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Is oblique scanning laser ophthalmoscope applicable to human ocular optics? A feasibility study using an eye model for volumetric imaging

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Abstract

Oblique scanning laser ophthalmoscopy (oSLO) is a novel imaging modality to provide volumetric retinal imaging without depth sectioning over a large field of view (FOV). It has been successfully demonstrated in vivo in rodent eyes for volumetric fluorescein angiography (vFA). However, engineering oSLO for human retinal imaging is challenging because of the low numerical aperture (NA) of human ocular optics. To overcome this challenge, we implement optical designs to (a) increase the angle of the intermediate image under Scheimpflug condition, and (b) expand the magnification in the depth dimension with cylindrical lens to enable sufficient sampling density. In addition, we adopt a scanning-and-descanning strategy, resulting in a compact oSLO system. We experimentally show that the current setup can achieve a FOV of $\sim 3 \times 6 \times 0.8 \text{ mm}^3$, and the transverse and axial resolutions of 7 and 41 μm , respectively. This feasibility study serves an important step for future in vivo human retinal imaging.

Graphical Abstract

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Keywords

human eye model; large field of view; oblique scanning laser ophthalmoscope; volumetric retinal imaging

1 | INTRODUCTION

The development of modern optical retinal imaging methods has revolutionized the clinical practice of ophthalmology and vision science. Each technique has its unique strength and is best suited for certain retinal pathologies [1–5]. Among them, optical coherence tomography (OCT) provides 3D structural images of the retina and choroid with micrometer-scale resolution. OCT angiography (OCTA) has emerged as an important tool in imaging 3D microvasculature over a large field-of-view (FOV) non-invasively [6]. One major limitation of OCT is that it detects the scattering signal, and thus is insensitive to fluorescent contrasts [7]. On the other hand, fluorescence-based imaging methods, such as fluorescein angiography (FA), indocyanine green angiography (ICGA) and autofluorescence imaging are valuable in providing other functional information in clinics. However, all these fluorescence-based methods have limited capability in volumetric retinal imaging.

One common method to image fluorescent contrasts is scanning laser ophthalmoscope (SLO) [8]. By using a flying laser beam, SLO permits imaging at a low light intensity and avoids the amount of unwanted light exposure outside the focal volume. To improve the

resolution and enhance the contrast, confocal gating was used in SLO to block the diffusive light [9]. In order to observe finer retinal structures, parallel confocal line was adopted in SLO systems, and a lateral resolution of 3.91 μm for the human eye was reported [10]. Although the lateral resolution of SLO-based approaches has gained considerable improvement, the depth sectioning ability is restricted. Even with an optimized pinhole size, the axial resolution for the human eye is 200–250 μm due to the aberration when the pupil is fully dilated [11, 12]. Apparently, this resolution is insufficient to provide depth discrimination within the retina for volumetric imaging [13].

With the application of adaptive optics technique, the way we image the retina has been changing [14]. As adaptive optics corrects the wavefront error caused by the aberration of the imperfect optics in the human eyes, both the lateral and axial resolution of an adaptive optics scanning laser ophthalmoscope (AOSLO) could approach the diffraction limits. The lateral resolution of AOSLO was revealed to be 2 to 5 μm , and the axial resolution was 37 to 84.2 μm for the human retina [15–23]. Even though the axial resolution was improved, the volumetric imaging by AOSLO in the human retina was plagued by the need for the plane-wise depth sectioning and a small FOV, typically within 1° to 2°.

To enable volumetric fluorescence retinal imaging, we recently developed a novel method, called oblique scanning laser ophthalmoscope (oSLO). It is a variant of single objective light sheet microscopy [24–28], essentially a scanned light sheet microscopy in the eye. It breaks the coaxial alignment of the emission and excitation in the conventional SLO, by using the asymmetric, off-axis excitation and detection. As the off-axis excitation light creates an angle with the optical axis of the eye, a tilted 2D cross section of the retina can be illuminated and imaged. By sweeping the excitation light, the volumetric retinal imaging can be achieved without any depth sectioning.

We have demonstrated the success of oSLO in rats and mice in vivo over ~30°FOV with a sufficient depth resolution to resolve the stratified microvascular plexus [29, 30]. However, it is challenging to apply the same oSLO design directly to human eyes because of the low numerical aperture (NA) of the human ocular optics. To overcome this challenge, we redesigned the optics under Scheimpflug condition to increase the angle of the intermediate image so that the system has a sufficient light collection efficiency. A pair of cylindrical lens was used in the remote focusing system to maintain a sufficient sampling density in the depth dimension. In addition, we adapted a scanning-and-descanning strategy, resulting in a more compact system setup comparing to our previous oSLO design [25]. With the new oSLO system, we demonstrated the feasibility of oSLO in a realistic human eye model and characterized the lateral and depth resolution of 7 μm and 41 μm , respectively, and a ~10°FOV.

2 | EXPERIMENT SETUP AND METHODS

2.1 | The overall design of oblique scanning laser ophthalmoscopy for the human eye

The schematic of oSLO for the human eye is shown in Figure 1A. The 3D modeling in Figure 1B was used to validate the feasibility of the overall optical and mechanical design.

Figure 1C is a photograph of the actual oSLO system. The setup is compact that can easily be transformed for clinical use in the future.

The light source (LS) is a 488 nm laser with a 30 mW maximum power. The laser was first coupled into a single-mode fiber and collimated by an $f=10$ mm lens (L1). To maintain a confocal alignment of the excitation and detection path over a large FOV, we used a focus tunable lens (L2) to dynamically adjust the focal position of the excitation light during imaging. A galvanometer mirror (GM1) was used to steer the excitation light to form a raster laser line. A right-angle prism mirror (M1) was used to separate the excitation from emission light, which was marked in different colors (blue for excitation; green, yellow, and light green for emission). On one side of the mirror, the excitation light was first relayed to the slow scanning galvanometer mirror (GM2) by a 1:1 relay lenses (L3: $f=50$ mm, L4: $f=50$ mm), and further to the pupil entrance by another 2:1 relay lenses (L5 $f=50$ mm and L6: V40LC, Volk lens, $f=25$ mm). To form an oblique light sheet in the retina, the excitation light was offset from the optical axis by ~ 3 mm at the pupil entrance, resulting in an oblique angle of the light sheet to be $\sim 10^\circ$ at the retina [29]. GM2 was used to scan the light sheet through the retina, and also descanned the emission light. At the same time, the fluorescence emission from the retina was mapped back through the relay lenses (L3–L6) and turned to the back pupil plane of OL1 (UplanFL N 20 $\times$ /0.75) by the prism mirror (M1). An emission filter F1 (500–550 nm) was placed in front of OL1. A stationary and tilted intermediate image of the light sheet could then be created after OL1. The optical axis of the remote focusing system (OL2: UplanFL 20 $\times$ /0.5, L7: $f=50$ mm, L8: $f=10$ mm, and L9: $f=50$ mm) OP4 was angled to focus the tilted image onto the camera sensor (BFS-U3–51S5M-C, Point Grey). The intersection angle of OP3 and OP4 could be precisely adjusted by a mechanical stage (TS) with 3 degrees of freedom, as can be seen in Figure 1C. The choice of this angle was to keep a balance between the FOV and the emission light collection efficiency.

As shown in Figure 1C, the human eye model was placed at the front of L6 with a distance of ~ 22 mm, allowing a pleasant working distance. The power of the laser beam projected on the cornea was limited within 0.5 mW, which was below the maximum permissible exposure (MPE) limit established by the American National Standards Institute and other international standards [31, 32]. The exposure time of the camera was set to 10 ms, permitting a frame rate of 100 fps.

2.2 | Optics design for low NA oSLO by using a cylindrical lens pair

Figure 2A is a simplified sketch from Figure 1A. As the excitation light enters the model eye, the light sheet on the retina has an oblique angle α with respect to the optical axis, represented by the tilted *Object 1* in Figure 1A. Due to the small NA of the human eye, the tilt angle α is $\sim 10^\circ$. The tilted *Object 1* has a conjugate intermediate *Image 2* between OL1 and OL2. The final *Image 3* is captured by the remote focusing system. The intermediate *Image 2* also has a tilt angle β with respect to the optical axis, according to the Scheimpflug condition [33]. If β is the same as α at $\sim 10^\circ$ for $M=1$, OL1 and OL2 will be aligned almost perpendicularly at $\sim 80^\circ$ and therefore the light collection efficiency will be dismal for any practical application.

To increase β , the transverse magnification from *Object 1* to *Image 2* was designed to be $M_X = 0.2$, and the corresponding axial magnification is $M_Z = M_X^2 = 0.04$ [34]. The heights of *Object 1* (h) and *Image 2* (h') in transverse direction have the relationship of $h/h' = M_X$; the depths of *Object 1* (z) and *Image 2* (z') in the axial direction are $z/z' = M_X^2$. Then the relationship of α and β can be derived as:

$$\frac{\tan(\beta)}{\tan(\alpha)} = \frac{h'}{z'} \times \frac{z}{h} = M_X \quad (1)$$

Therefore, β can be significantly increased to be $\sim 41^\circ$. As a result, the rotating angle of the remote focusing system can be reduced to $\sim 49^\circ$, dramatically improve the light collection efficiency. Because of the axial magnification is the square of that of the transverse magnification, the intermediate *Image 2* was heavily compressed in the axial direction. A kitten face is used in Figure 2A to vividly illustrate the unbalanced magnification in two dimensions.

To achieve a unidirectional magnification, we inserted an anamorphic telescope composed of two groups of cylindrical lenses within the remote focusing system (marked with hollow arrows) to magnify the depth dimension by $5\times$ and have no power in lateral dimension, as shown Figure 2A,B. As a result, the overall magnifications from *Object 1* to *Image 3* were corrected to be consistent in all directions within the light sheet plane.

2.3 | The design of the human eye model

We used a realistic eye model to test the feasibility of the new oSLO design on the human retina. The schematic is shown in Figure 3A,B, and it was modified from a commercial product (OEM-7, Ocular Instruments, Bellevue, WA). The model consists of the cornea, crystalline lens, aqueous humor, vitreous humor, and an artificial retina. As depicted in Figure 3A, the retina was emulated by a spherical shell of 0.5% agarose gel attached to the bottom of the eye model. Fluorescent microspheres with $3.1\ \mu\text{m}$ diameter were embedded in the gel to characterize the imaging resolution. To simulate the scattering properties of the human retina, $2\ \mu\text{m}$ diameter polystyrene microspheres were added to the gel at a final concentration of 0.14%. The scattering coefficient μ_s and the anisotropic factor g were calculated to be $21.4\ \text{cm}^{-1}$ and 0.84 at 488 nm by Mie theory[35]. The human retina is reported to have the scattering coefficient in the range of $100\ \text{cm}^{-1}$; and meanwhile is highly forward directed with $g \sim 0.97$ [36, 37]. Therefore, the concentration of the polystyrene microspheres takes into account of the scattering directionality and matches the reduced scattering coefficient $\mu_s' = \mu_s(1-g)$ to be $3.4\ \text{cm}^{-1}$. To prevent the agarose gel from detachment, a 3D printed plastic fixture was used to hold the gel in place. On the bottom of the plastic fixture, a 6 mm diameter clearance hole was designed for imaging, as shown in Figure 3B.

To measure the physical dimension of the artificial retina, OCT imaging was performed on the eye model. The theoretical resolutions in axial and transverse direction for our OCT setup were ~ 5.6 and $\sim 10.2\ \mu\text{m}$ [38], respectively, providing a sufficient measurement accuracy. Figure 3D–F were two OCT B-scans, and the *en face* projection of the artificial

retina. The boundary of the clearance hole and the entire thickness of the retina can be visualized. The diameter of the clearance hole is used to calibrate the transverse dimension. The thickness of the artificial retina was measured to be ~0.6 mm.

2.4 | Data acquisition and image processing

The synchronization of the camera trigger, the galvanometer GM1, and GM2 was modified from our previous publications [29]. Briefly, the camera was triggered to acquire a 2-D B-scan fluorescent image every 10 ms. In every trigger period, the fast scan (GM1) was controlled by triangular waves with 2 ms period and 50% duty cycle to create a scanned light sheet. The slow scan (GM2) was driven by a ramping wave to sweep through the retina. The focus of the excitation was adjusted by the tunable lens with a second ramping wave, allowing confocal alignment of excitation and detection. The amplitude of the focus change was determined by experimentally measuring the focal shift along the whole trip of the slow scan. In order to recover the physical geometry, the 3-D matrix was sheared by 10° using affine transformation to correct the oblique illumination angle.

3 | RESULTS

To verify whether this optical design could unfold the compressed depth information and balance the magnifications in the transverse and axial direction, an imaging target was fabricated, as shown in Figure 4A. The fluorescent microspheres of 3.1 μm diameter were immobilized in 0.5% agarose gel, which was sandwiched between the four glass slides forming three layers of agarose gel. The thickness of each layer is 1.2 mm. To image this imaging target, the human eye model in Figure 1A was replaced by a lens with $f = 25$ mm. Figure 4A,B are two cross-sectional images captured without and with cylindrical lens (L7 and L8), respectively. There were three agarose gel layers from left to right side of the image. The image of the microspheres is sharp and clear in the left-most layer while it gradually becomes blurred toward the right-most layer. This is because the left-most layer of the agarose is within the depth of focus of the excitation light while the other two layers are out of the depth of focus. The depth information in Figure 4A was dramatically compressed. On the contrary, the depth dimension in Figure 4B was magnified by using a pair of cylindrical lenses. As can be seen from the comparison in Figure 4A, B, the width of the phantom image in Figure 4B is approximately 5 times that of the image in Figure 4A, which validated our optical design. The overall magnifications in the lateral and axial direction are consistent to be ~0.9. As the lens in front of the phantom has a slightly different focal length with the realistic eye, the magnification is slightly different from the previous calculation. This result still serves as a proof-of-concept in our optical design.

In order to experimentally characterize the resolution and the feasibility of the proposed oSLO with the human ocular optics, the artificial retina was imaged.

Figure 5 shows the representative oSLO images acquired from the artificial retina. The maximum intensity projections on three planes were displayed in Figure 5A–C. The FOV covered half of the clearance hole of the fixture, achieving $\sim 6 \times 3 \text{ mm}^2$ area. The edge of the 6 mm clearance hole can be seen in Figure 5A. The length of the FOV is sufficient to cover the macular area, typically ~5 to 6 mm in diameter. The FOV on the fast axis is currently

limited by the size of the camera sensor size. Figure 5D–F are the zoomed-in views from Figure 5A–C. The axial resolution appeared consistent throughout the depth of 0.6–0.7 mm, which is sufficient to penetrate the entire retina for a typical thickness <0.5 mm. Meanwhile, the lateral resolutions were well maintained over the entire FOV.

Due to the small diameter, the fluorescent beads served as point sources to characterize the point spread function (PSF) for our oSLO system. Three representative beads labeled in Figure 5D–F were randomly selected to characterize the 3D PSF. Maximum intensity projection was applied to each of the three beads along x , y , and z directions with the corresponding results shown in Figure 6A. Figure 6B–D are intensity profiles plotted through the center of each bead in three directions. To get more accurate intensity profiles, the intensity data of all three beads in each direction was fitted to Gaussian curve. The FWHM of fitted curve are 6.5 μm , 7 μm , and 41 μm in x , y , and z directions, respectively.

4 | DISCUSSION AND CONCLUSION

We have proposed a new oSLO system for volumetric fluorescent retinal imaging with the human ocular optics. We have assessed its feasibility and resolution using a realistic eye model and an artificial retina. The dimension of the artificial retina is precisely measured by OCT. The resolution of the current oSLO setup was assessed to be 6.5 μm , 7 μm , and 41 μm in x , y , and z directions, respectively, over a FOV of $3 \times 6 \times 0.8$ mm in x - y - z . The maximum thickness of the human retina at the macula is about 350 μm , and the overall thickness is 200–300 μm . With the current depth resolution of 41 μm , the proposed oSLO system would provide fair depth discrimination ability to separate anatomical retinal layers. The fast scan x dimension is currently limited by the size of the camera sensor and magnification, which can be improved by larger sensor size and proper magnification. With 10 ms exposure time, the current frame rate is 100 fps. By limiting the power of the incident laser beam to 0.5 mW, the imaging procedure is safe for the human eye and compliant to laser safety standards. Therefore, we conclude that the proposed oSLO is feasible for volumetric fluorescence imaging in the human retina, providing depth resolution that has been challenging for all the existing fluorescence-based retinal imaging modalities.

The design of the magnification ratio in the detection path, as well as the adoption of cylindrical lenses, overcome the challenge raised by the low NA in the human eye. Firstly, the tilted image can be increased from 10° to 41° allowing enough space for the subsequent optical components; secondly, as shown in Figure 2, the heavy compression in the axial direction is optically expanded, and the overall symmetrical magnification is achieved. Due to the small NA of the human eye (equivalent to a small NA objective lens in a microscopic setting), the depth resolution of oSLO is fundamentally limited at several tens of microns [39]. Nevertheless, we demonstrated a depth resolution ~ 41 μm without correcting any aberrations, sufficient to separate retinal circulations by different layers, and from choroidal circulation.

There are several revenues to further improve our human eye oSLO system. Firstly, the efficiency of the emission collection is limited by the tilted objective lens OL2 in the proposed image system. Applying water immersion objective lens OL2, such as the method

described in single objective light sheet microscopy [27], would improve the collection efficiency. Secondly, although 10 ms exposure time was demonstrated in the proposed method, the frame rate was limited by the data transfer speed of the currently used camera. This speed can be dramatically improved by using a high-speed scientific CMOS camera. Thirdly, our system was built up with off-the-shelf lenses and suffered from the aberration of the human eye such that it did not reach the theoretical limit. Improvements can be achieved by optimizing the off-axis performance and correcting the aberrations. Finally, all images are shown as acquired, without reconstruction and deconvolution. Additional image processing, such as a model-based or inter-plane deconvolution, can be used to improve the resolution [40].

To conclude, a novel oSLO design intended for the human eye is presented for the first time. The feasibility of the proposed method is demonstrated using a human eye model for volumetric fluorescence retinal imaging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Experiment setup of oSLO for the human eye. A, The system schematic. L: lens; OL: objective lens; F: filter; M: mirror; GM: galvanometer mirror; LS: light source, OP: optical axis, TS: translation stage. B, The 3D modeling of oSLO system. C, The photography of the actual oSLO system. oSLO, oblique scanning laser ophthalmoscope

FIGURE 2.

The schematic of oSLO by using cylindrical lenses for human ocular optics. A, The optical design to correct both the angle and aspect ratio of the image. B, The correction of the compressed image by the unidirectional optical power of the cylindrical lens pair. oSLO, oblique scanning laser ophthalmoscope

FIGURE 3.

The preparation of the human eye model and characterization of the artificial retina. A, The 3D assembly and the inner structure of the human eye model. B, A cross-section and a top view within the 3D human eye model. C, Photography of the actual human eye model. D-F, The X - Y , X - Z , and Y - Z cross-sectional images captured by an OCT device. Bar = 1 mm. (g) The zoom-in image of the artificial retina from the 3D model in panel B. OCT, optical coherence tomography

FIGURE 4.

The verification experiment for the optical design based on cylindrical lens. A, 3D model of the image target with three fluorescent layers. B, A cross section of 3D model showing the inner structure of the image target. C, Cross-sectional image captured without cylindrical lenses. Bar = 0.25 mm. D, Cross-sectional image captured with cylindrical lenses. Bar = 0.25 mm

FIGURE 5.

Feasibility and resolution characterization of oSLO for the human eye. A-C, Maximum intensity projection of the 3-D volumetric fluorescent image in X - Z , Y - Z , and X - Y planes. D-F, Zoomed-in view of the square area A, B, and C in panel A-C, respectively. Bar = 0.25 mm. oSLO, oblique scanning laser ophthalmoscope

FIGURE 6.

Intensity profile of the three representative beads. A, Maximum intensity projection of three representative beads along x , y , and z directions with their locations marked in panel D-F of Figure 5. Bar = 10 μm . A-C, The intensity profile through the center of the three beads in x , y , and z direction. oSLO, oblique scanning laser ophthalmoscope