High speed adaptive optics ophthalmoscopy with an anamorphic point spread function

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Abstract: Retinal imaging working with a line scan mechanism and a line camera has the potential to image the eye with a near-confocal performance at the high frame rate, but this regime has difficulty to collect sufficient imaging light while adequately digitize the optical resolution in adaptive optics imaging. To meet this challenge, we have developed an adaptive optics line scan ophthalmoscope with an anamorphic point spread function. The instrument uses a high-speed line camera to acquire the retinal image and act as a confocal gate. Meanwhile, it employs a digital micro-mirror device to modulate the imaging light into a line of point sources illuminating the retina. The anamorphic mechanism ensures adequate digitization of the optical resolution and increases light collecting efficiency. We demonstrate imaging of the living human retina with cellular level resolution at a frame rate of 200 frames/second (FPS) with a digitization of 512 × 512 pixels over a field of view of 1.2° × 1.2°. We have assessed cone photoreceptor structure in images acquired at 100, 200, and 800 FPS in 2 normal human subjects, and confirmed that retinal images acquired at high speed rendered macular cone mosaic with improved measurement repeatability. © 2018 Optical Society of America under the terms of the OSA Open Access Publishing Agreement

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1. Introduction

The introduction of adaptive optics (AO) to ophthalmoscopy has enabled a revolutionary ability for studying retinal structure and function in the living human eye [1], particularly for investigating photoreceptor spatial packing structure due to the capability of resolving individual photoreceptor cells and rendering the photoreceptor mosaic [2–9]. Photoreceptors, including cones and rods, are essential cells for visual phototransduction. The macular cones in human eye are critical for high acuity vision [10]. The spatial packing density and geometry (regularity) of the cones are basic metrics for assessing the fundamental limit of human vision [11–16] and the impact of ocular diseases [4, 8, 9, 17, 18]. With the biometrics obtained from high resolution retinal imaging [19, 20], AO ophthalmoscopy has provided quantitative microscale assessment of the photoreceptor packing structure in healthy and diseased eyes [8, 9, 21–27]. These achievements are very promising to enabling sensitive evaluation of therapeutic efficacy at the cellular level and facilitating development of novel treatment for photoreceptor degeneration [28–32]. To achieve this potential, the AO imaging system must render the retinal structure truthfully, i.e. with high fidelity so that it can accurately measure photoreceptor structure and its change over time [33–35]. However, this endeavor has been challenged by the rapid and continuous movement of the living human eye, even when fixating [36]. The human eye motion involves complex modes such as tremors, drifts, and microsaccades. The movement frequency can be up to 100 Hz and even more [37, 38]. These features dictate the necessity of high speed imaging that can be resistant to the motion impact.

Photoreceptor spatial distribution has been mostly studied using two en face AO imaging modalities, namely, AO flood illumination funduscopy [1,39–43] and AO scanning laser Ophthalmoscopy (AOSLO) [44–49]. Only the AO flood illumination funduscopy possesses the speed to acquire an image with a ‘snap-shot’ during which (1 - 4 ms) the retina is momentarily stationary thus the image is free of motion artifact. However, this modality has an intrinsic drawback; the light scattering from different retinal depths due to the flood illumination nature compromises the image contrast. To solve this problem, a confocal imaging system as the AOSLO that can reject the out-of-focus scattering [44] is much desirable. However, the AOSLO typically operates with a ‘flying point’ scanning mechanism that requires two coordinated scanning mirrors to deflect the imaging light thereby projecting a 2-D raster scanning onto the retina. The frame rate of this scheme is constrained by the frequency of the fast scanner, typically a resonant one. With the frequency up to 16 KHz of a fast scanner, the AOSLO can operate at a frame rate about 30 frames/second (FPS) only (for a frame consisting of 512 image lines) [9]. Even by making use of the flying-back path of the fast scanning, the frame rate can only be up to 60 FPS [50]. Thus current AOSLO image acquisition is rather slow compared with the eye’s movement, consequently, leading to image distortion and displacement not only in successive frames but also within a single frame [36, 51, 52]. Cooper et al have found that the intraframe distortion may significantly impair the accuracy and repeatability of cone mosaic metrics assessed by AOSLO [52].

High speed and confocal retinal imaging may be achieved by the line-scan mechanism [53–56]. A disadvantage of this regime is that the imaging resolution and contrast are reduced by light interference in the imaging line direction, i.e., the non-confocal direction. To mitigate this problem, we have developed a proof-of-concept system of adaptive optics parallel confocal scanning laser ophthalmoscope (AO-PCSO) that used a digital micro-mirror device
(DMD) to modulate the imaging light (line) thereby reducing light interference in non-confocal direction [56]. With a similar imaging field of view to that of current AOSLO, the AO-PCSO achieved an image acquisition speed of 100 FPS, which is a large improvement in comparison to AOSLO but is still insufficient for avoiding the artifact caused by high frequency eye movement (~100 Hz) [55]. The imaging speed of the AO-PCSO is mainly limited by the intrinsically low light collection efficiency of the line scan mechanism [54]. As illustrated in Fig. 1, to adequately digitize the optical resolution, the point spread function (PSF) of the imaging system (typically in the form of the Airy disk) should be sampled by at least $4.88 \times 4.88$ pixels (the pixel size equals to one half of the Abbe resolution) [57]. Under this condition, in a line-scan system using a camera with a line image chip [54–56], the camera collects a small portion of the Airy disk only. Most of the light is outside of the line imaging chip [Figs. 1(a) and 1(b)]. By contrast, if the optical system is designed with a small Airy disk that covers one pixel only so that all light can be used to form the image [Fig. 1(c)], the optical resolution in the line image chip direction will be compromised by insufficient digitization. Thus, there exists an intrinsic incompatibility between (adequate) digitization and light collection efficiency in high resolution line scan imaging system using symmetric optics and a line camera. In other words, it is impossible to fully utilize the imaging light encircled in the Airy disk while meet the digitization requirement of the optical resolution [53–56]. To solve this conflict, the PSF would be ideally stretched along the line image chip thereby allowing the most imaging light to be collected by the line camera in the scanning direction. Meanwhile, along the line imaging direction, the PSF can be digitized with sufficient pixels [Fig. 1(d)]. Such a PSF may be realized using an anamorphic imaging system.

![Fig. 1. The incompatibility between adequate digitization of optical resolution and light collection efficiency in a line-scan ophthalmoscope and proposed solution. (a) A diagram of image formation in a line-scan ophthalmoscope. The retinal structure illuminated by the light line is imaged by a line camera. (b) The Airy disk, i.e., the point spread function (PSF), formed by the imaging system on the line imaging chip of the camera. To ensure sufficient digitization of the optical resolution, the PSF must be sampled by $4.88 \times 4.88$ pixels [57]. Under this condition, a line imaging chip can only collect a small portion of the PSF, resulting in significant imaging light loss and poor image signal to noise ratio. (c) When the PSF is focused within one pixel, the optical resolution is digitized insufficiently. (d) As a technical solution, the PSF of is designed with an elliptic form instead of a circular one.](image)

In this paper, we present a technical solution for resolving the conflict between digitization and light collection efficiency that hinders high speed imaging. We have developed an AO line scan ophthalmoscope with an anamorphic imaging mechanism that generates an elliptic PSF [Fig. 1(d)] thereby increasing imaging light collection efficiency. We demonstrate improvement of imaging fidelity of the cone photoreceptor structure achieved by this high speed near-confocal imaging system.

2. Method

2.1 Design of the AO ophthalmoscope with an anamorphic PSF

The optical system, shown in Fig. 2, was designed based upon the AO-PCSO, which has been described in a previous letter [56]. The instrument consists of three major modules: light
Light delivery, scanning optics & AO: The imaging system employs a superluminescent diode (SLD) (Broadlighter S795-HP, Superlum Ltd., Ireland) with a central wavelength of 795 nm and a spectral bandwidth of 15 nm. Light emitting from the fiber tip of the SLD is first collimated and then focused by a cylindrical lens (CL0) to form a light line on the DMD (Texas Instruments, DLP 0.55 XGA Series 450 DMD, Dallas, TX), which modulates the light to create a line of point sources. The width of the light line generated by the cylindrical lens is 27 \( \mu \text{m} \). The modulated beam is relayed by lens L1 and fed into the scanning optics by a beam splitter BS1 (with a reflection ratio of 20%). Then the light is relayed to the deformable mirror (DM, Hi-Speed DM97-15, ALPAO SAS, France), the galvanometric scanner (GS), and finally the eye by the telescopes formed by a series of spherical mirrors (S1-S6), generating a 2D scanning raster on the retina. Light scattered back from the retina following the ingoing path transmits (80% of the light) through the BS1 into the image acquisition arm.

The AO consists of a beacon light (SuperK, NKT Photonics AS, Denmark) for wavefront sensing \((\lambda = 730 \text{ nm})\), which is fed into the scanning optics by a beam splitter BS2 (with a reflection ratio of 10%) and a dichroic filter (DF). The ocular wave aberration is measured by a custom Shack-Hartmann wavefront sensor (WS) and corrected by the DM which has 97 actuators whose stroke can be up to 30 \( \mu \text{m} \). The WS measures the wave aberration by 193 sampling points over a pupil of 6.75 mm (diameter). The control algorithms were adopted from previous development [58–61]. The AO closed loop frequency is 50 Hz. In most eyes, the root mean square of the wave aberration can be compensated to be as low as 0.04 \( \mu \text{m} \), less than 1/14 of the wavelength of the light for wavefront sensing.

Anamorphic imaging: The retinal image is formed by an anamorphic mechanism [62, 63], which consists of a pair of cross-cylindrical lenses CL1 and CL2. CL1 (with a focal length of 200 mm) was placed with its cylinder axis parallel to the line camera, and CL2 (with a focal length of 50 mm) was placed with its axis orthogonal to CL1, as shown in the box of Light collection of Fig. 2. The imaging system was optimized using optical design software (Zemax, Zemax LLC, Kirkland, WA) to ensure the combination of CL1 and CL2 focusing the image of the retina on the line camera. The anamorphic ratio, determined by the power of the 2 cylindrical lenses, is 4. The anamorphic design allows the PSF to be shaped with orthogonally decoupled magnifications.

The system was designed with a pupil diameter of 6.75 mm, which theoretically yields a lateral resolution 2.01 \( \mu \text{m} \) (by Abbe criterion) or 2.40 \( \mu \text{m} \) (by Rayleigh criterion) [64], allowing for resolving the smallest cone photoreceptors in the fovea and rod photoreceptors whose size is about 2-3 \( \mu \text{m} \) [16]. With the anamorphic imaging, the PSF of the imaging system has the form of an ellipse with a major axis of 70.32 \( \mu \text{m} \) (along the line camera) and a minor axis of 17.58 \( \mu \text{m} \) (perpendicular to the line camera). The pixel size of the line camera is 10 \( \mu \text{m} \times 10 \mu \text{m} \). The camera was set with the vertical dual line binning mode, i.e., the effective pixel size is 10 \( \mu \text{m} \times 20 \mu \text{m} \). Thus, the PSF covers 7 pixels along the line image chip (with its major axis) and 0.88 pixel perpendicularly (the scanning direction). The line camera serves as a confocal aperture in the scanning direction whose width is 1.14 times of the minor axis of the PSF, shown in the box of Light collection of Fig. 2. This design ensured sufficient digitization of the optical resolution in the imaging direction (the line camera) [57] and collected most light of the PSF.
Fig. 2. The DMD based high speed AO ophthalmoscope with anamorphic imaging. The Top row shows the anamorphic PSF of the system across the imaging field (left edge, middle, and right edge). The ellipses indicate diffraction limited PSF. SLD: Superluminescent diode. DMD: Digital micromirror device. CL0-CL2: Cylindrical lens. DF: Dichroic filter. BS1, BS2: Beam splitters. L0-L4: Lenses. S1-S6: Spherical mirrors. GS: Galvanometric scanner. FM: Flat mirrors. WS: Wavefront sensor. DM: Deformable mirror. The top right part of the light delivery box shows configurations of DMD. The “on” and “off” states of the micromirrors are represented by solid and hollow squares, respectively [56]. The Light collection module shows the elliptic PSF formed by anamorphic imaging on the image chip of the line camera (spL2048-140km, Basler Co., Germany).

**DMD dynamic modulation of imaging light:** The DMD has $1024 \times 768$ micromirrors which are in a square shape of $10.8 \mu m \times 10.8 \mu m$. Each micromirror has two states, “on” or “off”, corresponding to two angular positions (+12° and −12°) tilting along the diagonal of the micromirror. The “on” or “off” state of each micromirror can be programmed individually to modulate the incidental light. A line of microreflectors (each can consist of one or multiple micromirrors, as illustrated in Fig. 2), which were positioned to be conjugate to the retina and the camera, were programmed to be “on” to modulate the light into a line of point sources. The diagonal length of the image of a single micromirror on the retina is 1.39 µm, which is 0.56 times of Airy disk radius. In our previous study, the DMD was programmed into a line of microreflectors consisting of a series of $2 \times 2$ ‘on’ micromirrors, during imaging all these microreflectors remained stationary [56], i.e., the light modulation was static. This strategy partially reduced light convolution along the imaging line. To further reduce light interference in this direction, we tested an advanced modulation strategy. As shown in Fig. 2, during a line...
acquisition period, a series of microreflectors separated with a lattice distance ($\delta - \eta$) were turned ‘on’ simultaneously for a time $t$ (which was determined by the refreshing rate of the DMD). Then the adjacent microreflectors separated with the same distance were turned ‘on’ sequentially along the light line formed by the CL0. Where $\eta$ is the size of a microreflector and $\delta$ is the lattice distance between two simultaneously ‘on’ microreflectors. The arrangement of the ‘on’ microreflectors with large distance ($\delta - \eta$) may eliminate the light interference completely in the imaging line direction [65, 66].

2.2 Characterization of spatial resolution

We evaluated the spatial resolution in a model eye constituted by an achromatic lens with a focal length of 18.3 mm and a USAF target (R1DS1P, Thorlabs Inc., NJ). The latter was placed at the focal plane of the former. The lateral resolution was assessed by the intensity profiles of the calibration lines on the USAF target in both horizontal and vertical directions. The axial resolution or the depth discrimination ability was estimated using the method introduced by Romero-Borja et al [45, 67]. A special model eye was made with a mobile retina, which was a diffuse reflector attached on a micrometer head (DRV304, Differential Micrometer Drive, Thorlabs Inc., Newton, NJ). Initially, the model retina was adjusted to be on the focal plane of the model eye, and then it was moved away from the focal plane by the micrometer drive. The image intensity of the model retina reduced with the increase of the displacement. The average intensity of the model retinal image was recorded and plotted against the axial distance that the model retina was moved from the best focus. The full width at half maximum (FWHM) of the intensity-distance plot was considered as the axial resolution of the imaging system. The axial resolution in the human eye was then calculated from the measurement of the model eye with the human eye’s parameters (Gullstrand reduced eye model).

2.3 Assessment of the benefit of high speed imaging

To assess the improvement of the accuracy for measuring photoreceptor structure, we acquired images of the same retinal area in the same subject at the frame rates of 100 FPS, 200 FPS, and 800 FPS.

The study involved human subjects. It followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board for Human Use (IRB) at the University of Alabama at Birmingham. Written informed consent was obtained from the participants after the nature and possible consequences of the study were explained. Two subjects (32 and 50 years old) with normal healthy retinae were enrolled and cone structure was assessed in 2 eyes.

Following the method reported by Cooper et al [20, 52], we assessed the repeatability of cone metrics obtained in images of the same retinal area registered with different reference frames. In the Cooper’s study, retinal images taken with a flood illumination AO fundus camera (with an image acquisition time of 4 ms) were considered as free of motion artifact [20, 52]. Because we did not have an AO flood illumination funds camera, to obtain an image that is ‘perfectly’ motion artifact free, we acquired the images at 800 FPS with a reduced field of view consisting of 128 image lines. At this speed, a frame was acquired within 1.25 ms and the between-frame time was reduced to $\frac{1}{4}$ of that the 200 FPS imaging. Although the acquisition time for a frame of the 800FPS images was equal to that for the corresponding portion of the frame of the 200 FPS imaging, the reduced between-frame time may mitigate artifacts in successive frames caused by high speed rotation or torsion of the retina, which have been difficult to be eliminated by post image registration. Thus, the images taken at 800 FPS served as a quasi artifact-free reference for estimation of the ‘perfection degree’ of images taken at 200 FPS.

The power of imaging light ($\lambda = 795$ nm) was 0.5 mW, which was 0.24 times of the ANSI maximum permissible exposures (MPE) under the condition of 1 hour continuous exposure.
The power of the wavefront sensing light ($\lambda = 730$ nm) was 25 µW, which was less than 1/10 of the ANSI MPE. The composite MPE for multiple laser exposure was 0.34 [68]. The pupil of the participant was dilated with 1.0% tropicamide and 2.5% phenylephrine hydrochloride. The subject’s head was aligned and stabilized using a head-mount with a chin-rest. A LCD screen (Raspberry Pi 7” LCD Display) presented a flashing green light dot serving as a fixation target that directed the subject’s view direction.

Retinal images at 100 FPS and 200 FPS were acquired over a field of view of $1.2^\circ \times 1.2^\circ$ with a digitization of $512 \times 512$ pixels. Because the imaging frame rate was limited by the maximum line rate of the camera, the images at 800 FPS were achieved by reducing the line number in a frame from 512 to 128 with a correspondingly shrunk field of view of $1.2^\circ \times 0.3^\circ$ and a digitization of $512 \times 128$ pixels. The line images in the three speed settings were acquired with the same line exposure time (8.5 µs/line), thus retinal images acquired at different frame rates had same imaging SNR.

The images were registered and averaged with custom software [36]. Images taken at different retinal locations were manually montaged on a cell-to-cell basis in the overlapping area to create a montage using image processing software (Photoshop, Adobe System Inc., Mountain View, CA). The image pixel size was computed from the image of a precisely calibrated dot grid placed at the retinal plane of the model eye.

In the Cooper’s study, to assess the repeatability of the cone structure measured from the image registered with different reference frames, they selected the reference frames with minimal intraframe distortion by ‘experienced experts’ [20, 52, 70]. To examine the advantage of the high speed imaging, we arbitrarily used the first 12 frames of the video in turn as the references to register the rest frames. We thus obtained 12 registered retinal images with different references. A total of 36 images (12 images for each frame rate) were aligned in ImageJ (Version 1.51n, National Institute of Health, USA) to crop a region of interest (ROI) of the same retinal area of 50 µm x 50 µm. The coordinates of all cones in the ROI were obtained using a method described previously [71]. First, a Gaussian filter of 1 pixel radius was applied to each image to reduce noise. Then the “find maxima” function of ImageJ was used to determine the location of each cones with manual checking. With the cone coordinates, Voronoi tessellation was generated using a custom program written with Matlab (R2016b, MathWorks, Inc. Natick, MA). Only the Voronoi cells fully contained within the ROI (i.e. bounded) were considered in metric calculation. We assessed 6 metrics of the cone mosaic in the ROI [20].

1. Cone density (CD): the ratio of the number of bound Voronoi cells to the total area of the bound Voronoi cells in the ROI.
2. Percentage of six-sided cells (PSSC): the ratio of the number of Voronoi cells with six sides to the total number of bound Voronoi cells within the ROI.
3. Intercellular distance (ICD): the average distance between a given cone and all of its neighbors. The ICD reported for each ROI is the average ICD for all of the cones with bound Voronoi cells in that ROI.
4. Nearest neighbor distance (NND): The distance between a given cone and its closest neighbor. The NND reported for the ROI is the average NND for all of the cones with bound Voronoi cells in that ROI.
5. Nearest neighbor regularity (NNR): the ratio of the mean NND for all of the cones with bound Voronoi cells in the ROI to the standard deviation (SD) of the NND for all of the cones with bound Voronoi cells in that ROI.
6. Voronoi cell area regularity (VCAR): the ratio of the mean area of the bound Voronoi cells in the ROI to the SD of the area of the bound Voronoi cells in that ROI.
3. Result

3.1 Improvement of imaging light collection efficiency

Figure 3(a) shows a raw single frame of the retinal image acquired with a conventional imaging detection, in which an achromatic spherical lens with a focal length of 200 mm was used to focus the imaging light onto the camera. Figure 3(b) is a single frame of the same retinal area with the anamorphic imaging. The mean intensity of Fig. 3(a) is 19.2, whereas the mean intensity of Fig. 3(b) is 69.2. Both images were acquired at 200 FPS. The anamorphic imaging increased the light collection efficiency by 2.60 times. The image brightness and definition are thus significantly improved, as shown in Fig. 3(c). The dynamic range of image acquired with anamorphic imaging spreads more widely thereby rendering finer detail of the retinal structure.

![Fig. 3](image)

Fig. 3. Improvement of brightness and definition of retinal imaging by anamorphic detection. (a) A single frame of retinal image acquired without anamorphic detection (see Visualization 1 for the video). (b) A single frame taken at the same retinal location with anamorphic detection (see Visualization 2 for the video). (c) The corresponding normalized histograms of retinal images taken without and with anamorphic detection. The histogram of the image acquired without anamorphic detection is truncated due to insufficient photons. The images were acquired at the eccentricity of 0.8° nasally. The images size is about 300 µm on a side. All images were taken at 200 FPS.

3.2 Spatial resolution

Figure 4 shows the USAF target images (groups 6 and 7) acquired with DMD ‘all on’ and ‘2 × 2 on’ settings. The lines of element 6 in group 7 are resolved in both images. The lateral resolution is 2.19 µm in the human eye (the relaxed condition of the Emsley reduced eye model). The normalized intensity profiles of the lines of element 6 in group 7 imaged with the DMD ‘all on’ setting have shallower troughs [Figs. 4(a) and 4(b)] than those obtained with the DMD ‘2 × 2 on’ setting [Figs. 4(c) and 4(d)]. The trough value in the horizontal direction was reduced from 0.28 to 0.12 (by 57%), whereas in the vertical direction, the trough value
was reduced from 0.46 to 0.35 (by 24%). The deepening of the troughs indicates the improvement of the lateral resolution by DMD modulation of the imaging light. Overall, the vertical intensity profiles have shallower troughs than the horizontal intensity profiles. This is due to the light interference in the illumination line (oriented vertically), even with DMD modulation. With the DMD ‘2 × 2 on’ configuration, the anisotropy of the lateral resolution (the ratio of the vertical trough to the horizontal trough) was improved from 0.61 to 0.34 (smaller is better).

Figure 4 illustrates the axial resolution under different settings of static and dynamic DMD modulation. Under the configuration of each individual microreflector consisting of 2 × 2 micromirrors (η = 2), with increase of the spatial duty cycle δ/η from 2 to 16, the axial resolution improved from 112.9 µm to 105.8 µm [Fig. 5(a)]. A smaller microreflector (η = 1) that corresponds to 0.56 of the Airy disk on retina did not make significant improvement of the axial resolution [Fig. 5(b)]. The axial resolution achieved under settings of the DMD ‘all on,’ ‘2 × 2,’ and ‘1 × 1’ was 122.2 µm, 113.3 µm, and 109.8 µm, respectively.

Dynamic modulation has the potential to improve the axial resolution and has not implemented and tested in previous study. It required that all microreflectors scanned sequentially to cover an image line. The DMD refreshing frequency is 17241 Hz. With a spatial duty cycle of the microreflector δ/η, the highest line frequency can be 17241/(δ/η) Hz. For a frame consisting of 512 lines, the highest framerate is 33.7/(δ/η) FPS. Because δ/η > 1,
the frame rate is < 33.7 FPS. Thus, our experiment proved that dynamic modulation indeed improved the axial resolution, but the slow refreshing rate of the DMD limited the imaging speed. The high speed retinal images presented in this paper were therefore all acquired with the DMD ‘2 × 2’ static modulation.

Fig. 5. Axial resolution. (a) Axial resolution measured with DMD static and dynamic modulation. The configuration that \( \eta = 2 \) indicates that each microreflector consists of \( 2 \times 2 \) DMD micromirrors. \( \delta/\eta \) is the spatial duty cycle of the modulation. The setting that \( \eta = 2 \) and \( \delta/\eta = 1 \) (the brown line with crosses) is essentially the static DMD ‘2 × 2 on’ state. Data measured in the DMD ‘all on’ setting are plotted for comparison (the blue line with diamonds). (b) Axial resolution measured in dynamic DMD modulation with different microreflector configurations. The configuration that \( \eta = 1 \) indicates that each microreflector consists of 1 DMD micromirror. The axial resolution measured in the ‘DMD all on’ setting (red asterisk) is drawn for comparison.
3.3 Retinal imaging

Figure 6 shows cone photoreceptors acquired from a human subject with normal healthy retina. Except the very center of the fovea (< 0.1°), cones are resolved showing a contiguous mosaic [Figs. 6(a) and 6(b)]. Rod photoreceptors [Fig. 6(c)] were imaged in the macula. To access the depth discrimination ability in human retina, we imaged the retinal capillaries at 4° eccentricity nasally in three depths with an interval distance of 0.1 diopter (D) (~36.4 μm), as shown in Figs. 6(d)-6(f). With change of focusing plane, images acquired at different depths reveal different retinal vasculature, demonstrating the ability of depth discrimination.

Fig. 6. Photoreceptor and retinal capillary imaging. (a) Retinal image acquired at the fovea (see Visualization 3). (b) Retinal image acquired at the eccentricity of 1.8° nasally (see Visualization 4). All cells are cone photoreceptors. Images in (a) and (b) are in linear grey scale. (c) Retinal image acquired at the eccentricity of 5° nasally, revealing cones (larger and brighter dots) and surrounding rods (smaller dots). The image is in logarithmic scale (see Visualization 5, raw video is in linear grey scale). All images were taken from an eye of a subject with normal retinal health, and registered from a set of 100 successive frames. (d) - (f) Retinal capillaries imaged at different depths. The numbers on top right corners of the panels indicate the imaging light defocus power (in diopter: D) induced by the deformable mirror while AO was correcting the ocular wave aberration. Zero D corresponds to the plane of the inner segment layer of the cone photoreceptors. The capillaries were extracted using the standard deviation of a sequence of 50 successive images [50]. All images were acquired with a frame rate of 200 FPS. Scale bars represent 50 μm.
3.4 Repeatability of cone metrics assessed in retinal images acquired with different frame rates

As shown in Figs. 7-9, the CD and the ICD assessed in images acquired at three frame rates (100 FPS, 200 FPS, and 800 FPS) exhibit minor difference (< 0.5%). However, the coefficients of variation (CV) of the CD and the ICD measured in the image acquired at 100 FPS are larger than those assessed in images taken at 200 FPS and 800 FPS. The PSSC and the NND assessed in images taken at 100 FPS are considerably smaller than those measured in images taken at 200 FPS and 800 FPS. The difference of the PSSC in images taken at 3 frame rates can be directly perceived in the Voronoi diagrams. The NNR and the VCAR in images acquired at 100 FPS are poorer than those in images acquired at 200 FPS and 800 FPS. To avoid the impact of rods appearance on cone packing geometry, we assessed the repeatability of cone mosaic metrics at the retinal areas of 1° and 2° away from the foveal center nasally.

Fig. 7. The repeatability of cone mosaic metrics assessed in images acquired with different frame rate at 1° eccentricity nasally (Subject 1). (a), (b), and (c) are Voronoi diagrams of the ROI in images acquired at 100 FPS, 200 FPS, and 800 FPS, respectively. Blue Voronoi cells are 6-sides, and red ones are not. (d) Cone density (CD) repeatability under different frame rates. (e) Percentage of six-sided cells (PSSC) repeatability. (f) Inter cellular distance (ICD) repeatability. (g) Nearest neighbor distance (NND) repeatability. (h) Nearest neighbor regularity (NNR) repeatability. (i) Voronoi cell area regularity (VCAR) repeatability. Error bars indicate 1 standard deviation (SD). Numbers above the bars are the coefficients of variation (CV) from 12 series measurement of this metric.
Fig. 8. The repeatability of cone mosaic metrics assessed in images acquired with different frame rate at 1° eccentricity nasally (Subject 2). (a), (b), and (c) are Voronoi diagrams of the ROI in images acquired at 100 FPS, 200 FPS, and 800 FPS, respectively. Blue Voronoi cells are 6-sides, and red ones are not. (d) Cone density (CD) repeatability under different frame rates. (e) Percentage of six-sided cells (PSSC) repeatability. (f) Inter cellular distance (ICD) repeatability. (g) Nearest neighbor distance (NND) repeatability. (h) Nearest neighbor regularity (NNR) repeatability. (i) Voronoi cell area regularity (VCAR) repeatability. Error bars indicate 1 standard deviation (SD). Numbers above the bars are the coefficients of variation from 12 series measurement of this metric.
4. Discussion

The major novelty of the present work is the anamorphic imaging design of the line scan AO ophthalmoscope. The anamorphic imaging mechanism solved the intrinsic incompatibility between digitization and light collection efficiency in high resolution line scan retinal imaging system, thereby enabling high resolution AO imaging of the living human retina at 200 FPS which is more than 6 times faster than current AOSLO. Meanwhile, the anamorphic AO ophthalmoscope has been demonstrated possessing similar spatial resolution and field of view to those of conventional confocal AOSLO. The increased imaging light collection ensured the retinal image (a frame) to be acquired within a time close to the ‘snap shot’ exposure of the flood-illumination AO-fundus photography thus the artifact induced by rapid and continuous eye motion has been effectively reduced.

The purpose of the present work was not to merely increase the imaging light collection efficiency or image signal to noise ratio. Rather, it was to solve fundamental problems that impeded AO high resolution and high speed imaging, i.e., adequate digitization properly rendering the optical resolution and sufficient imaging light collection. Generally, to
maximize light collection in an imaging system using a line camera, a digitization strategy that one pixel covers the PSF as shown in Fig. 1(c) may be adopted for low resolution imaging, but it is not suitable for AO ophthalmoscopy. In fact, the PSF of an AO retinal imaging system has reached the size of the smallest retinal cells such as the cones in the foveal center or the rods in the living human eye by AO correction for the ocular wave aberration. Obviously, it is inappropriate to render a cone or rod with one pixel. According to Pawley [57], the PSF should be digitized by 4.88 pixels practically. Under this condition, much portion of the PSF would fall out of the line image chip, resulting in significant loss of imaging light. The anamorphic imaging provides orthogonally decoupled optical magnifications from the retina to the camera in the scanning direction and the camera sensor direction thereby meeting both requirements of digitization and light collection.

The anamorphic imaging design represents a new technical advance in comparison to our previous development [55, 56] and brings in new benefits. In a demonstration of the feasibility of high speed retinal imaging, the AO line scan ophthalmoscope (AOLSO) had to be configured with a hyperconfocal setting so that the optical resolution can be rendered properly [55]. The width of the confocal gate served by the line imaging chip was of 0.28 times of the Airy disk (diameter). This hyperconfocal gate resulted in a significant light loss thereby leading to poor imaging SNR. Moreover, imaging resolution and contrast in the direction of the line camera were reduced by light interference due to non-confocal nature (in this direction). In the successive development, i.e., the AO-PCSO, we used a DMD to modulate the imaging light along the illumination line and thereby reduced the light interference [56]. To compensate the significant light loss caused by the DMD modulation, the width of the confocal slit of the AO-PCSO was designed to be 0.45 times of the Airy disk (diameter) so that more light could be collected. However, with this design the pixel size on the retinal plane was 1.11 µm. The PSF was digitized by 4 pixels, less than the sampling law required number of pixels. Furthermore, limited by the SNR, the image could be acquired at a frame rate of 100 FPS only. In the instrument presented in this study, anamorphic imaging shapes the PSF into an elliptic form. The light outside of the line image chip is ‘compressed’ into the image chip thus the light collection efficiency was increased by 2.6 times, ensuring retinal images acquired at 200 FPS with enhanced SNR, as shown in Fig. 3. Meanwhile, the orthogonally decoupled magnification allows for sufficient digitization of the optical resolution in the line imaging direction. The PSF was digitized by 7 pixels and the pixel size is 0.67 µm. In summary, the present instrument possesses an imaging speed equal to that of our previously developed high speed AOLSO but has improved image contrast and lateral resolution due to DMD modulation that reduces the light interference in the line camera. Compared with the AO-PCSO, this instrument achieved a higher imaging speed (200 FPS vs. 100 FPS) due to the anamorphic imaging light collection.

The performance of the near confocal anamorphic imaging has been tested and shown to be comparable to that of conventional confocal AOSLO, in terms of spatial resolution, digitization, and field of view. The lateral resolution was tested as 2.19 µm, which is between the Abbe criterion (2.01 µm) and the Rayleigh criterion (2.40 µm) under the imaging system configuration (with the imaging light wavelength of 795 nm, the relaxed condition of the Emsley reduced eye model with a pupil size of 6.75 mm in diameter). Thus, the system has achieved diffraction limited lateral resolution. The axial resolution is 113.3 µm. By contrast, the axial resolutions of the AOLSO with a confocal aperture of 0.28 of the Airy disk was 88.7 µm [55] and the AO-PCSO with a confocal aperture of 0.45 of the Airy disk (diameter) was 84.2 µm [56]. Thus, due to using a large confocal aperture (1.11 times of the PSF’s minor length), the system developed in this study exhibited reduced ability of depth discrimination. Nevertheless, the overall spatial resolution is comparable to that of the confocal AOSLO using a similar size of pinhole [45, 67]. In vivo imaging has demonstrated corresponding depth discrimination ability in imaging of the retinal capillary, as evidenced by Figs. 6(d)-6(f).
One of the main motivating factors for the present development was to improve imaging fidelity of the retinal structure. The impact of the rapid and continuous eye motion is especially pronounced in high resolution retinal AO imaging because the image is acquired within small field of view (typically 1° - 2° inside the human eye) and with high magnification. In recent studies [20, 52], Cooper et al identified substantial variation in the repeatability of AOSLO measured cell-to-cell spacing, cell density, percentage of 6-sided Voronoi cells, and Voronoi cell area regularity, ranging from 4.6% to 13.2% [52]. In particular, the Voronoi cell area regularity derived from the AOSLO images was significantly different from that measured from a flood-illumination AO-fundus camera whose images were thought free of intra-frame motion artifact. In this study, imaging at 200 FPS and 800 FPS was demonstrated with overall improved repeatability of cone structure measurement in comparison to imaging at 100 FPS, as assessed by the coefficient of variation using different reference frames. Retinal images taken at higher speed revealed better regularity of cone packing geometry, as measured by the nearest neighbor regularity, Voronoi cell area regularity, and the percentage of six-sided cells in the ROI. The cone mosaic metrics obtained in images acquired at 200 FPS are much closer to those measured in images taken at 800 FPS than those measured in images taken at 100 FPS. These data indicate a more effective reduction of the intra-frame distortion in images taken at 200 FPS and imply the inadequacy for overcoming distortion using a frame rate of 100 FPS. In future work we will statistically assess the improvement of the repeatability of the cone mosaic measurement at 100, 200 and 800 FPS in more subjects.

The development was also inspired by the needs for in vivo studying physiological function of the human retina. High speed AO flood illumination funduscopy has been demonstrated to characterize light-evoked oscillation signals in cone photoreceptor after a visual stimulus [41], to measure the velocity of red blood cells in retinal capillary [42], to assess photopigment bleaching and associated phototransduction in individual cones [43], to study bleaching dynamics of individual foveal cone photoreceptors [72], to evaluate flicker-induced functional hyperaemia in human retinal capillaries [73], and to estimate capillary reactivity of the human retinal microvasculature during acute gas breathing provocations [74]. It has been suggested that direct imaging red blood cells flowing in retinal capillaries may require imaging speed beyond 200 FPS [75]. With further development and optimization, we expect that the instrument developed in this study may provide adequate temporal resolution for investigating dynamic physiological activities of the living human retina.

There are important limitations of the present study that may be addressed in future development. First, current system exhibited reduced axial resolution in comparison to the highly confocal system reported in previous study due to an enlarged confocal gate size. Future design with an optimal aperture size may improve the axial resolution. Second, DMD dynamic modulation may potentially compensate this degradation. In an attempt to improve the spatial resolution by further reducing light interference along the image direction, we tested the dynamic modulation in which individual DMD elements were turned ‘on’ sequentially so that the image was formed by a true point light source. However, the refreshing speed of current DMD make it impractical for high speed imaging. DMD dynamic modulation has not yielded the axial resolution that we have expected, it has indeed demonstrated improvement. With future faster DMD available and a more flexible control, we will fully investigate the strategy using dynamic modulation to further improve the spatial resolution. Third, we acknowledge that the comparison between the 200 FPS imaging and the 800 FPS imaging may not completely estimate the ‘perfectness’ of the 200 FPS imaging. A direct comparison with the images acquired by an AO flood illumination fundus camera in future study may provide a more convincing assessment of the quality of the images acquired at 200 FPS. Our ultimate goal is to overcome the motion artifact in high resolution retinal imaging. We believe that the motion artifacts will be effectively minimized with increased
imaging speed. We will further increase the image acquisition speed through advanced optical design and use of state-of-art imaging sensors.

5. Conclusion

With the anamorphic mechanism and the DMD modulation of the imaging light, we have demonstrated an AO ophthalmic instrument featuring high speed image acquisition and near confocal imaging performance. High speed high resolution retinal imaging offered by this instrument are able to improve imaging fidelity of cone structure in the living human eye, and hold the potential to expand the functionality of AO ophthalmoscopy from imaging static retinal structure to investigating rapid time-varying functional activities.

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