.

4. CONCLUSION

We developed an OCT-based system capable of achieving histology grade volumetric resolution for in-vivo clinical applications. We demonstrated the capabilities of GD-OCM to resolve the microstructures of the cornea, which can represent a relevant tool for corneal imaging and disease diagnosis. A better understanding of in vivo ECs microanatomy through non-invasive imaging is crucial to identify anomalies at the early stage before the disease become symptomatic. Also, the imaging system could be suitable for in vivo analysis of the different aspects of tissue bioengineering considered as a fundamental component in tomorrow’s treatment for the most diseases. In addition, the ability of imaging with high-definition the edge of soft contact lenses could help designers to finely analyze the edge for further development. In the medical perspective, as GD-OCM can provide high-resolution images of the microstructures of the cornea, it may also be used to study the interaction between the contact lens, the ocular surface, and the tear film, which can provide insights into the impact of long term use of contact lenses. The capability of GD-OCM is currently being extended in a wide range of application in material engineering, tissue engineering, and biomedical research.

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