Development of Multi-modal and Super-resolved Retinal Imaging Systems

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biomedical Engineering in the Graduate School of Duke University

2016
ABSTRACT

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Abstract

Advancements in retinal imaging technologies have drastically improved the quality of eye care in the past couple decades. Scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) are two examples of critical imaging modalities for the diagnosis of retinal pathologies. However current-generation SLO and OCT systems have limitations in diagnostic capability due to the following factors: the use of bulky tabletop systems, monochromatic imaging, and resolution degradation due to ocular aberrations and diffraction.

Bulky tabletop SLO and OCT systems are incapable of imaging patients that are supine, under anesthesia, or otherwise unable to maintain the required posture and fixation. Monochromatic SLO and OCT imaging prevents the identification of various color-specific diagnostic markers visible with color fundus photography like those of neovascular age-related macular degeneration. Resolution degradation due to ocular aberrations and diffraction has prevented the imaging of photoreceptors close to the fovea without the use of adaptive optics (AO), which require bulky and expensive components that limit the potential for widespread clinical use.

In this dissertation, techniques for extending the diagnostic capability of SLO and OCT systems are developed. These techniques include design strategies for miniaturizing and combining SLO and OCT to permit multi-modal, lightweight handheld probes to
extend high quality retinal imaging to pediatric eye care. In addition, a method for extending true color retinal imaging to SLO to enable high-contrast, depth-resolved, high-fidelity color fundus imaging is demonstrated using a supercontinuum light source. Finally, the development and combination of SLO with a super-resolution confocal microscopy technique known as optical photon reassignment (OPRA) is demonstrated to enable high-resolution imaging of retinal photoreceptors without the use of adaptive optics.
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1. Introduction

1.1 Scanning Laser Ophthalmoscopy

The scanning laser ophthalmoscope (SLO) is capable of producing high contrast retinal images by raster scanning a laser spot and detecting backscattered light through a confocal pinhole [1-3]. High contrast images are achieved because both the method of raster scanning and the use of a confocal pinhole allow for the minimization of optical cross talk, defined as unwanted light scattered from areas outside the focal volume [4].

SLO systems have been widely adapted for various clinical applications. The earlier diagnostic applications of SLO included detection of the imaging biomarkers of diabetic retinopathy [5], age-related macular degeneration [6], and glaucoma [7]. More recent generations of SLO have enhanced and extended application of this imaging modality. For example, ultra-wide-field scanning laser ophthalmoscopes are used to evaluate ischemia in retinal diseases such as retinal vein occlusion [8]. On another front, combined imaging of SLO and spectral-domain optical coherence tomography with separate [9] or shared [10] light sources has been demonstrated for enhanced image aiming, guidance, and motion tracking as well as optimal classification of disease imaging biomarkers. Finally, integration of adaptive optics with SLO has enabled visualization of individual cone photoreceptors including those at the fovea where they are most closely packed [11-13], and more recently rod photoreceptors [14], which are smaller than foveal cone photoreceptors.
1.2 Combined SLO and OCT in Ophthalmology

Scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) are complementary retinal imaging modalities that can assist in the diagnosis of retinal pathologies. OCT, like SLO, utilizes raster scanning and confocal detection but also employs coherence gating in the axial direction, which allows for high resolution depth sectioning [15]. Current-generation clinical spectral domain OCT (SD-OCT) systems utilize rapid acquisition along the depth axis (depth-priority scanning) at 20 - 50 kHz line rates to produce high-resolution 2-D cross-sectional images (B-scans) near video rate; however, 3-D volumes comprising hundreds of B-scans require several seconds to acquire. Thus, SD-OCT volumes are typically compromised by patient motion artifacts, particularly in the direction perpendicular to B-scan acquisition [16, 17]. In contrast, current-generation SLO systems utilize rapid acquisition along the lateral axis (lateral-priority scanning) at multi-kHz rates to obtain 2D en-face retinal images also near video rate. Thus, OCT and SLO provide complimentary lateral image information at different time scales such that when combined, rapidly acquired 2-D en-face SLO images may be used to register volumetric OCT B-scans to correct for patient motion within an OCT volume.

The combination of SLO and OCT was first explored by Podoleanu et al. [18] and further developed by various groups [10, 19-23] in either simultaneous or sequential SLO-OCT imaging. Some of these imaging systems have been translated to the clinic as table-
top systems mounted to a patient positioning frame like those used in modern slit-lamps. However, due to the physical size and design of these tabletop systems, imaging is limited to patients who are able to sit in an upright position and fixate for several minutes.

1.3 Multi-color SLO imaging

SLOs are able to achieve superior contrast and axial sectioning capability compared to fundus photography. However, SLOs typically use monochromatic illumination and are thus unable to extract color information of the retina, which is critical in fundus photography and indirect ophthalmoscopy for evaluation of retinal pathologies such as neovascular age-related macular degeneration [24].

The introduction of color imaging to the SLO was first demonstrated by Manivannan et al. [25] by using red, green, and blue lasers sequentially to acquire red, green, and blue reflectance images of the retina on a single detector. Simultaneous color imaging with three color lasers and three detectors [26, 27] and quasi-simultaneous color imaging using pulsed red, green, and blue lasers and a single detector [28, 29] were also demonstrated and shown to obtain color fundus images that resembled color fundus photography but with higher contrast. Recently, an SLO employing adaptive optics has demonstrated color imaging using narrowband red, green, and blue filters on a supercontinuum source with each color obtained sequentially [30]. All of these techniques utilized multiple lasers or narrowband colors for illumination and are therefore not capable of generating “true color” fundus images [31]. As Bartsch et al. explains in [31],
the combination of red, green, and blue lasers can appear to an observer as white light, but the reflectance of objects illuminated by such light consists of only 3 discrete wavelengths. Conversely, in fundus photography, the illumination spans the full visible spectrum. Therefore, the majority of the spectral information used in fundus cameras to determine color is lost in a 3 narrowband source illumination approach. Since the retina is known to have continually varying spectral reflectivity that spans the visible spectrum [32], sampling only 3 narrow bands of wavelengths does not provide enough information to reconstruct the “true” color of the retina and may even miss certain features of the retina detectable with the full visible spectrum [31]. Also, the previous color SLO techniques (except for [30]) did not compensate for the longitudinal chromatic aberration (LCA) of the human eye, which is known to introduce a chromatic difference of refraction of ~ 2 diopters (D) over the visible spectrum [33]. As a result, without an achromatizing lens, images from each color channel are acquired at different depth sections of the retina, thus introducing errors in the combined color image.

1.4 **Optical photon reassignment confocal microscopy**

Optical photon reassignment (OPRA) confocal microscopy is an all-optical (free from image reconstruction) confocal imaging technique with a relatively open pinhole that is able to achieve the lateral resolution improvement of confocal microscopy that uses an infinitely small pinhole [34, 35]. Confocal microscopy with an infinitely small pinhole achieves a full-width-at-half-maximum (FWHM) resolution of $\frac{0.37 \lambda}{NA}$ while widefield
microscopy has a FWHM resolution of $\frac{0.51\lambda}{NA}$ given diffraction-limited optics, uniform illumination at the objective, no Stoke’s shift, and a circular aperture [36]. However the improvement in lateral resolution for confocal microscopy quickly disappears as the pinhole size increases (see Figure 1 below) such that the lateral resolution improvement of confocal detection completely disappears at pinholes of approximately one Airy disc diameter. While reducing the pinhole size to approximately one third of an Airy disc diameter allows for a lateral resolution near the infinitely small pinhole case, this results in a large reduction in SNR compared to that of widefield microscopy such that most microscopists adjust the pinhole size to at least one Airy disc diameter thus sacrificing lateral resolution for the sake of SNR [35]. The same compromise is often made in the case of SLO when imaging the retina [37, 38].

![Figure 1: The variation of the lateral full-width-at-half-maximum (FWHM) as a function of pinhole diameter in Airy Units (AU), which is the pinhole diameter normalized by Airy disc diameter.](image-url)
The optical photon reassignment technique assigns photons to the location of the maximum of the joint probability of the excitation and detection PSFs [34, 35]. This can be done computationally if an image is acquired at the pinhole plane of a confocal microscope for every excitation position on the sample [39]. However, since SLOs must scan the retina quickly to reduce the presence of motion artifacts, sampling rates of the detected photons at the pinhole plane are generally between 5 – 30 MHz [11, 37, 40], which is much higher than the detection bandwidth of current 2D sensors. Therefore, an all-optical method like OPRA is preferred for retinal imaging, OPRA has been achieved in confocal microscopy by de-coupling the scanning magnification of the object and the magnification of the scanning spot in two different but functionally equivalent methods: 1) by rescanning the emitted sample light onto a CCD over the same field of view as the sample (scan magnification of 1) after decreasing the detection spot magnification by a factor of two [34], 2) by rescanning the emitted sample light onto a CCD with double the field of view as the sample (scan magnification of 2) while having unity magnification of the detection PSF [35].

1.5 Specific Aims

Specific Aim 1: Handheld probe development with SLO and OCT

The first aim of this work begins with the determination of fundamental design equations for scanning retinal imaging modalities like SLO and OCT. This was done in part by using optical design software in order to create an SLO design that minimized cost and size
while maximizing imaging performance parameters like resolution, field of view, and signal-to-noise ratio (SNR) [37]. These principles were applied to design and create a combined SLO and OCT system in a handheld probe weighing 1.45 kg [40]. Additionally, motion estimation and correction algorithms were developed to correct for motion in OCT volumes. Finally, a second, much smaller handheld SLO and OCT probe weighing only 94 g was developed through the use of a single 2D microelectromechanical systems (MEMS) scanner and a custom optical design utilizing a converging beam before the scanner [41]. Pediatric imaging results from this smaller handheld probe demonstrate parafoveal cone visualization and quantification for the first time in young children. The work in this aim spans Chapters 2 to 4.

**Specific Aim 2: True-color scanning laser ophthalmoscopy**

The second aim of this work describes a technique to acquire true color retinal images with an SLO by adapting the technique for acquiring color images with cameras to confocal laser scanning systems [42]. This technique involves illuminating the eye with a collimated white light source, collecting with red, green, and blue color channels, and color calibrating the result. As part of this work, we also design a custom achromatizing lens to compensate for the average longitudinal chromatic aberration in human eyes to avoid retinal images from the different color channels to be from different depths within the retina. This aim is addressed in Chapter 5.
Specific Aim 3: Super-resolution SLO using optical photon reassignment

The final aim of this work describes a technique to achieve super-resolution in the lateral dimension without loss of SNR in scanning laser ophthalmoscopy by utilizing principles from the optical photon reassignment technique. We describe a unique method for implementing this technique through the use of a scanning mirror with an optical coating on both sides for both scanning and descanning light from the sample and rescanning light onto a camera. A system setup was constructed demonstrating super-resolved imaging of a USAF test target in an intermediate imaging plane of the system. Super-resolved retinal images were also acquired in a human volunteer demonstrating parafoveal cone photoreceptor imaging as close as 1.5° from the fovea. This aim is addressed in Chapter 6.
2. Optimization of SLO design

In this chapter, we first present fundamental SLO design equations and describe their use in arriving at a first-order design. Next, we explain and characterize our detailed lens based optical design that achieves near diffraction-limited resolution with minimized imaging artifacts. Finally, we show the imaging results of an experimental implementation of our SLO design. These results include a study measuring the relationship between throughput and sharpness as a function of pinhole size and a comparison between images taken from our optimized SLO design and a commercial SLO system.

2.1 SLO design parameters

A generalized SLO design is shown in Figure 2 which is useful for deriving basic relationships between design parameters and SLO performance. The generalized design includes separate optical pathways for illumination and collection through a common telescope whose function is to image the subject’s pupil plane into the optical scanner aperture. Although practical SLOs utilize paired scanners to construct a 2-dimensional raster scan, it is typically the faster scanner which limits performance due to electro-mechanical trade-offs between scan frequency, scanner aperture, and maximum scan angle [43, 44]. Thus we only include the limiting (i.e., fast) scan direction here and assume that the slow scanner is either placed close to or imaged onto it by use of a second telescope.
Fundamental SLO performance parameters considered include the maximum field of view (FOV), optical throughput (T), frame rate (FR), and resolution. Note that the maximum FOV parameter corresponds to the maximum square FOV since it is very likely that the slow scanner can match and even exceed the maximum scan range of the fast scanner. These performance parameters depend upon design parameters such as the subject eye's pupil diameter (P), the SLO telescope magnification (M), the pinhole size (PH), and the limiting scanner parameters, which are the fast scanner aperture (D), maximum optical scan angle (θ) and scan repetition frequency (freq). These basic parameters and components of the SLO design are shown and labeled in the generalized SLO schematic in Figure 2.

Figure 2: Generalized one-dimensional schematic of scanning laser ophthalmoscope (SLO) design showing both the illumination path (red) and collection path (blue) for zero scan angle. The purple lines indicate the chief rays of the optical return path at the extrema of the scan angle.
The maximum one-dimensional FOV of the SLO entering the eye is a function of the maximum optical scan angle and telescope magnification. Telescopes that de-magnify the object size also magnify scan angles so the maximum FOV can be described as simply the maximum optical scan angle after angular magnification:

\[ FOV = M \cdot \theta \]  

(1)

The throughput of the SLO is a function of the limiting (fast) scanner aperture and the collection beam diameter \((M \cdot P)\), which is in turn limited by the size of the pupil imaged by the telescope into the scanner plane. Reflected light which makes it back through the pupil but overfills the scanner aperture is clipped, reducing throughput and exposing the subject to unnecessary light exposure. We quantify throughput as the ratio of the fast scanner aperture and the collection beam diameter:

\[ T = \frac{\text{Fast scanner aperture}}{\text{Collection beam diameter}} = \frac{D}{M \cdot P} \]  

(2)

Interestingly, taking the product of throughput and maximum FOV gives an expression proportional to the scan-angle mirror-size product for the fast scanner \((\theta \cdot D)\) (Eqn. 3). Thus, the optimal fast scanner for SLOs is one with the largest scan-angle mirror-size product at a given scanning frequency.

\[ FOV \cdot T = \frac{M \cdot \theta \cdot D}{M \cdot P} = \frac{\theta \cdot D}{P} \]  

(3)

The frame rate of the SLO is limited by the desired number of lines per frame and the number of lines per unit time, which in turn is related to the scanning frequency of the
fast scanner. If only one sweep of the fast scanner is acquired, the number of lines per unit time is equal to the scanning frequency. If both front and back sweeps are acquired, the number of lines per unit time is twice the scanning frequency. The frame rate is then the ratio of the number of lines per unit time and the number of lines per frame:

$$FR = \frac{\text{lines per unit time}}{\text{lines per frame}} = \frac{(1 \text{ or } 2) \cdot \text{freq}}{\text{lines per frame}}$$  \hspace{1cm} (4)$$

The SLO, like any linear imaging system, has a resolution that can be described by the full width at half maximum intensity (FWHM) of the intensity point spread function (PSF) of the detected light from a point source object [45]. More specifically, the theoretical resolution of the SLO can be described similarly to that of a confocal scanning laser microscope [46] because the SLO is a confocal scanning laser microscope that uses the patient’s eye as the objective lens [47]. Thus, the equation for the PSF at the detector plane of the SLO ($PSF_{\text{det}}$) can be described as:

$$PSF_{\text{det}} = PSF_{\text{illum}} \cdot (PSF_{\text{coll}} \otimes PH)$$  \hspace{1cm} (5)$$

where $PSF_{\text{illum}}$ is the PSF of the illumination optics including the eye, $PSF_{\text{coll}}$ is the PSF of the collection optics including the eye and scattering properties of the retina, and $PH$ is a function that represents the transmission through the confocal pinhole of a certain size, shape, and position. Due to the direction-sensitivity of light reflected from retinal photoreceptors (referred to as the optical Stiles-Crawford effect), the pinhole size,
shape, and position can be designed to relatively increase the amount of signal from the photoreceptor layer thus improving photoreceptor visualization [48-50].

2.2 First-order design procedure

The design procedure of our SLO was driven by seven constraints that we set based on our desired application: a compact, low-cost, lens-based SLO system. The inputs/constraints and the resulting design decisions are presented in the diagram in Figure 3.

<table>
<thead>
<tr>
<th>Constraints</th>
<th>Design Decisions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compact as possible and scalable</td>
<td>Use a combination of a resonant scanner and galvanometer placed as close as possible</td>
</tr>
<tr>
<td>Low cost components</td>
<td>Use APD, custom amplifier, and off-the-shelf optics</td>
</tr>
<tr>
<td>Imaging speed ≥ 8 fps, ≥ 500×500 pixels per frame</td>
<td>Use 2 kHz resonant scanner</td>
</tr>
<tr>
<td>Maximum throughput</td>
<td>( D = 7 \text{ mm} ) ( \theta = 20^\circ )</td>
</tr>
<tr>
<td></td>
<td>( 1 = \frac{D}{M \cdot P} = \frac{7 \text{ mm}}{M \cdot 7 \text{ mm}} )</td>
</tr>
<tr>
<td></td>
<td>( FOV = M \cdot \theta = 1 \times 20 = 20^\circ )</td>
</tr>
<tr>
<td>Eye parameter ( P = 7 \text{ mm} )</td>
<td>( M = 1 )</td>
</tr>
<tr>
<td>Minimum imaging artifacts</td>
<td>Optimize optical design to reject imaging artifacts</td>
</tr>
<tr>
<td>Highest lateral resolution on the retina</td>
<td>Utilize optimal illumination beam diameter at pupil ( \sim ) 2.5 mm</td>
</tr>
</tbody>
</table>

Figure 3: First-order design procedure for compact SLO design

To make our system as compact as possible, we chose to use a combination of a resonant scanner and galvanometer with the scanners placed as close as to each other as mechanically possible without risking damage to the scanners (∼8 mm separation). To
minimize system cost, we chose to use off-the-shelf optics, to electronically filter with a low-cost, custom-fabricated amplifier and to detect with an avalanche photodiode (APD) instead of a photomultiplier tube (PMT). To achieve 8 frames per second (fps) imaging speed with 500 lines per frame, we used a 2 kHz resonant scanner (Electro-Optical Products Corp, Glendale, NY USA) and utilized both sides of the scan sweep to effectively scan at 4 kHz. The 2 kHz resonant scanner had a 20° peak-to-peak maximum optical scan range with an aperture size of 10 x 10 mm and a limiting aperture \( D \) of 7 mm due to the 45° tilt of the scanner. To maximize throughput \( T = 1 \) we used the expression for throughput \( T \) in Eqn. 2 and solved for the magnification of the telescope \( M \) assuming a maximum (dilated) pupil diameter of the eye \( P \) to be 7 mm. This gave a design magnification \( M \) of 1. Using this magnification, we determined that the FOV of our system would be 20° from Eqn. 1. To maximize lateral resolution on the retina, we chose to use a 2.5 mm illumination beam diameter at the pupil of the eye, which was determined to be the optimal beam diameter for lateral resolution based on the aberrations of 15 subjects as described by Donelly III et al. [51]. This illumination beam diameter infers an Airy disk radius at the retina (assuming ideal ocular optics) of 7 μm.

### 2.3 Optimized optical design

We optimized the SLO optical design using Zemax to achieve near diffraction-limited resolution across a 20° FOV. Achromatic doublet lenses were used to minimize chromatic aberration and lens splitting was utilized after the scanners to reduce spherical aberration.
An effective focal length of 50 mm was chosen for each set of lenses in the SLO telescope to balance device size and aberrations. The corneal reflection was minimized by constraining the system design such that the specular reflection from the cornea-air interface was well out of focus at the plane of the confocal pinhole, thus strongly rejecting this backscattered light. Lens reflections were not optimally minimized through the optics since they are stationary with the system and did not saturate the detector, amplifier, or digitizer and so could be removed through background subtraction.

An overview of the optimized optical design of our SLO is shown in Figure 4. Spot diagrams, Modulation Transfer Function (MTF) plots, and an off-axis PSF plot were determined using a recent eye model from Goncharov and Dainty [52] and are shown in Figure 5, Figure 6.a, and Figure 6.b respectively. The PSF plot was of a configuration demonstrating the largest FWHM of 7 μm. A fixation target to minimize patient eye motion was inserted by placing a dichroic mirror between the last two set of lenses before the eye (see Figure 4) in order to image the fixation pattern displayed by a 1 inch LCD screen onto the retina. The lens closest to the eye was mounted on a knob-adjustable rack and pinion linear translator designed to allow for ± 6 diopters of refraction correction. A photograph of the implemented SLO design is shown in Figure 7.
Figure 4: Schematic of the optimized SLO as described in the text with the confocal pinhole at the end of the optical path from the eye with the illumination input (red), backscattered collection of light from the retina (blue), and backscattered light from the cornea (green).
Figure 5: Object plane spot diagrams for nine configurations spanning a 20-deg field of view (FOV) square on the Goncharov and Dainty [10] model eye’s retina demonstrating near diffraction-limited resolution for the illumination path. The Airy disk radius was 7 μm. The Strehl ratios for the center, top-bottom, left-right, and corner configurations are 0.981, 0.975, 0.944, and 0.674, respectively.

Figure 6: (a) Modulation transfer function (MTF) of the SLO for configurations spanning 20-deg FOV on the retina. The black curve represents the diffraction limited MTF and the blue curves represent the MTFs for nine configurations spanning a 20 deg FOV on the retina. (b) Point-spread function (PSF) of the SLO at the retina for one of the outermost configurations. The largest full width at half maximum (FWHM) across the diagonal of the PSF was 7 μm and the box containing the PSF was 32 × 32 μm2. The
PSF appears different from the spot diagrams shown in Figure 3.4 because in the Zemax® optical design software, the PSF takes into account diffraction while spot diagrams do not.

![Image: Physical implementation of the described SLO design with subject at left. The focus adjustment knob is denoted by a yellow arrow.](image)

Figure 7: Physical implementation of the described SLO design with subject at left. The focus adjustment knob is denoted by a yellow arrow.

The SLO source was a superluminescent diode (SLD) (Superlum, Cork, Ireland) operating at 840 ± 25 nm. The detector was an avalanche photodiode (APD) (Hamamatsu, Shizuoka-ken, Japan) with fixed gain and a custom amplifier (40 dB gain and 2.75 MHz BW) was designed and used to amplify the detector signal such that the maximum signal amplitude from the retina plus that from lens reflections filled the dynamic range of the digitizer. The digitizer used was an NI PCI 6115 card (12-bit, 10 MS/s/ch) (National Instruments, Austin, TX USA) and scanners were controlled separately with an NI PCI 6711 card (12 bit, 1 MS/s/ch) (National Instruments, Austin, TX USA).

Raw images acquired with the PCI 6115 digitizer have a bit-depth of 12 bits (4096 gray levels), however after background subtraction the resulting image bit-depth was
normally reduced to ~11 bits (2048 gray levels). The resulting bit-depth for low reflectivity eyes (~25% – 50% reflectivity of normal) was reduced to ~9 – 10 bits (512 – 1024 gray levels). We were satisfied with this tradeoff between dynamic range and background subtraction, but alternatively one could forgo background subtraction and let the lens reflections saturate the detector to avoid reducing the bit-depth of the retinal image. This could be done by using a higher gain detector, applying a higher gain amplifier, or reducing the dynamic range of the digitizer (possible with the PCI 6115 card).

Custom software was developed in Labview (National Instruments, Austin, TX USA) for image acquisition, scanner control, background subtraction, image dewarping, image interweaving, and gamma correction. Images were dewarped and linearly resampled due to the sinusoidal waveform of the resonant (fast) scanner. Image processing was done in real time with MATLAB (Mathworks, Natick, MA USA) to provide correctly oriented, gamma corrected images at 8 fps. The steps of background subtraction, dewarping, image interweaving, and gamma correction are illustrated with sample data in Figure 8. Gamma correction was applied to enhance the contrast of features with intensity values closely spaced on a linear scale. The gamma correction algorithm used was:

\[
g(x, y) = f(x, y)^\gamma
\]  

(6)
where \( f(x, y) \) is the normalized original image’s pixel intensity as a function of position, \( \gamma \) is the gamma value applied for gamma correction, and \( g(x, y) \) is the gamma corrected image’s pixel intensity as a function of position [53].

Image sharpness was quantitatively measured with a simple variation of the image focus measurement technique by Kautsky et al [54], which is computed as the ratio of the L2 norm of the high-passed image region and the L2 norm of the low-passed image region as shown in Eqn. 7. We explain our method for separating a given image region into high and low-passed image regions in the Results section. The overall throughput was calculated by taking the sum of the pixels in the image region of interest as shown in Eqn. 8.

\[
\text{Sharpness Metric} = \frac{\|\text{Im}_{\text{HP}}\|_2}{\|\text{Im}_{\text{LP}}\|_2} \quad (7)
\]

\[
\text{Overall Throughput} = \sum_{i=1}^{n} \sum_{j=1}^{m} \text{Im}(i, j) \quad (8)
\]
Figure 8: Image processing steps for SLO image acquisition on a 6.7-deg FOV image. (a) Raw image containing both forward and backward sweeps of the resonant scanner. The lens reflections appear saturated because they are the brightest features in the image, and the image was normalized to enhance visualization of darker parts of the image. (b) Raw image after background subtraction. (c) Raw image after background subtraction, dewarping, and interweaving image from both resonant scanner sweeps into a single image. (d) Image in part (c) after gamma correction with $\gamma = 0.5$ showing nerve fiber bundles.

### 2.4 Imaging results

Single-frame SLO images of a normal human subject for two FOV’s (20° and 6.7°) and two digitally zoomed in FOVs (3.3° and 1.3°) from an original 6.7° FOV image are shown in Figure 9. The relative location of a retinal image is given by the eccentricity which is
defined as the distance in degrees between the fovea and the center of the image. All images exhibited minimum corneal reflection and, after background subtraction, minimum lens reflections. Imaging was done in slightly dimmed lighting with non-dilated pupils (~3 mm in diameter) and with an incident power at the eye of 580 μW, which is below the maximum permissible radiant power for SLO’s at 840 nm wavelength [55].

Figure 9: Unaveraged retinal SLO images (single frame each) from the described design at two optically zoomed in FOVs: 20 and 6.7 deg as shown in (a) and (b), respectively. The 3.3- and 1.3-deg FOV images in (c) and (d), respectively, were digitally zoomed from a 6.7-deg optically zoomed image and showed the cone photoreceptor mosaic. (a),
(c), and (d) were focused on the photoreceptor layer and (b) was focused on the nerve fiber layer. Image eccentricities are given in degrees under the FOV for each image.

Larger pinhole sizes were used to image larger FOVs when image resolution was limited by the sampling rate as opposed to the optical resolution. We describe pinhole size in terms of the times-diffraction-limited spot size (TDL), which is the pinhole size normalized with respect to the Airy disc diameter of the collection optics (TDL = Pinhole size/Airy disc diameter). A 100 μm pinhole (TDL = 3.31) was used for acquiring 20° FOV images since the resolution for that FOV was ultimately limited by the number of lines per frame and sampling rate of our digitizer. A 20 μm (TDL = 0.66) and 30 μm pinhole (TDL = 1.0) were used for obtaining 6.7° FOV images because the resolution for that FOV was optically limited and those pinhole sizes provided a good balance of resolution and SNR. Images were taken at 8 frames per second (fps) with 500 lines per frame and a pixel density of 1000 samples per line.

In the second set of experiments shown in Figure 10, a 6.7° FOV foveal image was taken with a 30 μm pinhole (TDL = 1.0) and five 0.5° square FOV patches at 0.8°, 2.3°, 3.2°, 3.7°, and 4.3° eccentricity from the foveal center were digitally zoomed to qualify how close to the fovea photoreceptors were resolved, which appeared to be at retinal eccentricities ≥ ~3.2°.
Figure 10: A 6.7-deg FOV SLO image of the fovea showing resolution of cone photoreceptors at retinal eccentricities ≥~3.2 deg. The large image is a single, un-averaged frame without background subtraction taken at 8 fps with a pinhole of 30-μm diameter or a times-diffraction-limited spot size (TDL) of 1. The cropped images span a 0.5-deg FOV.

In the third set of experiments, to determine the effect of the confocal pinhole on throughput and sharpness, five images containing a 0.4° area of the retina at 4.2° eccentricity of a subject’s right eye was imaged with 6.7° FOV (see Figure 11) for each of seven pinhole sizes. Pinhole sizes ranged from 10-100 μm in diameter with TDLs from 0.33-3.31. Image sharpness was quantified for each of the 0.4° areas using the image focus measurement technique described previously (see Eqn. 7). The filter used to separate the image region into low and high-passed image regions was Gaussian with a 50% cutoff at
the spatial frequency $1/12.2 \, \mu m^{-1}$. Spatial frequency was calculated by assuming the eye’s second nodal point is approximately 16.5 mm from the retina [56], which implies that a 1° retinal region would be approximately 288 $\mu m$ wide. Since the average cone spacing at 4.2° eccentricity is at a higher spatial frequency (approximately $1/8 \, \mu m^{-1}$ [57]) than the 50% spatial frequency cutoff of our filter ($1/12.2 \, \mu m^{-1}$), we expect higher values of sharpness (at the expense of throughput) as the contrast and resolution of our system improves through the use of smaller pinhole sizes. The plot of the sharpness measurements and the observed throughput are shown in Figure 12.

Figure 11: The location of the portion of the retina utilized for the sharpness metric across varying TDL spot sizes is shown by the red box marked in this 6.7-deg FOV image and is at approximately 4.2 deg eccentricity. This image was taken with a TDL of 1.3 and at a 7.2-deg eccentricity.
Figure 12: Plot of sharpness and throughput for varying TDLs at the location specified in Figure 11. Red circles delineate TDLs used for retinal imaging outside of this experiment. Red circles with subscripts 1 and 2 correspond to TDLs 0.66 and 1.0, respectively, and were used to image at low FOVs where resolution was determined by optical resolution. The red circle with subscript 3 corresponds to a TDL of 3.31 and was used to image at high FOVs where resolution was determined by digital sampling frequency not optical resolution.

Finally, we compared the imaging results of our optimized SLO design to that of a commercial SLO system, the Heidelberg Spectralis (Heidelberg Engineering, Inc., Vista, CA). With our optimized SLO design, both 20° and 6.7° FOV images were taken at 8 fps with 500x1000 pixels per image. With the Heidelberg Spectralis, 20° and 15° FOV images were taken at 6.8 fps with 1024x1024 pixels per image and 8.8 fps with 768x768 pixels per image, respectively, using Heidelberg’s “High-resolution” setting. We would like to note that a 6.7° FOV with the Spectralis is not possible so the 15° FOV setting, which is the lowest FOV setting on the Spectralis, was used instead. Results from the comparison are shown in Figure 13.
Figure 13: Comparison of images from our optimized SLO design and a commercially available SLO (Heidelberg Spectralis). (a), (b), and (c) each show an 18.5-deg FOV image cropped from a 20-deg FOV image. (a) Single frame from our optimized SLO design. (b) Single frame from the Spectralis. (c) One-hundred-frame average from the Spectralis. (d), (e), and (f) each show a 3.3-deg FOV image taken at a 6.3-deg eccentricity. (d) Single frame from our optimized SLO design cropped from a 6.7-deg FOV image. (e) Single frame from the Spectralis cropped from a 15-deg FOV image. (f) One-hundred-frame average from the Spectralis cropped from a 15-deg FOV image.

2.5 Summary

We have demonstrated a simple, compact optical design for a SLO that produces near diffraction limited illumination on the retina across a 20° FOV with minimized imaging artifacts. With the experimental implementation of our design, we demonstrated fast, high SNR, high resolution retinal imaging to visualize micron scale anatomical structures of the retina in vivo. At lower FOVs, by adjusting the focus to the respective retinal layers,
we were able to visualize nerve fiber bundles throughout the retina, and photoreceptors at eccentricities ≥ ~3.2° with TDL’s ≤ 1, without the use of adaptive optics. The theoretical resolution of our system (7 µm) supports the resolution of detected individual cone photoreceptors starting at approximately 3° eccentricity [57]. In practice, we were able to visualize photoreceptors near this eccentricity (see Figure 10).

Using various pinhole sizes, we quantified the relationship between retinal image sharpness and the times-diffraction-limited spot size (TDL). Experimental sharpness measurements (see Figure 12) showed that as the confocal pinhole decreased in size, image sharpness increased while throughput decreased. However, pinholes smaller than 0.5 TDL resulted in very low SNR so sharpness appeared to decrease rather than increase.

Through an experiment comparing our optimized SLO design to the Heidelberg Spectralis, we have shown that our design demonstrates an improvement in both image quality and resolution. This improvement is especially noticeable at a 6.7° FOV, in which our system can resolve parafoveal cone photoreceptors in a single frame, which is not possible with either a single frame or a 100 frame average via the Spectralis.

While adaptive optics based SLO designs have superior resolution, this comes at the expense of cost, size, and system complexity. We have demonstrated high-quality retinal imaging of micron scale anatomical features of the retina with a significantly more compact and affordable SLO. Our optimized optical design for the SLO may also be
extended to optical coherence tomography (OCT) systems, in which the sample arm optics are nearly identical to SLO optics.
3. Handheld simultaneous SLO and OCT system

In this chapter, we present a handheld probe and system design that acquires high signal-to-noise ratio (SNR) SLO and OCT images simultaneously with patient exposures safely below the ANSI limit [58]. At reduced fields of view (FOVs), we show that the SLO can image parafoveal cones without adaptive optics at a retinal eccentricity of 11° in subjects with good ocular optics. We also demonstrate lateral motion correction of OCT B-scans based on motion estimates determined from concurrently acquired SLO images using a modified version of the patch-based cross correlation image registration technique [59-66]. Finally, we demonstrate axial motion correction utilizing a significantly modified version of the automated segmentation-based OCT volume registration technique [67].

3.1 System design

The SLO and OCT sources were superluminescent diodes (SLDs) operating at 770 ± 8 nm (Inphenix, Livermore, CA) and 840 ± 35 nm (Superlum, Moscow, Russia), respectively. The detector for the SLO was an APD (C5460, Hamamatsu, Shizuoka-ken, Japan) with fixed gain, and the detected signal was low-pass filtered from DC - 2 MHz (Model 3945, Krohn-hite Corporation, Brockton, MA) and acquired at 5 MS/s using the NI PCI 6115 card (12-bit) (National Instruments, Austin, TX). The SLO signal was low-pass filtered with a cutoff frequency of 2 MHz (just less than half the sampling frequency) in order to avoid aliasing artifacts in the SLO image. The SLO scanners consisted of a 4 kHz resonant scanner (Electro-Optical Products Corp, Glendale, NY) and a 5 mm aperture
galvanometer (Thorlabs Inc, Newton, NJ) placed as close as mechanically possible without risking damage to the scanners (~8 mm). The horizontal or fast scanner operated at 4.24 kHz, but both the forward and backward sweep of the scanner were utilized to effectively operate at 8.48 kHz. The vertical or slow scanner of the SLO operated at a rate of 16 Hz to record frames at 16 fps. Each image of the SLO consisted of 530 x 580 pixels. The OCT scanners consisted of a commercially available, mounted XY galvanometer set with 3 mm aperture mirrors (Cambridge Technology Inc, Bedford, MA). The integration time per A-scan was 50 μs with 2048 pixels per A-scan. For imaging, we used 500 A-scans per B-scan, 500 B-scans per volume, and a delay equivalent to 50 A-scan integration times per return path (fly-back) of the galvanometer mirrors between B-scans. This corresponded to a fast scan of 40 Hz and a slow scan of ~0.073 Hz giving ~220 SLO images per OCT volume. The SLO scanners were controlled separately with an NI PCI 6711 card (12 bit) (National Instruments, Austin, TX) and the OCT scanners were controlled with the NI PCI 6115 card. Custom software developed in Labview (National Instruments, Austin, TX) and software used in commercially available Bioptigen SDOCT systems (Bioptigen Inc, Durham, NC; see author disclosures in acknowledgments section) were used on the same computer for real-time display of SLO and OCT images. An 80:20 fiber coupler was used for the OCT interferometer and a commercial spectrometer (SD800, Bioptigen Inc, Durham NC) was used as the OCT detector. The OCT axial resolution and 6 dB falloff range were measured to be 7 μm (in air) and 1.1 mm, respectively.
3.2 Probe optical design

The optical design and system optimization for both SLO and OCT sub-systems of the handheld probe were completed using optical design software (Radiant ZEMAX LLC, Redmond, WA). Given the six design considerations mentioned previously, mechanical constraints, and commercially available lenses, the optical design was optimized by minimizing spot sizes spanning the FOV at intermediate image planes and along the retina of a model eye. An overview of the optimized optical design of the handheld system is shown in Figure 14. The SLO and OCT beam paths begin separately and are scanned with separate scanners. The beams were combined at a dichroic mirror just prior to the objective lens. The dichroic mirror was placed in this location for two main reasons: 1) the dichroic mirror was designed to work at an angle of incidence of 45° which, after scanning, only occurs between the lenses of the telescope after the scanners, and 2) there was no other location that the dichroic mirror would mechanically fit without increasing device size. Unity beam magnification was employed for both the SLO and OCT systems when imaging the scanners onto the eye’s pupil. The beam diameter at the pupil of the eye was ~2.5 mm for both the SLO and OCT. The telescope prior to the eye consisted of 2.54 cm diameter lenses with an effective focal length of 37.5 mm. This focal length was chosen to give a working distance of ~25 mm and have sufficient room for the dichroic mirror and OCT fold mirror. Since the f-numbers of the lenses are small given the 20° scan range and the bandwidths of the light sources are relatively wide, careful consideration was taken
in choosing a combination of commercially available lenses to minimize both spherical and chromatic aberration. Our solution was to use achromatic doublet lenses to reduce chromatic aberration and split the lens with a 3 to 5 ratio in refractive power in order to gradually refract the scanned beams and thus reduce spherical aberration. For the confocal pinhole, we used a multimode fiber with a diameter equal to approximately 1.6 times the Airy disc diameter, which has been demonstrated to give a good balance between image sharpness and throughput [37, 38]. To achieve a confocal pinhole of this size given a 3 mm collection pupil (undilated), a 50 mm focal length collection lens and a 50 μm diameter multimode fiber was utilized.

Figure 14: A side view schematic of the handheld SLO-OCT design. All optical components are labeled and described in the legend. The SLO source is a 770 nm SLD with 15 nm bandwidth. The OCT source is an 840 nm SLD with 70 nm bandwidth. The optical paths shown are the superposition of both the illumination and collection paths. The illumination beam diameters for both SLO and OCT were ~2.5 mm. The collection beam diameter for the SLO was larger due to the larger NA and size of the collection fiber, and because backscattered light from the retina fills the pupil in the return path.
Nearly diffraction-limited performance of 7 and 7.5 μm was achieved in Zemax for nine points spanning a common 20° FOV at the back of a model eye for the SLO and OCT systems, respectively (Figure 15). The eye model was created based on parameters determined in a study by Goncharov and Dainty [52].

![Spot diagrams for the SLO (A) and the OCT (B) illumination on the retina spanning a 20° FOV. SLO and OCT are nearly diffraction limited at 7 and 7.5 μm (the Airy disk radii), respectively (Airy disk is shown by black circle on spot diagrams). Spot diagrams are color coded for 3 wavelengths spanning the bandwidth of the respective sources. SLO spot diagrams have increased astigmatism compared to OCT spot diagrams due to the transmission through the tilted dichroic mirror.](image)

**3.3 Probe mechanical design**

The opto-mechanical design for the system was developed in Solidworks (Dassault Systèmes SolidWorks Corp, Concord, MA) and is shown with and without its outer casing in Figure 16. Custom lens tubes, lens spacers, and mirror mounts were designed and fabricated to accommodate the closely spaced optics of the system and minimize its footprint. The internal skeleton and other structural components were made of aluminum.
to simplify fabrication and maintain low weight. Alignment pins were used at the junctions between mechanical components to facilitate assembly. Zemax was used to determine the maximum permissible positional error of optical components, and a mechanical tolerance stack analysis was performed to ensure that the design specifications were satisfied. The minimum number of adjustments in the design to optimize performance at assembly and operation was determined, resulting in adjustability of the axial position of the objective lens, the XYZ position of the collection and illumination fibers, and the angular orientation of the resonant scanner. The lens closest to the eye (L6) was mounted on a rack and pinion linear motion system with a total travel of 23 mm to allow for refraction correction of ± 8 diopters. The OCT collimation optics and the SLO collimation and collection optics were adjusted for lateral beam placement using XY translation stages and axial beam placement by sliding the optics along cage rods. The resonant scanner was attached to the main body of the handheld probe via an L-bracket with curved slots to adjust the rotation of the scanner while maintaining the correct center of rotation. The outer casing was 3D printed (Dimension 1200es, Stratasys, Ltd., Edina MN) with Acrylonitrile Butadiene Styrene and consisted of two halves that joined together as a snap fit and could be secured with four set screws. The fabricated setup is shown in Figure 17 both mounted to a slitlamp-type patient interface configuration with a chin rest for alignment as well as in handheld operation. A rubber eyecup was attached to the last lens of the handheld to aid in keeping the eye a working distance away from
the lens. The handheld probe weighed 1.45 kg and was 25.4 cm long x 10.9 cm wide x 15.5 cm tall.

Figure 16: Solidworks design of the handheld SLO-OCT probe. A) Side view showing the internal components of the probe. B) Isometric view of probe with case.

Figure 17: The handheld SLO-OCT probe. A) Tabletop mountable configuration on a patient positioning system from a Carl Zeiss slit-lamp. B) Handheld use of probe.

3.4 Image processing

The lateral motion of the patient’s retina during combined SLO-OCT operation was determined in post-processing after data acquisition by registration of SLO images. First, background measurements taken prior to SLO and OCT image acquisition were
subtracted from SLO and OCT images in order to remove any static lens reflections and artifacts due to dust or scratches on optical elements. Then SLO images were preprocessed using a multi-scale coupled Laplacian of Gaussian (LoG) and Gabor filtering technique similar to that described by Estrada et al. [68]. LoG and Gabor filtering were applied to enhance the vessel contrast of fundus images and thus improve the accuracy and robustness of image registration [68, 69]. A LoG filter is a Gaussian filter convolved with a Laplacian filter and can be used to isotropically enhance edges while smoothing the image to reduce noise. Images were convolved with two LoG filters with kernels of size 100 x 100 pixels with standard deviation values of 1.5 and 3 pixels, respectively. The maximum response was kept at each pixel for the two LoG filtered images and the resulting image was further filtered with Gabor filters to enhance vessel-like structures.

A Gabor filter is a Gaussian filter modulated by a sinusoid and can be used to enhance edges of a predefined length at the orientation of the sinusoid. Gabor filters are defined with the following five parameters: 1) standard deviation of the Gaussian along the sinusoid ($\sigma_x$), 2) standard deviation of the Gaussian orthogonal to the sinusoid ($\sigma_y$), 3) spatial frequency of the sinusoid ($v$), 4) phase of the sinusoid ($\psi$), and 5) orientation of the sinusoid ($\theta$). We reduced the Gabor filter to just two parameters ($\sigma_x$ and $\theta$) by setting $\sigma_y$ to 8 pixels, $\psi$ to 0°, and $v$ to $1/(2\sigma_x)$. We then applied 30 Gabor filters with kernels of size 100 x 100 pixels to our LoG filtered image with $\sigma_x$ equal to 2, 5, and 8 pixels, respectively, at 10 orientations spanning 180°. The maximum response was kept at each pixel for the 30
Gabor filtered images and, in the resulting image, pixels with intensities less than 5% of the maximum intensity were set to zero by thresholding.

Preprocessed SLO images were then registered using a modified version of the patch-based cross correlation technique [59-66]. Each preprocessed SLO frame was divided into 15 horizontal strips (14 strips with 35 x 580 pixels each and 1 strip with 40 x 580 pixels). All strips were cross-correlated to a reference frame (usually the first acquired frame) in order to determine the (x,y) displacements of each strip. Since frames were acquired at 16 fps, images were divided into 15 strips to obtain 240 lateral motion estimates per second. Image rotation was assumed negligible per strip but was determined per frame. This was done by setting the initial or reference frame to have 0° rotation, rotating each successive SLO frame with a set of 20 rotations spanning ± 0.5° about the rotation of the previous frame, cross correlating with the reference frame, and keeping the rotation value resulting in the highest cross correlation peak. This range and step size for rotations was chosen because, in practice, the rotation between successive frames was much less than ± 0.5° and a rotation of an SLO image by a single step size (0.05°) was indistinguishable from no rotation. Blinks were detected by monitoring when the average pixel intensity in the unprocessed SLO image fell below 5% of the average pixel intensity in the reference SLO image. Both SLO and OCT frames corresponding to blinks were discarded from the raw data. After patch-based cross correlation was completed for a set of SLO data, the full motion field of each SLO image was found by
spline interpolation of the estimated motion. Each individual line of an SLO image was warped using this motion field to correct for motion artifacts.

The motion estimates from SLO image registration were then applied to correct for lateral motion artifacts in the simultaneously acquired OCT volumes. Since the OCT scanners spanned the same FOV as the SLO scanners, the OCT motion was determined by multiplying the SLO motion by the ratio between the OCT lateral pixel dimensions and the SLO lateral pixel dimensions. The bulk lateral displacement between OCT volumes was determined by taking an en-face summed voxel projection (SVP) of each lateral motion-corrected OCT volume, interpolating gaps in the SVPs up to 4 × 4 pixels in size where data was not captured, preprocessing the interpolated SVPs with LoG/Gabor filters, and cross correlating the filtered SVPs to get the lateral motion and rotation between volumes. Composite SVPs were created by shifting and rotating the individual lateral-motion-corrected SVPs by the previously calculated bulk lateral displacement and rotation, averaging the overlapping pixels containing data, and interpolating gaps in the composite SVP with the same method described for interpolating individual SVPs.

To correct for axial motion during the OCT volume acquisition, we utilized a significantly modified version of the automated segmentation-based volumetric image registration technique [67], which incorporates the orthogonal scanning technique also utilized in other publications [70, 71]. Orthogonal acquisition was accomplished by taking OCT data in two modes: 1) with the horizontal scanner as the fast scanner (X-fast) and 2)
with the vertical scanner as the fast scanner (Y-fast). In our new method, first the internal limiting membrane (ILM) of the retina in each B-scan for both X-fast and Y-fast OCT volumes was automatically segmented through preprocessing and thresholding. Preprocessing involved median filtering the image with a 25 x 25 pixel kernel and convolving with 20 directional filters with kernels of size 30 x 20 pixels spanning 180°. Directional filters were created by simply rotating kernels with values of 1 and -1 for the top and bottom halves of the kernel. The maximum response for each pixel in the B-scan was obtained after convolution with all directional filters in order to enhance edges with different orientations. Individual A-scans in the preprocessed B-scans were then thresholded to find the first retinal border in the image, which corresponds to the ILM. The result was smoothed for all A-scans per B-scan to allow for a continuous segmentation.

After segmentation, lateral motion-corrected OCT volumes were overlapped to create the initial composite OCT volume. Two X-fast and two Y-fast B-scans near the corners of the composite OCT volume were then axially translated and rotated such that the segmented ILM in each B-scan overlapped axially for points of lateral overlap. Remaining X-fast B-scans were then also registered by axial translation and rotation such that the segmented ILM overlapped axially at points of lateral overlap in the two initial Y-fast B-scans. The same was done for the remaining Y-fast B-scans with respect to the two initial X-fast B-scans to complete axial registration of the composite OCT volume.
Regions in the composite volume with overlapping A-scans were averaged to improve SNR.

### 3.5 Imaging results

SLO and OCT images were acquired simultaneously spanning a 20° FOV at 16 and 40 fps, respectively (as shown in A and B of Figure 18). Background measurements taken prior to imaging were subtracted from both SLO and OCT images to remove static artifacts like lens reflections and scratches or dust on optical elements. The SNR of the OCT system was determined by measuring the maximum signal from an A-scan when the optical beam was reflected from a mirror divided by the background noise level of the system. To avoid saturation of the spectrometer, neutral density filters were added into the sample arm and the maximum A-scan signal detected was compensated to account for this additional loss. The SNR of our OCT system was 100 dB for 50 µs integration time and 300 µW illumination power at the sample. The SLO, at smaller FOVs, was shown to visualize parafoveal cones at a retinal eccentricity of 11° without adaptive optics (as shown in C-E of Figure 18). The average cone spacing at 11° eccentricity is approximately 12.4 µm [57] which is ~1.8 times the diffraction-limited spot size of the SLO arm. The radiant flux incident on the eye for the SLO and OCT were under the ANSI limit at 300 µW each, which comprised 56% and 41% of the thermal hazard maximum permissible exposure limit [58].
Figure 18: A) SLO image (single frame) with red line representing location of B-scan. B) Single B-scan taken simultaneously with the SLO at 40 fps. C) Foveal SLO image (single frame) indicating the position where the SLO was optically zoomed to visualize parafoveal cones. D) Optically zoomed retinal image (5 frame average) via reduction of scan range to a 2.5° FOV at location shown by red box in C) at an 11° eccentricity. E) Digitally zoomed image at location shown by blue box in D) with a 1.5° FOV showing the cone photoreceptor mosaic.

SLO images were preprocessed with LoG/Gabor filtering and thresholding as shown in Figure 19 prior to image registration. Preprocessed SLO images were registered using our modified version of the patch-based cross correlation technique and the motion field generated from registration was used to de-warp SLO images as shown in Figure 20.
Figure 19: Single frame SLO image (A) and the corresponding LoG/Gabor filtered and thresholded SLO image (B).

Figure 20: SLO patch-based registration. A) SLO reference frame to which all images are registered. B) Another SLO image from the same subject. C) Patch-based registration of SLO image in B) to the reference frame in A). The voids have been
colored in green to better visualize the regions where no information was obtained. D) Spline interpolated motion determined from the patch-based registration in C) is applied to each line in the SLO image from B) and empty voids within the frame are linearly interpolated.

Plots of SLO motion estimation are shown in Figure 21 for an X-fast and Y-fast OCT volume. SVPs are shown before and after lateral motion correction, combination of X- and Y-fast SVPs, and interpolation in Figure 22.

Figure 21: SLO motion estimation with X-fast (A-C) and Y-fast (D-F) OCT. A slow horizontal drift is visualized in the X-fast motion estimation with a large saccade captured at around the 13 second point. Both vertical and horizontal drifts are apparent in the Y-fast motion estimation with two microsaccades captured at around the 0.5 and 11 second points.

OCT axial motion was determined using our significantly modified version of the automated segmentation-based volumetric image registration technique. A depiction of this procedure and the resulting registered OCT volume are shown in Figure 23 below.
Figure 23: Steps for axial registration with an X- and Y-fast OCT volume. A) The initial four B-scans taken near the corners of the composite volume. Red dots indicate points of lateral overlap on the surface of each initial B-scan. B) The initial four B-scans after axial translation and rotation. Note that the red dots at points of lateral overlap now overlap axially as well. C) The registration of an additional two X- and Y-fast B-scans. D) Final registered volume rendering of all X- and Y-fast B-scans.

3.6 Summary

We have demonstrated an SLO-OCT handheld probe with ~7 μm resolution spanning a 20° FOV across the retina. Parafoveal cone imaging was shown using the SLO arm of this device using a 2.5° FOV at an 11° eccentricity without adaptive optics. SLO motion estimation was achieved using a patch-based cross correlation approach using 15 strips to allow 240 motion estimates per second. OCT lateral registration was demonstrated using
the motion estimation from simultaneously acquired SLO images. OCT axial registration was accomplished using a registration technique utilizing orthogonal fast scan directions and the segmentation of the ILM of the retina. Lateral and axial motion correction were both fully automated and allowed for automatic generation of motion corrected OCT volumes. The use of this technology will provide a compact and cost-effective solution for high SNR, motion-corrected imaging of patients that are supine, under anesthesia, or unable to position. These include infants with variety of retinal diseases such as retinopathy of prematurity [72-74], albinism [75], nystagmus [76], Shaken baby syndrome [77], and for imaging young children.
4. Ultra-compact switchable SLO and OCT design

In this chapter, we present a new ultra-compact, dual-modality SLO/OCT handheld probe design capable of parafoveal cone photoreceptor visualization with a weight of only 94 g, which is over an order of magnitude lighter than the prior handheld SLO/OCT designs [40, 42], and similarly far lighter than commercially available OCT-only handheld systems, which weigh 1.5 kg or heavier [78]. This design utilizes a combination of three significant innovations that enabled the significant reduction in system size. First, a novel telescope design employing converging rather than collimated light on a scanner was used to reduce the length of the system’s telescope compared to standard 4f telescope designs. Second, a single high-speed 2D micro-electromechanical systems (MEMS) scanner was used for both SLO and OCT imaging as an alternative to two sets of larger galvanometer-based optical scanners. Finally, custom lens designs were created to minimize device size while correcting for monochromatic and chromatic aberrations in the optical system (including those of the average human eye).

4.1 Converging-at-scanner telescope design

Conventional SLO and OCT retinal systems employ one or a series of standard 4f imaging telescopes to image the scanner plane into the patient’s pupil [3, 79]. Figure 24 illustrates conceptually a novel telescope design which employs light converging on the scanner to enable a substantial reduction in the telescope length. The beam enters the telescope converging at a distance $r$ from the scanner. There are two lenses, L1 and L2, with focal
lengths \( f_1 \) and \( f_2 \), respectively. The layout is similar to a traditional 4\( f \) imaging telescope, except the distance between the lenses \( d \), is decreased by the factor \( f_1^2 / r \) to compensate for the initial defocus. As \( r \) is increased to infinity, the input beam becomes collimated and \( d \) becomes \( f_1 + f_2 \) as in a 4\( f \) telescope. Because the chief rays of the scan all start a lateral distance \( f_1 \) from the principal plane of L1, they travel parallel to the optic axis between the lenses, and thus converge at a lateral distance \( f_2 \) after the principal plane of L2. Additionally, the same lateral and angular magnifications based on the length of the lenses apply as for a 4\( f \) telescope (\( f_2 / f_1 \) and \( f_1 / f_2 \) respectively). Thus, a 4\( f \) imaging configuration is easily converted to a converging-at-scanner design without change in angular range or spot size.

Figure 24: Diagram of the converging-at-scanner telescope. Relevant lengths and angles are labeled (\( r \), convergence distance from the scanner; \( \theta_{in} \), optical scan range of the scanner; \( \theta_{out} \), magnified optical scan range prior to the pupil, L1, first lens of telescope; L2, second lens of telescope; \( f_1 \), focal length of L1; \( f_2 \), focal length L2; \( d \), distance between L1 and L2). Multiple beams show the different optical paths throughout the extent of
the optical scan range of the scanner. Chief rays are denoted by dashed lines. Dotted circle indicates location of beam convergences.

By approximating L1 and L2 as thin, paraxial lenses, the total length of the telescope \( l \) can be derived as:

\[
l = f_1 + f_2 + d = f_1 + f_2 + \left( f_1 + f_2 - \frac{f_1^2}{r} \right)
\]

(9)

By normalizing this expression by the total length of a 4\( f \) telescope, \( 2f_1 + 2f_2 \), we get the following general expression for the fractional length of the telescope \( L \):

\[
L = 1 - \frac{1}{2R(1+M)}
\]

(10)

where \( R = r/f_1 \) (normalized convergence distance) and \( M = f_2/f_1 \) (lateral magnification of the telescope). A plot of the fractional reduction in telescope length, is shown in Figure 25.
Figure 25: Plot of fractional length reduction vs. normalized convergence distance, $R$ for converging-at-scanner telescopes with magnification ranging from 0.1 to 10.

The largest fractional reduction in telescope length possible with this technique is 0.5, which is the point when there is no spacing between the telescope lenses. The drawback of this technique, however, is that by using a converging beam before the scanner, the intermediate image plane becomes curved thus introducing field curvature. Therefore, unless field curvature is desired, in practice it is important to design this kind of telescope with a field flattener lens.

4.2 System and optical design

The ultra-compact switchable SLO/OCT handheld probe schematic and optical design are shown in Figure 26. For both SLO and OCT imaging, light from an 840 ± 35 nm superluminescent diode (SLD) (Superlum, Moscow, Russia) was directed through an
80/20 coupler to an input fiber port on the handheld probe. Light to the handheld probe was collimated by an off-axis parabolic mirror and then focused by an achromatic lens prior to a 1 mm diameter 2D MEMS scanner (Mirrorcle Technologies Inc., Richmond, CA) such that the beam at the scanner had a 0.8 mm 1/e² diameter with a 25° angle of incidence. After the MEMS scanner, a telescope comprised of a combination of custom (L2 and L4) and commercial (L3) lenses was used to magnify the beam at the scanners to create a 2.3 mm beam at the pupil of the eye with a 7° field of view (FOV). A commercially available plano-concave lens (L3) (012-0050, OptoSigma, Santa Ana, CA) was placed soon after the intermediate focal plane of the probe to remove most of the field curvature in the system. Prior to this lens, the curvature, thickness, glass type and position of a custom biconvex lens (L2) was optimized in optical design software (Radiant Zemax LLC, Redmond, WA) to remove residual field curvature and ensure that the principal plane of the L2 and L3 lens pair was a focal length away from the scanner. The last lens of the telescope, an asymmetric triplet (L4), was optimized to provide a telescope magnification of 2.8, minimize induced monochromatic aberrations, and correct for chromatic aberrations (including the longitudinal chromatic aberration of the eye) across the illumination spectrum. The schematics and specifications for the custom lenses (L2 and L4) are given in Figure 27. The lenses were manufactured by Optimax Systems, Inc. using readily available glasses from the Ohara catalogue. Lenses were AR-coated with a single layer MgF₂ coating to minimize lens reflections for 850 nm light at a 0° angle of incidence.
Figure 26: Ultra-compact SLO/OCT handheld probe schematic and optical design. All optical components are labeled and described in the legend. Blue and red text in the schematic correspond to components used for imaging in SLO or OCT mode, respectively.

Figure 27: Custom lens designs used in the ultra-compact SLO/OCT probe. A) Custom biconvex lens design (L2 in Figure 26). B) Custom asymmetric triplet lens design (L4 in Figure 26). All glass types are labeled on the schematics and dimensions are given to the right of the lens schematics.
The resulting optical design achieved a near-diffraction limited lateral resolution of 8 µm over a 7° FOV on a model eye [52], which was modified as described in [42] to incorporate the effects of dispersion for different ocular media including that of the gradient-index crystalline lens. Geometric spot diagrams demonstrating the performance of the optical design are shown in Figure 28. Backscattered light from the eye was collected through the same fiber used for illumination and directed to a spectrometer (SD800, Bioptigen Inc, Durham, NC) when imaging in OCT mode or an avalanche photodiode (C5460, Hamamatsu, Shizuoka-ken, Japan) when imaging in SLO mode. The remaining end of the 80/20 coupler was connected to a beam dump or a reference arm when imaging in SLO or OCT mode, respectively. Switching between SLO and OCT modes was done manually, but could be automated using electromechanical or electro-optical switches to allow sequential SLO and OCT imaging on a frame-by-frame basis.
Figure 28: Spot diagrams (color coded for 3 wavelengths spanning 805 – 870 nm) for both SLO and OCT illumination on the retina of a model eye spanning a 7° FOV.

SLO images were acquired at 14.8 frames per second (fps) with 500 lines per frame and 675 pixels per line by using a 5 MHz digitizer, applying a 3.7 kHz sinusoidal waveform to the fast axis of the MEMS scanner, and utilizing both the forward and backward sweeps of the sinusoidal scan as separate lines in the frame. For OCT imaging, B-scans were acquired at 40 fps with 500 A-scans/B-scan and 2048 pixels/A-scan, by using a 20 kHz A-scan rate (limited by the speed of the spectrometer) and a 40 Hz sawtooth waveform to the fast axis of the MEMS scanner. The OCT axial resolution and 6 dB falloff range were measured to be 7 μm (in air) and 1.1 mm, respectively.
4.3 Mechanical design

All components of the mechanical system were designed and modeled in Solidworks as shown in Figure 29(A) and Figure 29(B). The handheld probe had a form factor of 7 x 6 x 2.5 cm and a weight of only 94 g, a factor of 15 less than our previous handheld SLO/OCT design [40]. The body of the probe was made from thin-walled 7075 aluminum alloy. Rotation of the outer bore allowed for easy focus adjustment of ± 3 D of refractive correction. Fast correction of patient refractive error was possible by using a 4 lead ACME threaded interface and a knurled pointer-and-thumb interface on the outer bore. A polytetrafluoroethylene (PTFE) ring provided a smooth sliding surface on the inner/outer bore interface. The PTFE ring applied radial tension on the outer bore to prevent the objective from unintentional translation. The radial tension was supplied by ~20% compression of a neoprene foam ring, where both rings were fixed longitudinally to the inner bore by a set of flanges. Custom spacers and pertinent reference surfaces were tolerated following the results of an opto-mechanical tolerance stack analysis to ensure that the assembled probe would satisfy design specifications when manufacturing errors are considered. A highly customized spacer with tangential and toroidal interfaces was used to support the close proximity of the multiscale lens pair located after the MEMS scanner while minimizing stress-induced distortions of the optical wavefront. After assembly of the probe, an L-shaped key was attached to the larger end of the outer bore
to prevent the outer bore from being unintentionally removed when adjusting the focus.

The fabricated probe in handheld configuration is shown in Figure 29(C).

![Figure 29](image)

**Figure 29**: Solidworks design images and photograph of the fully assembled probe. A) Photorealistic rendering of the probe. B) Section view showing the interior design. C) Assembled probe in handheld configuration.

### 4.4 SLO image calibration

Acquired image distortions in SLO operation, primarily due to the use of resonant scanning in the fast axis of the MEMS scanner, were calibrated and corrected by imaging a diffusely reflecting dot test target with 0.5 mm dot spacing (62951, Edmund Optics, Barrington, NJ) through a 60 mm focal length lens and applying transformations to dewarp the acquired images to match the known dot pattern. The calibration process
consisted of 4 main steps: 1) splitting the acquired image corresponding to a complete round-trip of the resonant lateral scanner into front and back sub-images, followed by reversing the back sweep image to match the orientation of the front sweep image, 2) applying sinusoidal dewarping at the frequency of the fast axis (3.7 kHz) to both images, 3) dewarping the front sweep image to match the back sweep image and interleaving the result with the back sweep image, 4) dewarping the interleaved image to match the expected dot spacing of the dot test target and cropping to remove regions in which little or no calibration information was obtained. A 4th order polynomial transform and a piece-wise linear transform were used to dewarp the images in steps 3 and 4, respectively. Transformations were calculated from the locations of the centroids of the dots in each image, which were detected by thresholding and using blob analysis software in MATLAB (MathWorks, Natick, MA). This calibration procedure was repeated for each scan range setting used for retinal imaging. Figure 30 illustrates the steps and results of this procedure for images acquired at the maximum scan range of the MEMS scanner, which produced an 8.8° x 6.4° or 6.4° x 8.8° FOV at the image plane depending on the orientation of the handheld probe. The maximum FOV was rectangular because the MEMS scanner underwent larger deflection angles along the resonant (fast) axis than the sawtooth (slow) axis given the same maximum input voltage. The RMS errors of the calibration at the maximum scan range setting were 0.24 and less than 0.001 pixels for the 4th order polynomial and the piece-wise linear transformations, respectively.
Figure 30: Results of the dewarping procedure on the dot test target for an 8.8° x 6.4° scan range. A) Raw image acquired including both directions of the sinusoidal fast scanner sweep B) Front sweep portion of the raw image. C) Back sweep portion of the raw image reversed to match the orientation of the front sweep. D) Sinusoidally dewarped front sweep image. E) Sinusoidally dewarped back sweep image. F) Image obtained by dewarping the front sweep image to match the back sweep image and interleaving the two images. G) Image obtained after dewarping the interleaved image in F) to match the known dot spacing of the test target and cropping to remove regions in which little or no calibration information was obtained.

4.5 Registration between OCT and SLO images

Since OCT and SLO operation was sequential, careful registration between the lateral dimensions of the OCT images and the de-warped and calibrated SLO images was critical for image fidelity. To accomplish this, the relative positioning between the two modalities
was determined by cross-correlating OCT summed voxel projections (SVPs) with calibrated SLO images of subject retinas. OCT images did not need to be dewarped because they were acquired at speeds within the linear response regime of the MEMS scanner. The processing steps employed for OCT SVPs are depicted in Figure 31 and include: 1) segmentation and extraction of the top (upper 40%) and bottom (lower 60%) of the retina (excluding the choroid) in B-scans (as shown in Figure 31(E)), 2) SVP creation for both of the extracted halves, and 3) subtraction of the top half SVP from the bottom half SVP to improve the contrast of retinal blood vessels and remove the common noise present in both SVPs. To extract the top and bottom halves of the retina, the retinal surface and retinal pigment epithelium were automatically segmented using graph theory and dynamic programming [80] and then the upper 40% of the region between segmentations was summed to create the top half SVP while the bottom 60% was summed to get the bottom half SVP. This method enhanced the contrast of blood vessels because the bottom half of the retina in OCT-B-scans contains the shadows of vessels while the top half contains the backscattered signal from vessels. The 2:3 ratio between the top and bottom half SVPs was determined empirically to maximize the contrast of blood vessels in the final processed SVP.

After vessel contrast was enhanced in OCT SVPs, Laplacian-of-Gaussian (LoG) and Gabor filtering (as described in [40]) was applied to both the vessel-enhanced OCT SVPs and SLO frames to generate binary vessel maps as shown in Figure 32. These vessel
maps were then cross-correlated to determine the lateral and rotational offset between the SLO frame and OCT SVP as shown in Figure 32(E), and to allow for the correct positioning of OCT B-scans relative to the SLO image.

Figure 31: Processing steps for OCT summed voxel projections (SVPs). A) Raw SVP obtained from the entire depth of OCT B-scans. B) Top half SVP obtained from the area between the dotted blue lines in E). C) Bottom half SVP obtained from the area between the dotted red lines in E). D) Vessel-enhanced SVP from the subtraction of B) from C) and rescaling to avoid negative pixel values. E) OCT B-scan at the location of the dotted lines in A-D). Scale bars represent 1°.
Figure 32: Processing and registration of SLO images and OCT vessel-enhanced SVPs. A) SLO image. B) LoG and Gabor filtered SLO image. C) Vessel-enhanced OCT SVP. D) LoG and Gabor filtered, vessel-enhanced OCT SVP. E) Superposition of the SLO image and OCT SVP after motion was estimated and corrected via the cross correlation of the LoG and Gabor filtered images in B) and D). Scale bars represent 1°.
4.6 Experimental setup

SLO and OCT retinal images were acquired sequentially spanning either a 3° x 3°, 6.4° x 8.8°, or 8.8° x 6.4° FOV. Different FOVs were obtained by either changing the orientation of the probe by 90° or varying the voltages supplied to the 2D MEMS scanner utilized for both SLO and OCT imaging modes. Background measurements taken prior to imaging were subtracted from both SLO and OCT images to remove static artifacts including lens reflections and scratches or dust on optical elements. Images were registered and averaged using an image processing program, ImageJ (National Institutes of Health, Bethesda, MD), with the Register Virtual Stack Slices plugin [81] for SLO images and the StackReg plugin [82] for OCT B-scans. The SNR of the OCT system was 101 dB for a 50 μs integration time and 685 μW illumination power at the sample. The irradiance incident on the eye from the handheld probe during either SLO or OCT imaging was under the ANSI limit [38] at 685 μW.

4.7 Measuring cone photoreceptor packing density

Cone packing densities were measured for either 1° or 0.5° FOV areas depending on the largest, vessel-free region visible within 3° FOV SLO images. To calculate cone densities, we manually counted cone photoreceptors in ImageJ software and divided by the area sampled. The retinal area sampled was determined by converting the visual angle or retinal FOV (in degrees) to retinal dimensions (in millimeters) by multiplying the FOV by a retinal magnification factor. The retinal magnification factor for a 24 mm axial length
eye is 0.291 mm per degree, and we estimated the magnification factor for other axial
lengths using a linear scaling based on each subject’s estimated or measured axial length
[83]. For subjects that did not have axial length measured, axial lengths were determined
by the average axial length given the subject’s age [84, 85] and refractive error [86, 87].

4.8 Human subjects research

The use of our experimental setup for in vivo imaging of awake adults and children
during an examination under anesthesia (EUA) was approved by the Duke University
Health System Institutional Review Board and adhered to the tenets of the Declaration of
Helsinki. Informed consent was obtained from all subjects or their guardians. For imaging
of children already scheduled for a clinically indicated ophthalmologic EUA, the
handpiece was placed in a single-use sterile plastic bag with an opening for the distal lens
tip. Data collected from this research were stored and managed in compliance with
guidelines from the Health Insurance Portability and Accountability Act.

4.9 Results

The ultra-compact SLO/OCT handheld probe provided complementary retinal
SLO and OCT imaging data in both adults and in children. Imaging results from an adult
human volunteer with -3 diopters of axial myopia were obtained with the ultra-compact
SLO/OCT handheld probe as shown in Figure 33. Representative SLO images with either
a 6.4° x 8.8° or 3° x 3° FOV are shown in Figure 33(B) and Figure 33(D-E), respectively. At
the smaller FOV, the SLO visualized parafoveal cones as close as a 3.6° eccentricity
without adaptive optics. Two insets from Figure 33(D) and Figure 33(E) are magnified by a factor of 2 to show further detail. As expected and quantified in the two insets, a decrease in cone packing (13300 cones mm\(^2\) down to 10100 cones mm\(^2\)) was observed radially from the center fovea [86, 88, 89]. A mosaic of twenty-five 6.4° x 8.8° FOV images was created using ImageJ with the MosaicJ plugin [90] to obtain a composite FOV of 26.4° x 18.4° (Figure 33(A)). In the mosaic image, blood vessels and capillaries are well visualized across the posterior pole and nerve fiber bundles are clearly visible near the bottom left region adjacent to the optic disc. OCT images within the same regions were acquired at a 6.4° FOV as shown centered on the fovea in Figure 33(C). As obtained in all subjects in this study, high quality OCT images allowed visualization of all of the retinal layers that are identified on conventional tabletop OCT systems (labelled in Figure 33(C)) [91].
Figure 33: Imaging results from a healthy adult volunteer obtained with the ultra-compact SLO/OCT handheld probe. A) SLO image mosaic with a 26.4° x 18.4° FOV generated from 25 SLO images with 6.4° x 8.8° FOVs. B) SLO image (5 frame average) taken with a 6.4° x 8.8° FOV at a 4.4° eccentricity. C) OCT B-scan (10 frame average) spanning a 6.4° FOV at the fovea with anatomical landmarks labeled (NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; ELM, external limiting membrane; IS/OS, inner segment/outer segment; IZ, interdigitation zone; RPE, retinal pigment epithelium). D) and E) SLO images (5 frame average) taken with a 3° FOV at a 5.8° and 3.6° eccentricity, respectively. 1° FOV zoomed insets (2×) are shown to the right of D) and E) showing cone photoreceptors with a density that decreases with eccentricity [86, 88]. Colored boxes and line on the mosaic indicate the locations of the SLO images and OCT B-scan in B-E). Arrow indicates a well-defined interdigitation zone in the OCT B-scan.
Images were also obtained with the ultra-compact SLO/OCT handheld probe during EUA from 13 children ranging from 14 months to 12 years of age. The children were imaged in a supine position. Representative images from the healthy eyes of a 25 month old toddler and 14 month old infant are shown in Figure 34(A-D) and Figure 34(E-H), respectively. Both subjects, had approximately emmetropic vision. SLO frames were acquired with either a 3° x 3° or 8.8° x 6.4° FOV while OCT B-scans were acquired with a 6.4° FOV. Similar to the adult volunteers, small FOV SLO images (as shown in Figure 34(B) and Figure 34(F)) visualized parafoveal cones, which, to our knowledge, is the first demonstration of cone imaging for this age group. The foveal photoreceptor layer of the 14 month old, as visualized in cross-sectional OCT imaging, lacks a well-defined interdigitation zone (IZ, interface between photoreceptor tips and the underlying retinal pigment epithelium, Figure 34(G) arrowhead). In the 25 month old, the interdigitation zone is more mature (Figure 34(C) arrowhead) and similar to that seen in the adult eye (Figure 33(C) arrowhead).
Figure 34: Imaging results from healthy eyes of a 25 month old toddler (A-D, frames with green border) and a 14 month old infant (E-H, purple border) in the operating room. A) and E) show 25 and 20 frame average SLO images, respectively, with an 8.8° x 6.4° FOV. B) and F) show 25 and 24 frame average SLO images with a 3° FOV at a 4.6° and 11° eccentricity, respectfully. B) is located in the region within the red dotted box in A) and F) is located in an inferior temporal region of the retina outside of the FOV of E). 0.5° FOV zoomed insets (2×) are shown below B) and F) visualizing cone photoreceptors for both subjects. C-D) and G-H) show 21, 10, 15, and 20 frame average OCT B-scans, respectively, with a 6.4° FOV. The B-scan in C) is located at the fovea and lies approximately along the blue dotted line in A). The B-scan in G) is also located at the fovea, which lies approximately along the orange dotted line in E). I) shows an image of the handheld probe in use in the operating room during examination under anesthesia on a 3 year old toddler. The physician’s hand holding the non-contact probe is stabilized on a wrist rest and on the fingers of the other hand. Arrows indicate the
interdigitation zone, which is well-defined in the 25 month old toddler in C) but not in the 14 month old infant in G).

Children with retinal pathologies were also imaged in the OR with the handheld probe as shown by the images in Figure 35. In Figure 35(A-D) we documented healthy macular vessels and retinal layers in a 2 year old toddler with familial exudative vitreoretinopathy (FEVR), a genetic eye disorder that disrupts the growth and development of blood vessels in the retina. In Figure 35(E-J), retinal images are of a 2 year old toddler with X-linked retinoschisis, a hereditary retinal degeneration characterized by vision loss and often widespread abnormal splitting of the retina’s neurosensory layers. The infant had a previous surgery involving the retinal surface. Resulting microscopic corrugations of a fine membrane wrinkling the retinal surface are perpendicular to the arcuate nerve fiber layer in Figure 35(E) and barely visible at the inner surface in cross section in Figure 35(H) (arrowhead) where deeper layers show the classic gaps found in retinoschisis. The membranes coalesce into a thicker surface membrane in Figure 35(G) which is elevated off the surface in an adjacent cross section (Figure 35(I)) and shown in 3D relative to the retinal surface in Figure 35(J). Figure 35(K-N) shows images acquired from a 12 year old child with a history of blunt trauma resulting in retinal damage at the center of the macula, a macular hole (Figure 35(M) in cross-section and Figure 35(N) in 3D). At the margin of the hole, gaps within the retina (cystoid spaces) and a separation and disruption of the outer borders of retina from the underlying tissue are visible (Figure
Away from the region of the hole, the nerve fiber layer (Figure 35(K)) and deeper retina (Figure 35(L)) appeared healthy.

Figure 35: Imaging results from children with eye disease: a 2 year old toddler with familial exudative vitreoretinopathy (FEVR, A-D, frames with green border), a 2 year old toddler with X-linked retinoschisis (E-J, frames with blue border) and a 12 year old child with a blunt trauma-induced macular hole (K-N, frames with red border). A-B) and K-L) show 15, 14, 10, and 20 frame average SLO images, respectively, with a 6.4° x 8.8° FOV. E) and G) show 30 and 15 frame average SLO images, respectively, with an 8.8° x 6.4° FOV. F) shows a 20 average SLO image with a 3° FOV. C-D), H-I), and M) show 3, 8, 15, 2, and 20 frame average OCT B-scans, respectively, with a 6.4° FOV. The OCT B-scan in C) was acquired from the location of the dotted orange line in A), which was determined by the registration between the SLO frame and OCT SVP as described
in Section 2E. J) and N) show volume renderings of the pathology visible in the B-scans depicted in I) and M), respectively. Green arrow in H) shows a subtle wrinkling of the epiretinal membrane above the inner retinal surface. Blue arrows in G), I), and J) reveal an elevated retinal membrane of the toddler with X-linked retinoschisis. Red arrows in M) and N) indicate the location of the macular hole in the child with blunt trauma.

4.10 Summary

We have demonstrated an SLO/OCT handheld probe weighing only 94 g with 8 µm lateral resolution, 7 µm OCT axial resolution, and a FOV up to 6.4° x 8.8°. High definition SLO and OCT images were acquired in both healthy adult volunteers and patients in the OR ranging from 14 months to 12 years of age with and without pathology. Parafoveal cone imaging was shown using the SLO arm of this device without adaptive optics using a 3° FOV in adults and for the first time in infants and toddlers. A mosaic with a composite FOV of 26.4° x 18.4° was successfully constructed from 25 SLO images with seamless blending after distortion correction. The constructed probe exhibits the potential for high SNR, high resolution SLO and OCT imaging in a single, ultra-compact device. The use of this technology will form the foundation for high quality, handheld imaging of patients that are supine, under anesthesia, or unable to position, and may prove particularly useful for examination of infants and young children with diseases such as retinopathy of prematurity [72-74], albinism [75], nystagmus [76], trauma or shaken baby syndrome [77].

We are heading towards a future where novel therapies may eventually be considered in even younger patients with retinal degenerations and other diseases that affect photoreceptor development and survival [92-94]. For disease staging and management, it
is likely to become even more critical to accurately determine photoreceptor density or other cellular disease effects across retinal layers, in addition to function, prior to and after initiation of novel therapies. Lightweight and portable high definition systems such as the one developed here, are likely to have a significant role in assessing cellular anatomy in the ongoing care of these pediatric patients in the future.
5. True color SLO and OCT handheld probe

In this chapter, we present a spectral domain (SD)-OCT and “true color” SLO handheld probe design with a spectrally reshaped supercontinuum white light source for the SLO illumination and an achromatizing lens to correct for the LCA of the eye. This is the first “true color” SLO, handheld color SLO, and color SLO with OCT system. Color calibration was achieved by imaging a 30 patch color test target with calibrated color values and applying a least-squares, polynomial color transformation to match the raw acquired colors from the SLO to the known color values of the test target. With this probe, we demonstrate “true color” SLO images taken simultaneously with OCT B-scans at 5° and 20° fields-of-view (FOV) with patient exposures safely below the ANSI limit [58]. We then compare the imaging results taken by our system with commercial SLOs that image with multiple colors.

5.1 System design

The “true color” SLO-OCT handheld probe was built from a modification of our prior handheld SLO-OCT design described in Chapter 3 [40]. A schematic layout of the system is shown in Figure 36 below.
Figure 36: Side view schematic of the color SLO-OCT handheld probe design. All optical components are labeled and described in the legend. The illuminating beam at the eye’s pupil was 2 and 2.5 mm for SLO and OCT, respectively. The collection beam diameter for the SLO was larger due to the larger NA and size of the multimode collection fiber, and because backscattered light from the retina fills the pupil in the return path. The multimode fiber shown in the schematic is used to transfer the collected light from the SLO arm into an RGB color separation module consisting of 2 dichroic filters and 3 photomultiplier tubes (PMTs). A filter is placed in front of the red channel’s PMT to remove any contribution due to the OCT light source that is returned through the SLO collection path.

The SLO source was a supercontinuum laser (NKT Photonics A/S, Birkerød, Denmark) that was filtered to transmit light between 430 to 700 nm. To make the output illumination spectrum more uniform and allow more even light collection among the 3 color channels, the spectrum was reshaped with 2 wavelength division multiplexers (WDMs) and 3 variable optical attenuators (VOAs) as shown in Figure 36. The illumination spectrum
before and after reshaping is shown in Figure 37. Deviations in the reshaped spectrum from a perfectly uniform spectrum resulted in color accuracy errors that were minimized by the color calibration procedure described in Section 5.3 (Color calibration).

The OCT source was a superluminescent diode (SLD) operating at 840 ± 35 nm (Superlum, Moscow, Russia). An 80:20 fiber coupler was used for the OCT interferometer and a commercial spectrometer (SD800, Bioptigen Inc, Durham, NC) was used as the OCT detector. The OCT axial resolution and 6 dB falloff range were measured to be 7 µm (in air) and 1.1 mm, respectively. OCT B-scans consisted of 2048 × 500 pixels and were acquired at 36.4 frames per second.

Sample reflectance signal from the SLO arm was collected confocally using a multimode fiber with a diameter equal to approximately 1.9, 2.3, and 2.7 times the Airy disc diameter for the red, green, and blue channels, respectively. This range of confocal pinhole sizes have been demonstrated to give a good balance between image sharpness and throughput [37, 38]. To achieve confocal pinholes of these sizes given a 3 mm collection pupil (undilated), a 50 mm focal length collection lens and a 50 µm diameter multimode fiber was utilized. The wavelength-dependent confocality of the SLO resulted in relatively tighter confocal gates for the higher wavelength channels, which affected the amount of light collected and the optical section thickness imaged by each color channel. Differences in light collection from each color channel were accounted for by the color calibration procedure, while differences in optical section thickness proved to be relatively
minimal. The theoretical full width at half maximum (FWHM) optical section thicknesses were calculated for this system according to equations described in [36] to be 317, 312, and 309 µm for the red, green, and blue channels, respectively.

After the multimode fiber, the collected light was split into three different color channels (430-495, 495-580, and 580-700nm) for photomultiplier tube (PMT) detection (H10721-20, Hamamatsu, Shizuoka-ken, Japan). Wavelength-dependent sensitivity of the PMTs and other wavelength-dependent components of the SLO were also accounted for via the color calibration procedure. SLO images consisted of 530 × 580 pixels and were acquired at 16 frames per second (fps).

Figure 37: The SLO illumination spectrum before (A) and after (B) reshaping the visible portion of the supercontinuum laser spectrum with 2 WDMs and 3 VOAs.
5.2 Optical design

The optical design for the OCT arm was unchanged from our previous handheld SLO-OCT design but the SLO arm had two major modifications to reduce chromatic aberration: 1) the collimating aspheric lens was replaced by a parabolic mirror collimator and 2) two identical custom achromatizing lenses were implemented with one placed after the illumination fiber collimator and the other before the detection fiber collimator at equal distances away from the eye. Two achromatizing lenses were used instead of one in a common path in order to prevent back reflections of the achromatizing lens from entering the SLO’s detection fiber. The idea of using an achromatizing lens to correct vision or improve retinal image quality was first presented in [33, 95-99], and the strategy of designing an achromatizing lens placed directly after a collimation lens was first demonstrated by Zawadzki et al. [100]. Similar to Zawadzki et al., our design strategy involved optimizing the performance of the achromatizing lens in the Zemax model of the SLO imaging system together with a model eye. The model eye we used was created based on parameters of a wide-field eye model with a simplified gradient-index crystalline lens of 30 year olds as determined in a study by Goncharov and Dainty [52]. The dispersion functions for different ocular media were not given in [52] so were modeled as suggested by Atchison and Smith [101] in the same form in Zemax as described by Jaeken et al. [102]. The achromatizing lens design was optimized to produce a zero-power symmetric triplet made from readily available stock glasses with surface
curvatures, thicknesses and optical material as variables. The lens was manufactured by Optimax Systems, Inc. using S-FPL51 (Ohara catalogue) and N-ZK7 (Schott catalogue) glasses, and is shown in the schematic on the left part of Figure 38. The LCA of the model eye (given in terms of chromatic focal shift) at the retina was calculated before and after implementation of the achromatizing lens and is shown on the right part of Figure 38. The achromatizing lens was designed to correct LCA from 400 – 900 nm instead of just the visible spectrum (400 – 700 nm) to allow LCA correction for broadband NIR sources also with the same lens.

![Figure 38](image_url)

**Figure 38:** A) Achromatizing lens (AL) design. Dimensions are given to the left of the schematic. B) Chromatic focal shift at the retina of the eye model from 400 – 900 nm after passing through all SLO optics (shown by blue line) and after correction with the AL (shown by red line). The maximum focal shift range is 950 and 7 μm for the blue and red lines, respectively.

The optical design performance of both the color SLO and OCT is given in the form of spot diagram plots in Figure 39. Although the achromatizing lens corrects for the LCA of the human eye, the transverse chromatic aberration (TCA) of the eye is left uncorrected...
and can be visualized by the spot diagrams in Figure 39(A-B) without and with the achromatizing lens, respectively. However, since the three color channels used for SLO collection each detect a much smaller portion of the SLO illumination spectrum, TCA can be partially corrected in post-processing by warping the images from two of the color channels to match the image from a single color channel. We demonstrate this in Figure 39(C), in which the green and blue channels are warped computationally via an affine transform to match the red channel, thus reducing the contribution of TCA to image blurring. Following this procedure, the design resolution of the color SLO with the achromatizing lens and correction of TCA in post-processing was aberration-limited at ~7.8 μm. The OCT design resolution was nearly diffraction limited at ~7.5 μm as can be visualized by the spot diagrams in Figure 39.
Figure 39: Optical design spot diagrams for the illumination on the retina of a model eye spanning a 20° FOV for the “true color” SLO without (A) and with (B, C) the achromatizing lens and for the OCT system (D). The green and blue color SLO channels were warped to match the red channel in (C), to obtain an aberration-limited resolution of 7.8 μm. OCT was nearly diffraction limited at 7.5 μm. Spot diagrams are color coded for 3 wavelengths spanning the bandwidth of the respective sources and Airy disks are shown by black circles.

5.3 Color calibration

The color calibration method we applied on the raw color SLO images is similar to that performed in color photography and is described by the flowchart in Figure 40 below. The equations for the various transforms shown in Figure 40 are given in [103].
Figure 40: Color calibration flowchart with the raw, un-calibrated color SLO red, green, and blue (RGB) images as the input and the calibrated sRGB color SLO images as the output.

The first step in this method is to apply a spectrum-to-XYZ transform to the raw SLO color image (SLO RGB) in order to transform the image into the Commission Internationale de l’Eclairage (CIE) XYZ color space. After this transform, the SLO image is in the XYZ color space but the reference white point of this space is given by the X, Y, Z coordinates of the SLO illumination source spectrum. To make the reference white point for the SLO image one of the standard illuminants used in color spaces, such as D50 (the white point of a 5000 K blackbody source), we applied a chromatic adaptation transform using the Bradford method, which was found to give excellent results during the development of the sRGB color space standard [104]. The SLO image was then transformed into the L*a*b* color space, which is a color space designed to appear more perceptually uniform [105]. This space is ideal for color calibration, because in this space, minimized error corresponds to minimized perceptual color difference. After color calibration in L*a*b* space, the image was transformed into sRGB space, which has a D65 white point (the white point of a 6500 K blackbody source), for viewing purposes.
For color calibration, images of a 30 patch color test target with known L’a’b’ (D50) color values (ColorGauge Nano Target, Image Science Associates, Williamson, NY) were acquired by the color SLO. This was done after translating the last lens in the color SLO to create an image plane ~17 cm after the last lens. After image acquisition, lens reflections and other stray light artifacts were removed by background subtraction and 100 images were averaged to increase the signal-to-noise ratio (SNR). The averaged image was then transformed into L’a’b’ space prior to applying a least-squares, polynomial calibration matrix. A single L’a’b’ value was determined for each color patch on the test target image by taking the median L’a’b’ value of the center 40 × 40 pixels of each patch. Median values were taken in order to remove the effect of outliers due to occasional specular reflections within patches. The error of the color calibration is given in terms of the CIE76 color difference ($\Delta E_{ab}^*$), which is the Euclidean distance between the known and measured L’a’b’ value for a given color patch and is given by the formula

\[
\Delta E_{ab}^* = \sqrt{(L_{\text{known}} - L_{\text{meas}})^2 + (a_{\text{known}} - a_{\text{meas}})^2 + (b_{\text{known}} - b_{\text{meas}})^2}
\]

where $L_{\text{known}}$, $a_{\text{known}}$, and $b_{\text{known}}$ are the known L’a’b’ values for each patch and $L_{\text{meas}}$, $a_{\text{meas}}$, and $b_{\text{meas}}$ are the measured L’a’b’ values for each patch. A single error value is determined for a given color calibration by taking the average CIE76 color difference over all 30 color patches. Results from the color calibration matrix on color SLO and Nikon
D3100 digital SLR images are shown in Figure 41(A) along with the equation for the color calibration matrix in Figure 41(B). Only up to 2nd order polynomial color calibration is shown, since higher order calibrations did not significantly improve the color accuracy while adding computational complexity.

Figure 41: Color calibration on color SLO and Nikon D3100 digital SLR images of a 30 patch color test target with known L*a*b* (D50) color values (A) using a least squares, polynomial calibration matrix, \( \hat{A}_{LS,n} \), described in (B). \( \mathbf{B} \) is a \( 3 \times 30 \) matrix with the known L*a*b* (D50) values for each of the patches of the color test target. \( \mathbf{X} \) is a \( (3n + 1) \times 30 \) matrix containing the L*a*b* (D50) values of a single pixel in the color image, where \( n \) is the order of the polynomial used for fitting. “Poly1” and “poly2” correspond to first and second order polynomial fits, respectively. The mean CIE76 color differences (Mean \( \Delta E_{ab}^* \)) across all 30 patches are given for each calibration order under the images in A).

5.4 Experimental setup

Color SLO and OCT images were acquired simultaneously spanning either a 5° or 20° FOV at 16 and 36.4 fps, respectively. Different FOVs were obtained by varying the voltages supplied to the scanners of both the SLO and OCT systems. Background measurements taken prior to imaging were subtracted from the color SLO and OCT images to remove static artifacts including lens reflections and scratches or dust on optical
elements. The SNR of the OCT system was measured to be 100 dB for 50 µs integration time and 300 µW illumination power at the sample. The irradiance incident on the eye for the color SLO and OCT were under the ANSI limit at 50 µW and 300 µW, which comprised 46% and 41%, respectively, of the ANSI maximum permissible exposure (MPE) limit [58]. The ANSI MPE limit calculation was done using the equations in [58] for an 8 hour exposure duration of a point source after correcting for the pupil constriction assumption embedded in the standard as described in [55]. No correction was done for the eye movement assumption embedded in the standard because the maximum assumed motion spans a visual angle of 5° [55], which is the minimum FOV used in this system.

All SLO images were averaged after motion correction, which involved preprocessing using LoG/Gabor filtering and registration via cross correlation as described previously in [40].

5.5 Imaging results

Imaging results from a human volunteer are shown in Figure 42 below. Due to the confocality of the color SLO, color images could be obtained at different depth sections as shown in Figure 42(A-C) by shifting the focus of the SLO arm. At smaller FOVs, the color SLO was able to visualize much of the microvasculature structure near the fovea (Figure 42(D)). The raw, un-calibrated red, green, and blue channel images used to create the color SLO image in Figure 42(B) are depicted in the color of their respective channels in Figure
Simultaneously acquired OCT images were averaged as shown in Figure 42(H) and an image of the color SLO and OCT handheld probe is shown in Figure 42(I).

Figure 42: Imaging results from a human volunteer taken with the handheld color SLO and OCT system. A-C) 100 frame average of calibrated (2nd order polynomial) 20° FOV color SLO images taken with the focus at different depth sections with A) focused on the nerve fiber layer (NFL), B) focused on the retinal pigment epithelium (RPE), and C) focused on the choroid. D) 90 frame average of calibrated 5° FOV color SLO images at the location indicated by the dotted red square in B). The raw, un-calibrated red, green, and blue (RGB) color channels of B) are shown by E), F), and G), respectively. H) 20 frame average OCT B-scan taken at the location indicated by the dotted blue line in B). I) Image of the handheld color SLO and OCT system operated in handheld mode.

Imaging results from a different human volunteer are shown in Figure 43. Images were acquired without (Figure 43(A-D)) and with (Figure 43(E-F)) the achromatizing lens to evaluate the effect of LCA and how well it was corrected by the achromatizing lens. With the achromatizing lens implemented, 5° and 20° FOV color SLO images were
acquired near the fovea and optic disc, respectively, and were averaged to get Figure 43(I) and Figure 43(J).

Figure 43: Color SLO imaging results taken from a human volunteer (different subject from Figure 3.33). Images (100 frame averages) taken without (A-D) and with (E-H) the achromatizing lens (AL). A) and E) are color calibrated (2nd order polynomial) color images, B) and F) are the red channel images, C) and D) are the green channel images,
and D) and H) are the blue channel images. Note the reduction of the specular reflections around vessels in H) as compared to D). I) 100 frame average of calibrated 5° FOV color SLO images at the location indicated by the dotted red square in E) visualizing parafoveal cones. J) 100 frame average of calibrated 20° FOV color SLO images taken of the optic disc.

Figure 44: Comparison between two commercial multiple color SLOs and the “true color” SLO. A) Image acquired by the Optos 200 TX ultra-widefield SLO (Optos, Dunfermline, Scotland). B) Image acquired by the Spectralis HRA+OCT (6 mode) system (Heidelberg Engineering, Heidelberg, Germany). C) 100 frame average from the “true color” SLO system. The images in A) and B) have been cropped to match the field of view of the “true color” SLO.

5.6 Summary

We have demonstrated a “true color” SLO and OCT handheld probe that implements a custom achromatizing lens and images both 5° and 20° fields-of-view of the retina. “True color” images of the retina were obtained with an SLO for the first time and at different depth sections of the retina. In addition, this is the first demonstration of simultaneous true color SLO and OCT image acquisition. Retinal images including the foveal region of the retina clearly depicted the yellow pigment of the macula, which is less visible with other commercial color SLO systems or fundus photography. The use of this technology may provide a compact solution for confocal color imaging of the retina combined with
OCT and may be used to further study and evaluate treatments that affect the macula pigment density and distribution.
6. Super-resolution SLO using optical photon reassignment

In this chapter, we present a methodology and system design for obtaining super-resolution in retinal imaging by combining the concepts of SLO with a recent microscopy technique known as optical photon reassignment (OPRA). The resolution improvement of the system setup was tested with a 1951 USAF target in an image plane prior to the retina to quantify the system’s capabilities. Retinal images were also acquired with and without the optical photon reassignment technique to demonstrate the capability of achieving in vivo super-resolution retinal imaging in the human eye. We then quantify the resolution improvement in the retina by analyzing the radially averaged power spectrum of the retinal images.

6.1 Optical photon reassignment concept

The OPRA principle is based on the insight that the most likely origin of the detected photons at a given point in time for a scanning laser confocal microscope is at the maximum of the joint probability density function of the excitation and detection point spread functions (PSFs) (as shown in Figure 45) [34]. The location of the maximum of the joint probability density function is exactly halfway between the centers of the excitation and detection PSFs for the case that the excitation and detection PSFs are the same. This can occur when the excitation and detection optics are identical and there is no Stoke’s shift. The center of the excitation PSF follows the location of the excitation spot scanning the sample while the center of a detection PSF is at the location of a point source in the
sample that lies within the excitation PSF created on the sample. This offset between the centers of excitation and detection PSFs is not accounted for in traditional scanning laser confocal microscopes because confocal microscopes always assign all detected photons at any given point in time to the nominal excitation position [34].

![Figure 45: Demonstration of optical photon reassignment concept. A) Plot showing the illumination PSF and four detection PSFs at different locations on the sample plane (color coded according to legend). Solid vertical line represents the imaged location of pixel 6 from the pinhole plane onto the sample plane. The detection PSF generated from a point source at pixel 3 on the sample plane (red curve) contributes the largest signal at pixel 6. B) Plot showing the illumination PSF, detection PSF at pixel 6, and the joint probability distribution from the two PSFs indicating a maximum at pixel 3, which is halfway between the detection PSF and the illumination PSF. Dotted vertical lines represent the borders of the pinhole when imaged onto the sample.

### 6.2 System design

To experimentally test our framework for optical photon reassignment in scanning laser ophthalmoscopy, for the fast-axis scanner, we used an 8 kHz resonant scanner
(Cambridge Technology Inc, Bedford, MA) that had a reflective coating on both sides of the mirror substrate to enable scanning and descanning of light from the sample and rescanning of light onto a camera. The slow-axis scanner used for both scanning light on both the sample and camera were the same type of galvanometer scanners (Thorlabs Inc, Newton, NJ) and were driven with scan waveforms with the same sample clock to ensure synced performance. A schematic of our optical system is shown in Figure 46. Light from a 770 nm superluminescent diode (SLD) (Inphenix, Livermore, CA) with a 15 nm bandwidth was directed through a single mode fiber to the system for imaging. A collimation lens (CL) was used to create a collimated Gaussian beam with a 2.8 mm 1/e² diameter. An iris after the polarization beam splitter was used to truncate the Gaussian beam to 1.4 mm in diameter, in order create a beam that closely approximates a flat top beam profile. This was done to maximize imaging resolution at the retina, which has a numerical aperture limited by the pupil of the eye. To minimize the contribution of lens reflections that are detected when imaging, polarization gating was applied with the use of a linear polarizer, polarization beam splitter, and quarter wave plate (QWP) in the manner shown in Figure 46. A pinhole with a 1.5 airy unit diameter (200 μm) was used to confocally gate the out-of-focus backscattered light such that lateral resolution was not affected in order to properly test the OPRA concept. The system was configured to achieve either widefield imaging resolution by using an intermediate transverse magnification of 1 or OPRA resolution (resolution improvement by factor of \( \sqrt{2} \)) by using an intermediate
transverse magnification of 2 between the descanned light from the sample and the rescanned light from the backside of the resonant scanner. The transverse magnification was altered between configurations by changing the position and focal length of lens, L6, in Figure 46 such that the collimated beam size on the backside of the resonant scanner was either unaltered (M = 1) or increased by a factor of 2 (M = 2) relative to the collimated beam size on the front of the resonant scanner. Light was rescanned across a 2D CMOS camera (Point Grey Research Inc, Richmond, BC, Canada) with 2048 x 1536 pixels and a global shutter that was synced to the frame rate of the scan pattern on the sample (16 fps).

Figure 46: Optical Photon Reassignment SLO Schematic. SLD: Superluminescent diode; CL; collimating lens; PBS: polarization beam splitter; M1 - 4: plano mirrors; LP: linear polarizer; L1 - 9: lens; RS: resonant scanner; G1, G2: galvanometer scanner; PH: pinhole; QWP: quarter wave plane.
6.3 Optical photon reassignment imaging of calibration chart

To experimentally verify the theory, we used a 1951 USAF test target as our sample and placed it in the intermediate focal plane between lenses L3 and L4 in the schematic in Figure 46. This was done as an alternative to imaging with a model eye in place of the human eye because the intermediate image plane between lenses L3 and L4 is a flat image plane whereas the image plane of the eye is curved. The imaging results for the target are shown in Figure 47 below when imaging using an intermediate magnification of 1 (M1), which is equivalent to widefield imaging, and when using an intermediate magnification of 2 (M2), which represents OPRA imaging assuming the illumination and detection PSFs are the same. The resolution was quantified for both M1 and M2 imaging by taking the derivative of the line profile in the X and Y directions at the locations of the red and yellow boxes on Figure 47 to get the horizontal and vertical point spread functions, respectively. Resolutions were determined in terms of FWHM. The FWHM resolution improvement observed in the X-direction and Y-direction were 1.37 and 1.32, respectively. This was slightly lower than theoretical, which is $\sqrt{2}$, but is likely due to the accumulation of small aberrations along the detection path due to the additional optics in this path compared to the illumination path that further broaden the detection PSF over the illumination PSF.
Figure 47: Widefield vs optical photon reassignment SLO imaging of a 1951 USAF test target. A) Equivalent widefield image acquired of USAF target, which was done using an intermediate telescope magnification of 1. B) OPRA SLO image acquired of USAF target with an intermediate telescope magnification of 2. C) Horizontal point spread function (PSF) acquired at location of red box in A) with FWHM of 23.34 μm. D) Horizontal PSF acquired at location of red box in B) with FWHM of 17.07 μm. E) Vertical PSF acquired at location of yellow box in A) with FWHM of 22.51 μm. F) Vertical PSF acquired at location of yellow box in B) with FWHM of 17.00 μm.

6.4 Optical photon reassignment imaging of human retina in vivo

To demonstrate the capability of OPRA for achieving resolution improvement in the human retina in vivo, we acquired images from an adult volunteer at a 2.3° eccentricity
relative to the fovea with an intermediate telescope magnification of 1 (M1) or 2 (M2) as shown in Figure 48. To allow for a fair comparison between the images acquired in these two modes, the M1 image was also histogram matched to the M2 image (as shown in Figure 48(B)) to ensure equivalent brightness and contrast. Retinal image mosaics consisted of 4 or 5 single frames with the FOV of each frame having dimensions of 1.2° by 0.8°. It is clear from comparing Figure 48(A-B) with Figure 48(C) that the OPRA SLO image contains significantly more highly resolved structural information than the equivalent widefield images. We further analyze the resolution improvement by looking at regions indicated by the colored boxes in Figure 48 and calculating the radially averaged power spectrums for each region as shown in Figure 49. A comparison in the radially averaged power spectrums reveal that the OPRA SLO (M = 2) consistently achieves more signal in the higher frequencies than in the equivalent widefield SLO images (M = 1) both with and without histogram matching. The amount of increase in signal from the higher frequencies of the power spectrum increases closer to the fovea, as expected, because there is higher frequency information in the sample due to tighter cone spacings closer to the fovea. Brown vertical lines in the plots of the radially averaged power spectrums correspond to the expected cone spacing given an average adult for each eccentricity [57]. The expected cone spacing matches well with the peak of the power spectrums for both Figure 49(A) and Figure 49(B) indicating that the parafoveal cone mosaic is well resolved for both widefield and OPRA SLO images at eccentricities greater
than 2° from the fovea. However, in Figure 49(C) and Figure 49(D) the peak of the power spectrum is noticeably less than expected cone spacing for the widefield SLO images but matches well for the OPRA SLO images suggesting that at this eccentricity, 1.4° from the fovea, the cones are not fully resolved for widefield SLO but are for OPRA SLO.

Figure 48: OPRA retinal image mosaics with a 1.7° x 2.2° FOV at a 2.3° eccentricity relative to the fovea. A) Equivalent widefield retinal image mosaic acquired with an intermediate telescope magnification of 1. B) Image in A) after histogram matching to the image in C). C) OPRA SLO retinal image mosaic acquired with an intermediate telescope magnification of 2. Green, blue, yellow, and pink boxes correspond to 2.6°, 2.0°, 1.4°, and 1.4° eccentricities relative to the fovea.
Figure 49: Comparison of radially averaged power spectrums from retinal sections shown by the colored boxes in Figure 48. Zoomed in views from the retinal sections are shown to the left of each radially averaged power spectrum. In the radially averaged power spectrum plots, the black and red curves correspond to when an intermediate magnification of 1 and 2 is used, respectively. The blue curve corresponds to the radially power spectrum of retinal image with an intermediate magnification of 1 after histogram matching has been applied. Subsets A), B), C) and D) correspond to eccentricities 2.6°, 2.0°, 1.4°, and 1.4° from the fovea at the locations of the green, blue, yellow, and pink boxes in Figure 48, respectively. Brown vertical lines in the radially averaged power spectrums correspond to the expected cone spacing for the average human adult for each retinal eccentricity [57].

6.5 Summary

We have demonstrated optically super-resolved retinal imaging by combining SLO with optical photon reassignment to achieved improved visualization of cone photoreceptors near the fovea. Comparisons of the radially averaged power spectrums for different
eccentricities relative to the fovea have revealed higher signal at the higher spatial frequencies for OPRA SLO compared to widefield SLO that increases with proximity to the fovea. The use of this technology may provide increased resolution capabilities for adaptive optics imaging or full-field OCT imaging of the retina and may be used achieve foveal cone imaging with adaptive optics without the need for pupil dilation.
7. Conclusions

The work in this dissertation describes efforts to improve the diagnostic capability of retinal imaging modalities, particularly scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT), to overcome limitations due to system size, monochromatic imaging, and resolution degradation from ocular aberrations and diffraction. Lightweight SLO/OCT handheld probes were demonstrated through the use of novel telescope designs, custom optics and mechanics, and the use of 2D microelectromechanical systems (MEMS) scanners. In addition, methods to correct for motion in OCT volumes in combined SLO/OCT devices were described and may help mitigate the challenges of OCT imaging when large patient motions or operator hand motions are a concern. A novel method for extending true color imaging to confocal retinal imaging systems was described with improved visualization of the yellow pigment of the macula, which is less visible with other commercial color SLO systems or fundus photography. Finally, an all-optical super-resolution retinal imaging technique for SLO was developed to achieve a $\sqrt{2}$ improvement in lateral resolution and enabled the visualization of parafoveal cone photoreceptors closer to the fovea compared to conventional SLO systems without loss in imaging speed or SNR.
References


Biography

Francesco LaRocca was born in Licata, Sicily on December 27th, 1988 and grew up in Columbia, South Carolina. He received his Bachelor’s of Science in Engineering with Distinction from Duke University in 2011, majoring in Biomedical Engineering with a minor in Japanese. He received the John T. Chambers fellowship from the Fitzpatrick Institute for Photonics at Duke University in 2011, a National Science Foundation Honorable Mention in 2012, the Retina Research Foundation Travel Grant Award for the Association for Research in Vision and Ophthalmology (ARVO) conference in 2013, and the Pascol Rol Award for Best Paper in Ophthalmic Technologies at the SPIE Photonics West conference in 2015. He received his Doctor of Philosophy degree in Biomedical Engineering at Duke University in 2016 after authoring 7 peer-reviewed journal papers, 2 submitted journal papers, 1 conference proceeding paper, 19 conference presentations and 1 patent application.

Peer-Reviewed Journal Publications

F. LaRocca and J. A. Izatt, "Super-resolution scanning laser ophthalmoscopy using optical photon reassignment", In preparation


**Conference Proceedings**

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