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Bessel-Gauss Beam Light Sheet Assisted Fluorescence Imaging of Trabecular Meshwork in the Iridocorneal Region Using Long Working Distance Objectives

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ABSTRACT

Glaucoma is one of the leading cause of blindness characterized by increased intra ocular pressure (IOP), visual field defects and irreversible loss of vision. Remedial intervention of glaucoma primarily aims at the reduction of IOP and subsequent examination concerning the related anomalies in the aqueous outflow system (AOS) especially with newer angle procedures. Thus, high resolution imaging of the iridocorneal angle (ICA) region comprising trabecular meshwork (TM) is extremely valuable to clinicians and vision analysts in comprehending the disease state for the efficacious analysis and treatment of glaucoma. Imaging of the AOS inside the eye using the digitally scanned Bessel-Gauss beam light sheet microscopy has been used in this study to obtain high resolution optical sections with minimal phototoxicity and photobleaching. This paper investigates the effect of long working distance objectives in obtaining high resolution TM images while offering non-contact and non-invasive approach in imaging. A series of experiments were conducted to optimize various imaging parameters using porcine eyes as test samples. Investigations carried out by illuminating both the anterior segment region and limbal region resulted in promising results. A delineated network of collagen fibers in a meshwork fashion can be clearly seen in the obtained images of the TM. The optical sectioning capability of this technique is demonstrated and the structural features match well with previous literature reports.

Keywords

Bio optics; Bessel-Gauss beam; Glaucoma; Iridocorneal angle; Trabecular mesh work

1. INTRODUCTION

High resolution imaging and ultra-sensitive detection techniques in biomedical optics are remodeling the medical landscape with newly developed clinical instruments [1-3]. Various methodologies and techniques have been developed over the last few decades towards resolution improvement, and aberration and curvature correction for bio optic applications [1, 4-8]. Vision researchers around the globe have been constantly striving to innovate better diagnostics and treatment methods for various eye ailments. Primary angle closure glaucoma is one of the major eye diseases causing blindness worldwide [9]. Glaucoma is an eye disease caused by irregularities in the ocular aqueous outflow system (AOS) causing an elevated intraocular pressure (IOP) that may result in the decay of retinal ganglion cells, resulting in loss of vision. Primary angle closure glaucoma may occur when the iris is pushed or pulled against the drainage channels at the iridocorneal angle (ICA) region [10]. The aqueous outflow system at ICA consists of the trabecular meshwork (TM), the Schlemm's canal and the collector channels [11]. Imaging ICA in high resolution is of great diagnostic value to clinicians for the assessment of the disease state. The main diagnostic techniques currently available for ICA visualization can be broadly classified into photographic (gonioscopic photography [12], EyeCAMTM [13], pentacam [14] etc.) and optical tomographic methods (Ultrasound biomicroscopy [15], optical coherence tomography (OCT) [16] etc.). All these imaging modalities allow visualization of ICA and help in the diagnosis and management of glaucoma. There are advantages and inadequacies in measurement reliability and repeatability associated with each of these techniques. The widely used clinical reference standard for ICA assessment is gonioscopy. However patient discomfort and physician compliance are two main concerns of this technique. Since this technique is quite tedious and requires cumbersome methods and expertise to record the data, ICA images from gonioscopy are often not documented. In addition multiple reflections from the gonioscopic lens or mirrors used along with the coupling gel affects the image quality of the gonioscopic system [17]. To be able to resolve the TM structures, a spatial resolution of about 1–5 μm is required. Another clinically effective, state of the art tool used in the assessment of ICA to investigate angle closure is OCT. Even though OCT can image features on a micrometer

scale, the low image contrast of the OCT images restricts the visualization of TM structures [18]. In other words, these commonly used techniques usually can't deliver high resolution images required for the visualization of the TM structures.

In this regard we consider light sheet fluorescence microscopy (LSFM), as a potential imaging solution that has the ability to produce high resolution optical images with minimal photobleaching and phototoxicity [19]. In conventional static LSFM, a stationary sheet of light is projected onto the sample plane utilizing a cylindrical optics. The fluorescence signal emitted by the sample is then imaged using an imaging optics placed perpendicular to the illumination axis. This technique can generate two dimensional optical sections of the samples under investigation with high resolution in large field of view. A thinner and high signal to noise ratio light sheet with an extended depth of focus can be realized by digitally scanning a Bessel-Gauss beam in one dimension [20]. The superiority of Bessel-Gauss beam based LSFM had been demonstrated by several research groups. [21, 22]. Here we present our recent advances in Bessel-Gauss beam light sheet microscopy for high resolution imaging of the ICA, in particular the trabecular meshwork. By incorporating long working distance (WD), high numerical aperture (NA) objectives, this method provides a non-contact and non-invasive technique for high resolution imaging of the TM.

2. EXPERIMENTAL

2.1 Preparation of samples

Figure 1. Absorption and emission spectra of aqueous fluorescein sodium solution

Randomly collected porcine eye samples (*Sus scrofa domestica*) were used as ex-vivo samples for the measurements. The samples were collected from a local abattoir and are transported & kept on ice till investigations began for maintaining the freshness. The samples were mounted on to a custom made eye holder and the anterior chamber of the eye samples was injected with 0.2 ml of 0.05% w/v fluorescein sodium (Bausch & Lomb, UK). The experiments were completed within 8 hours of demise. Proper precautions and regulations on bio safety were followed according to the regulations of Nanyang Technological University (NTU) and Agri-Food and Veterinary Authority (AVA) of Singapore. The absorption and emission spectra of aqueous fluorescein sodium solution are shown in Figure 1. The emission spectrum shown here is obtained by exciting the fluorescein solution at 488 nm,

corresponding to the excitation wavelength used for the measurements.

2.2 Optical setup for imaging

Figure 2. Schematic of the Bessel-Gauss beam light sheet microscopy used for the imaging of ICA

For the fluorescence excitation, a fiber coupled continuous wave (CW) laser diode of 488 nm emission wavelength (LUX-488 Omicron, Germany) was used. The beam was expanded and collimated to the required size with the help of an external beam expander. A 176° apex angle plano convex axicon lens (Thorlabs) was used for the generation of the Bessel-Gauss beam. The generated Bessel-Gauss beam was made incident on a two axis Galvano scanner and directed to the illumination objective using a tele-centric scan lens followed by a tube lens. An infinity corrected, long working distance (WD = 20 mm) apochromatic objective of 20X magnification was used (Plan Apo, NA 0.42, Mitutoyo, Japan) for sample excitation. The Bessel-Gauss light sheet was created by scanning the vertical mirror of the Galvano scanner. The imaging arm consisted of an identical infinity corrected long working distance objective and a tube lens ($f = 200$ mm, WD = 148 mm, Thorlabs) with the optical axis orthogonal to the excitation arm. A 60 mm macro lens (Nikon AF Micro Nikkor 60 mm) was used for an easier collection adjustment at the camera end. Scattered excitation laser was isolated with the help of a 488 nm notch filter in the imaging system. A 5.5 megapixel sCMOS camera (Andor

Neo, UK) was used for recording the images. The illumination laser power was always kept lower than the maximum permissible exposure (MPE) limit recommended by the American National Standards Institute (ANSI) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [23, 24]. The experimental setup used for the measurements is outlined in Figure 2.

3. RESULTS AND DISCUSSION

Compared to a conventional Gaussian beam, the Bessel-Gauss beam can maintain a focused spot over a relatively larger distance [25, 26]. Additionally, the use of self-reconstructing Bessel beam results in the improvement of the sample image contrast and the reduction in shadowing and scattering artifacts. The use of fluorescence dye enhances the contrast of the images further. In addition, the digitally scanned light sheet technique offers a better control over the intensity profile and size of the generated light sheet compared to conventional static light sheet microscopy.

Images obtained using the digitally scanned Bessel-Gauss beam light sheet from a porcine eye sample are presented in Figure 3. These images clearly show the ICA region and the trabecular meshwork can be distinctly identified in these images. The optical sectioning capability of this technique is presented in a series of images (Figure 3b - 3d). These images are obtained by progressively moving the detection objective towards the sample in steps of 50 μm .

Figure 3. Optically sectioned Images (at 50 μm steps) of porcine TM obtained using the Bessel-Gauss beam light sheet microscopy

4. CONCLUSION

An imaging system based on digitally scanned Bessel-Gauss beam light sheet for the high resolution visualization of the iridocorneal angle region in the eye is presented with illustrative examples. The results shows a delineated network of collagen fibers in a meshwork design. Structural details of the obtained TM images are found to be in good agreement with previous reports in literature [27-29]. The optical sectioning capability of this technique is also demonstrated. High resolution imaging of the ICA and TM can help in the early and accurate detection of glaucoma, which empowers clinicians and vision researchers to comprehend the pathology and diverse conditions of the eye disease for ensuing treatment and follow-up methodology. Improved direct high quality visualization of the TM region through this system can be used for Laser trabeculoplasty, and further glaucoma management.

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