Ultrahigh speed Spectral / Fourier domain OCT ophthalmic imaging at 70,000 to 312,500 axial scans per second

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Abstract

We demonstrate ultrahigh speed spectral / Fourier domain optical coherence tomography (OCT) using an ultrahigh speed CMOS line scan camera at rates of 70,000 - 312,500 axial scans per second. Several design configurations are characterized to illustrate trade-offs between acquisition speed, resolution, imaging range, sensitivity and sensitivity roll-off performance. Ultrahigh resolution OCT with 2.5 - 3.0 micron axial image resolution is demonstrated at ∼ 100,000 axial scans per second. A high resolution spectrometer design improves sensitivity roll-off and imaging range performance, trading off imaging speed to 70,000 axial scans per second. Ultrahigh speed imaging at >300,000 axial scans per second with standard image resolution is also demonstrated. Ophthalmic OCT imaging of the normal human retina is investigated. The high acquisition speeds enable dense raster scanning to acquire densely sampled volumetric three dimensional OCT (3D-OCT) data sets of the macula and optic disc with minimal motion artifacts. Imaging with ∼ 8 - 9 micron axial resolution at 250,000 axial scans per second, a 512 × 512 × 400 voxel volumetric 3D-OCT data set can be acquired in only ∼ 1.3 seconds. Orthogonal registration scans are used to register OCT raster scans and remove residual axial eye motion, resulting in 3D-OCT data sets which preserve retinal topography. Rapid repetitive imaging over small volumes can visualize small retinal features without motion induced distortions and enables volume registration to remove eye motion. Cone photoreceptors in some regions of the retina can be visualized without adaptive optics or active eye tracking. Rapid repetitive imaging of 3D volumes also provides dynamic volumetric information (4D-OCT) which is shown to enhance visualization of retinal capillaries and should enable functional imaging. Improvements in the speed and performance of 3D-OCT volumetric imaging promise to enable earlier diagnosis and improved monitoring of disease progression and response to therapy in ophthalmology, as well as have a wide range of research and clinical applications in other areas.

1.Introduction

An increasingly important tool for medical diagnosis and biomedical research, Optical Coherence Tomography (OCT) enables two and three dimensional visualization of the internal structure and morphology of tissue [1]. High sensitivity, large dynamic range, and micron level resolution imaging are achieved with OCT by interferometric detection of backscattered light from the sample. In ophthalmology, OCT can perform non-invasive structural and quantitative

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OCIS codes: (170.3880) Medical and biological imaging; (170.4500) Optical Coherence Tomography; (170.4470) Ophthalmology.
imaging of the retina and anterior segment, which enables the identification of pathologies for
disease diagnosis or monitoring responses to therapy [2].

The earliest implementations of OCT used low coherence interferometry with time domain
detection in which the echo delay of backscattered light was measured by mechanically
sweeping a mirror in a reference arm [3,4,5]. Commercial ophthalmic OCT instruments using
this time domain approach operated with imaging speeds of 400 axial scans per second and
acquired individual cross-sectional OCT images of the retina. The development of Fourier
domain detection allowed dramatic improvements in imaging speeds, which made acquisition
of three dimensional data sets feasible.

Fourier domain OCT can be implemented either with a swept laser source [6,7] or spectrometer
based system [8,9]. Both Fourier methods not only offer faster imaging speeds, but also a
fundamental sensitivity advantage [10,11,12] when compared to time domain methods. Swept
source ophthalmic imaging has been demonstrated in research systems with axial scan rates
typically in the 10 kHz - 50 kHz range. Examples of such demonstrations include systems with
an axial scan rate of 18.8 kHz centered at 1050 nm [13], 43.2 kHz at 855 nm [14], 43 kHz at
840 nm [15], 16 kHz at 850 nm [16], and 30 kHz at 1050 nm [17]. Ultrahigh speed swept source
retinal imaging was recently performed using a Fourier domain model locked (FDML) laser
operating at 1050 nm with an axial scan rate of 236 kHz [18] and at 1060 nm with an axial
scan rate of 249 kHz [19]. OCT imaging speeds of 5 MHz using a stretched pulse super-
continuum source [20] and 60 MHz using optical demultiplexers [21] have been demonstrated
in the laboratory, but do not have adequate sensitivity at the maximum allowed incident light
levels for ophthalmic imaging applications. Swept source OCT systems have reduced
sensitivity roll-off with imaging depth when compared to spectrometer based systems [16,
18] and can readily operate in the 1050 nm wavelength range, which may have advantages for
deeper tissue penetration [22,23]. Swept source systems require rapidly tunable, narrow
linewidth lasers, but have the advantage that they use high speed A/D converters and single
point detectors, rather than bulky spectrometers.

Most of the current generation ophthalmic OCT systems use spectral / Fourier domain detection
based on a spectrometer design and there are currently no FDA approved swept source systems
commercially available. Spectrometer based systems can make use of superluminescent diode
(SLD) light sources with broad bandwidths for high axial resolutions, as well as leverage the
existing infrastructure of economically priced high speed line scan cameras and frame grabbers
used in machine vision. High-speed retinal imaging using spectral / Fourier domain detection
was first demonstrated in 2002 at an acquisition rate of 1 axial scan every 19 ms [24]. High
speed acquisition of 29,000 axial scans per second with better than 6 μm axial image resolution
was demonstrated in 2003 [25]. Ultrahigh image resolutions of 2.1 to 3.5 μm in the retina were
achieved at acquisition rates of 10,000 to 16,000 axial scans per second in 2004 [26,27,28].
 Manufacturers began introducing spectrometer based commercial instruments for ophthalmic
imaging beginning in 2006. Specifications vary, but most commercial systems have axial
resolutions of 5-7 μm and imaging speeds of 25,000 axial scans per second.

The sensitivity and dynamic range requirements for ophthalmic OCT imaging are quite
demanding because the incident power is limited by safety considerations. This governs the
selection of suitable linear sensor arrays for spectral / Fourier domain OCT. The standard sensor
for spectrometer based systems has been a low noise, high sensitivity, and high dynamic range
CCD line scan camera. Examples include the e2v (previously known as Atmel) Aviiva SM2
CL.2014 (2048 pixels with 28 kHz maximum line rate), SM2 CL.4010 (4096 pixels at 14 kHz
maximum line rate), M4 CL.2014 (2048 pixels at 53 kHz line rate), Basler L104K (2048 pixels
with a 29.2 kHz maximum line rate), and others. Line scan rates for this class of camera are
generally in the 10 kHz to 55 kHz range. Using such cameras, ophthalmic spectral / Fourier
domain OCT has been demonstrated in research systems at an axial scan rate of 29 kHz with 2048 camera pixels [28,29,30], 75 kHz with 512 camera pixels [31], and 12 kHz with 4096 camera pixels [32]. It has been recognized for some time that CMOS technology has the potential to achieve faster sustained imaging speeds than CCD technology because it is possible to directly integrate digital communication circuitry, gain stages, A/D converters, and photosensitive pixels on the same chip. However, CMOS has traditionally suffered from lower sensitivity and higher noise than CCD [33], reducing its suitability for OCT. Recent advances in CMOS imaging technology and sensor architecture are beginning to address these issues with a next generation of high speed line scan cameras.

This paper presents and compares several ultrahigh speed OCT system designs based on a recently developed CMOS line scan camera (Sprint spL4096-140k from Basler Vision Technologies) that exhibits exceptional sensitivity and noise characteristics while running at high speeds. The OCT system designs were chosen to investigate trade-offs in acquisition speed, sensitivity, sensitivity roll-off, resolution and imaging depth. The designs investigated differ in number of pixels used on the camera, light source/spectrum, spectrometer design, ophthalmic imaging module optics, and line rate/exposure time. Acquisition speeds for the four configurations tested range from 70,000 axial scans per second to 312,500 axial scans per second. A first configuration demonstrates improved sensitivity roll-off with imaging depth by using a high resolution spectrometer configuration while imaging at a speed of 70,000 axial scans per second. A second configuration demonstrates that it is possible to simultaneously achieve a higher axial resolution and larger spectrometer pixel count than any currently available commercial ophthalmic OCT system, while imaging at over 100,000 axial scans per second. A third configuration images at 250,000 axial scans per second, which is an order of magnitude faster than commercial systems, while maintaining good sensitivity performance. A fourth configuration images at 312,500 axial scans per second, which is to the best of our knowledge the fastest reported in vivo imaging of the human eye by any OCT method. Even higher speeds are possible by trading off resolution, sensitivity and imaging depth. Each configuration is characterized through optical testing and the trade-offs between acquisition speed, sensitivity, sensitivity roll-off, and resolution demonstrated with in vivo imaging of the fovea and optic disk in the human retina.

The high imaging speeds obtained with this new camera technology facilitate densely sampled, raster scanned, volumetric acquisition with minimal motion artifacts. Data sets of the fovea and optic disk show detailed fundus images with no visually detectable eye motion in the transverse plane. Registration scans that are oriented along the slow axis scan direction are used to correct for residual axial motion between B-scans, resulting in densely sampled three dimensional data sets which preserve the true global topography of the retina. Multiple volumetric data sets acquired over time can then be precisely registered and used to longitudinally track subtle changes in the retinal structure and promise to improve the monitoring of disease progression or response to therapy. The extremely fast imaging speeds also enable repeated sequential imaging of densely sampled volumes at a rapid refresh rate. This capability enables the visualization of small structures without the need for eye tracking, as well as the visualization of dynamic processes. As an example, we show that high speed image acquisition enables the visualization of individual photoreceptors (cones located at approximately 5 degrees peripheral to the fovea) in some areas of the retina without using a bite bar or adaptive optics. Registering the images of the cones between sequential volumes allows measurement of ocular motion in the transverse and axial directions. We then show how a similar repeated volumetric imaging technique can be used to image the capillary network surrounding the inner nuclear layer (INL) of the retina. Finally, we summarize and discuss the results of this study in the context of how increased acquisition speeds might improve clinical performance, functional imaging research, and investigation of disease pathogenesis. While
this manuscript focuses on ophthalmic applications of high speed OCT imaging, this technology promises to have applications in many other clinical and biomedical research areas.

2. Experimental apparatus

The overall layout of the experimental apparatus is shown in Fig. 1. In order to illustrate design trade-offs between speed, resolution, imaging range and sensitivity roll-off, four system configurations were studied. The systems demonstrate: (A) low sensitivity roll-off and long imaging range at 70,000 axial scans per second, (B) high speed and high resolution imaging at 106,382 axial scans per second, (C) ultrahigh speed imaging with good image quality at 250,000 axial scans per second, and (D) ultrahigh speed imaging, approaching acceptable sensitivity performance limits, at 312,500 axial scans per second. The general operating principle is the same for all design configurations, but individual components were selected as appropriate for the purpose of each experiment. Detailed descriptions of each configuration are listed in Table 1. The major subsystems of the spectral / Fourier domain OCT imaging system are the broadband light source, fiber optic coupler, reference arm, ophthalmic patient interface module, and spectrometer.

A light source consisting of either a multiplexed super luminescent diode (SLD) source or a custom built, femtosecond Titanium Sapphire laser was used to generate a broadband spectrum. The SLD source was a Broadlighter T870 commercially available from Superlum. A circulator was used with the Broadlighter as an isolator to protect the SLDs from back reflections. Only the two long wavelength SLDs were used for design configuration A in order to achieve high spectral resolution as well as match the spectrometer bandwidth, resulting in 3.7 mW of power exiting the fiber after the circulator with a 144 nm full width at half maximum (FWHM) bandwidth centered at 892 nm. All three SLDs were used for design configuration B to achieve a wide bandwidth for ultrahigh resolution, resulting in 4.6 mW of power exiting the fiber after the circulator with a 181 nm FWHM bandwidth centered at 873 nm. A custom built femtosecond Titanium Sapphire laser was tuned and filtered to create an adjustable bandwidth light source for configurations C and D. When the femtosecond laser was used, light was coupled into a long length of fiber to dispersively broaden the femtosecond pulses, reducing their peak intensity. Average power out of the fiber was 16 mW with a 33 nm FWHM bandwidth centered at 846 nm for configuration C and 16 mW with a 27 nm FWHM bandwidth centered at 845 nm for configuration D. The source power was attenuated in all design configurations by adjusting the length of the air gap at the fiber connector to reach safe levels when imaging the eye in vivo. The attenuated source was split by a fiber optic coupler with either a 50/50 or 90/10 coupling ratio depending on the configuration. Given the short exposure times required for the high speed operation of configurations C and D, a 90/10 fiber optic coupler was used in order to increase the amount of light returning from the sample to the spectrometer. This approach increases the instrument sensitivity, but requires high power light sources to achieve the desired incident power level on the sample because the fiber optic coupler passes only 10% of the light from the source to the sample arm. A 50/50 fiber optic coupler was used for the high resolution and longer exposure time configurations A and B to accommodate the lower output power of the multiplexed SLD light source.

A portion of the light from the fiber optic coupler was directed to the ophthalmic patient interface module, which consists of a collimating lens, a pair of galvanometers for 2-axis beam steering, a scan lens, and an ocular lens. The subject's eye is positioned such that the eye's pupil is coincident with the pivot point of the collimated beam exiting the ophthalmic patient interface module. By selecting an appropriate focal length of the ocular lens, the projected spot size on the cornea could be changed from a $1/e^2$ diameter of either 1.6 mm or 2.3 mm, as measured by a beam profiler (DataRay WinCamD). Imaging of the fovea and optic disk was performed with a 1.6 mm beam diameter for configurations A-C because beams of this size are relatively...
easy to align during ophthalmic imaging (important for clinical usage) and because the lateral resolution is not significantly degraded by aberrations of the eye at this beam size. A beam diameter of 2.3 mm was used for design configuration D in order to increase the light collection efficiency of the instrument to compensate for the extremely short integration time of 2 μs. This higher numerical aperture configuration had a shorter depth of field, but also provided improved transverse resolution on the retina, up to limits imposed by ocular aberrations [34]. For the small volume, rapid repeated imaging presented in Sections 4.3 and 4.4, a 2.3 mm beam size was used with design configuration C to achieve higher transverse resolution for resolving individual cone cells and capillary flow.

The reference arm consists of a set of polarization controllers, a collimating lens, neutral density filter to control reference arm power, BK7 prisms for dispersion compensation of glass in the ophthalmic patient interface module, a 20 mm long cuvette filled with water to balance the dispersion of the intraocular fluid, a focusing lens, and silver coated retro-reflecting mirror. The spectrometer consists of a collimating lens, a diffraction grating (1200 lp/mm), a focusing lens, and the Basler Sprint CMOS line scan camera. The spectrum, which contains the encoded depth reflectivity information, is measured by the camera and transmitted digitally to a high speed frame grabber (National Instruments PCIe-1429) in a PC running the Windows XP operating system. The galvanometers are driven by a digital to analog board residing in the PC. A LabVIEW program running on the PC provides a user interface and live preview, as well as coordinates the frame grabber, galvanometers, and file I/O during operation.

The Basler Sprint CMOS camera contains two rows of 4096 pixels and can be operated in a variety of different modes. For all results presented in this paper, the full 12 bit resolution was used and the top and bottom rows were vertically binned to create pixels with an effective pixel dimension of 10 μm wide by 20 μm tall in a mode called “line averaging”. When using a subset of the total number of pixels, the highest frame rate is achieved with the camera when the spectrum is centered on the sensor array, which required repositioning the camera for each configuration shown and starting the sensor readout at the pixel numbers listed in Table 1.

3. System performance comparison

The performance specifications for each of the four design configurations are summarized in the bottom four rows of Table 1. Design configurations A through D use between 4096 to 576 pixels. Configuration A has a measured sensitivity of 94 dB at a camera exposure time of 13.0 μs, corresponding to an imaging speed of 70,000 axial scans per second (a fixed 1.2 μs readout overhead increases the line period to 14.2 μs and applies to all configurations). By reducing the number of pixels read, configurations B, C and D achieve progressively faster imaging speeds with an associated decrease in the exposure time and sensitivity. Configuration D achieves imaging with a measured sensitivity of 89 dB at an exposure time of 2.0 μs, corresponding to 312,500 axial scans per second. Sensitivity measurements were performed using a back reflecting silver mirror placed after the ophthalmic patient interface module. A neutral density filter was placed between the mirror and ophthalmic patient interface module to attenuate the light, matching levels occurring during in vivo imaging. The system sensitivity was measured at an incident power level of 750 μW.

Figure 2(a) shows the normalized spectrum as measured by the CMOS camera for each design configuration and Fig. 2(b) shows the resulting axial point spread function (PSF) obtained by placing a silver mirror at a depth of approximately 0.2 mm from the zero delay. The significant amplitude modulation exhibited by the SLD light source generates relatively large sidelobes in the PSF that would normally be visible in, and compromise, images displayed with a log10 intensity scale. Spectral shaping [35, 36] by digitally processing the spectrum to approximate a Gaussian envelope before Fourier transformation can reduce the height of the
sidelobes, as shown in Fig. 2(c). Spectral shaping was not applied for configurations C and D, which are shown as dashed lines in Fig. 2(c) for comparison, because the spectrum had an approximate Gaussian envelope at the source. The height of each PSF has been normalized to unity in all plots. The resolution of each system was determined in air by measuring the full width at half max (FWHM) of the point spread function. Resolution estimates for imaging in tissue were calculated assuming n=1.33 for the index of refraction. Note that spectral shaping resulted in a degradation of resolution as well as a decrease in the system sensitivity, as shown in Table 1. Sensitivities for configurations A and B were reduced by 1 dB through spectral shaping, resulting in 93 dB and 91 dB, respectively.

Spectral / Fourier domain OCT systems exhibit a roll-off in sensitivity with imaging depth due to optical resolution limits, finite pixel width [37], aliasing at high spatial frequencies, and interpixel cross-talk [38] in the spectrometer. Sensitivity roll-off characterization of each configuration was performed by setting equal power in the reference and sample arms, placing a silver mirror after the ophthalmic patient interface module, then translating a retro-reflecting mirror in the reference arm by 0.1 mm increments. The sensitivity roll-off and axial resolution change with depth are shown in Fig. 3(a) and Fig. 3(b), respectively (note that while spectral shaping adversely affects the absolute sensitivity, it does not significantly affect the sensitivity roll-off characteristics in this case, so only the results from the shaped spectrum for configurations A and B are shown for clarity). The sensitivity roll-off in designs B through D is approximately 20 dB at 2 mm (in air) and is comparable to that previously reported for ultra-high resolution spectral / Fourier domain systems [28]. Although differing in axial resolution due to the difference in light source bandwidths, designs B through D exhibit the same spectral resolution at the spectrometer, and hence demonstrate similar sensitivity roll-off performance.

An improvement in the sensitivity roll-off can be achieved with a high resolution spectrometer design. However, spectral bandwidth is reduced as the spectral resolution is increased for a given number of camera pixels in a spectrometer. Thus, in order to preserve the axial resolution while increasing the imaging depth range and reducing sensitivity roll-off, it is necessary to improve the spectrometer resolution while maintaining a broad spectral bandwidth. Design configuration A accomplishes this by using a longer focal length spectrometer focusing lens that increases the linear dispersion in order to illuminate and use a larger number of camera pixels. The high spectral resolution spectrometer design of configuration A reduces the sensitivity roll-off and almost doubles the available imaging depth range compared with designs B through D, while maintaining high axial resolution and imaging at 70,000 axial scans per second. In ophthalmic imaging, the improved sensitivity roll-off performance is important for imaging deep structures, such as the optic nerve head, as well as for obtaining usable data, without sensitivity loss, in patients who have axial eye motion during imaging.

4. In vivo ophthalmic imaging results

The four OCT systems as described were used to acquire in vivo images of the fovea and optic disk in the human retina. Study protocols were approved by the investigational review board of the Massachusetts Institute of Technology. Written informed consent was obtained prior to the study. Retinal imaging was performed with an incident average power of 750 μW, consistent with safe retinal exposure as determined by the American National Standards Institute (ANSI) [39] and with exposure levels used in commercial ophthalmic OCT imaging instruments. Imaging was performed using several different scanning and acquisition protocols.

4.1. In vivo ophthalmic imaging B-scan comparison

Figures 4 and 5 show 500 axial scan (transverse pixels) and 2000 axial scan images of the fovea and optic disk, respectively. Design configurations A through C used a 1.8 mm beam diameter on the cornea, while design configuration D used a 2.3 mm beam diameter to increase the light
collection efficiency of the instrument, as explained in Section 2. All images in Fig. 4 and Fig. 5 are shown with a log10 intensity grey scale with thresholding to reduce noise. For comparative purposes, the lower threshold value was chosen such that the mean value of the background noise was the same for all images. The upper threshold value was set to be 0.98 times the peak intensity value in the data set. As an example, for the 2000 axial scan images shown in Fig. 4, the resulting grey scale coding spans 52.3 dB for configuration A, 48.8 dB for configuration B, 48.5 dB for configuration C, and 46.5 dB for configuration D. In all cases, the 2000 axial scan images show improved contrast when compared to the 500 axial scan images for any given design configuration. This improvement in contrast is due to an effective increase in signal to noise ratio from averaging of adjacent lines.

As expected from the sensitivity roll-off characterization measurements shown in Fig. 3(a), images obtained with configuration A at 70,000 axial scans per second demonstrate superior imaging at a given depth when compared to configurations B through D, as indicated by better visualization of the choroidal structure and deeper penetration into the optic nerve head. With significantly higher axial resolutions than design configurations C and D, images obtained with design configurations A and B show distinct bright points associated with the capillaries around the inner nuclear layer (INL) in Fig. 4 and clear axial separation between the photoreceptor layers in Fig. 5. Design configuration B exhibits the highest resolution, however, the increased sensitivity roll-off restricts the imaging depth, making structures in the choroid less visible than in configuration A. Configuration C trades off resolution to achieve higher imaging speed by reading a smaller number of pixels in the spectrometer. The ultrahigh speed images obtained with configuration C at 250,000 axial scans per second have lower axial image resolution and more noise than configurations A or B, but maintain clear visibility of the photoreceptor and inner retinal layers. The fastest acquisition images obtained with configuration D at 312,500 axial scans per second and a 2 μs exposure time exhibit noticeably more noise and less detail compared to the other configurations, but still show clear retinal structure in both the fovea and optic disk.

**4.2. Dense homogeneous volumetric imaging and axial motion correction**

Prior to the development of high speed Fourier domain OCT detection techniques, the conventional approach for clinical ophthalmic imaging was to collect individual cross sectional OCT images of the retina. This approach undersamples the retina, raising the probability of missing focal disease pathology, as well as making it difficult to longitudinally correlate imaging results from visit to visit. With the development of high speed spectral / Fourier domain OCT, it became possible to acquire three dimensional volumetric data sets using raster scanning. To date, the majority of spectral / Fourier domain systems have had acquisition speeds of ∼25,000 axial scans per second. These imaging speeds are still too low to enable dense, volumetric OCT data acquisition in ophthalmic applications, because the imaging time is limited by ocular motion.

Using the high speed design configuration C, which acquires 250,000 axial scans per second, it is possible to acquire dense homogeneous volumetric data sets an order of magnitude faster than most current generation spectral / Fourier OCT instruments. Figures 6(a) and 6(b) show select cross sectional images from a 512 × 512 × 400 voxel volumetric 3D-OCT data set of the fovea and disk, respectively. The data sets were obtained by raster scanning to collect 512 sequential B-scans, consisting of 512 axial scans each, with 400 pixels in depth, in a total time of 1.3 seconds. Figures 6(c) and 6(d) show the corresponding OCT fundus images constructed from the 3D-OCT data by summing the linear intensities along the axial direction for each transverse point in the image. OCT cross-sectional images with arbitrary orientation can be extracted from the volumetric 3D-OCT data set and the OCT fundus images enable the precise and reproducible registration of these images to fundus features.
The OCT fundus images have no noticeable discontinuity of retinal features and show that there are minimal motion artifacts in the x-y plane at this very fast acquisition speed. However, because of the extremely fine axial resolution, the volumetric data set is still susceptible to axial eye motion artifacts. This is apparent in the cross-sectional OCT image which was extracted from the foveal volumetric data set and shown in Fig. 7(c). Since the data is acquired by raster scanning in the horizontal (nasal - temporal) direction, axial eye motion is still visible along the “slow” vertical (superior - inferior) direction. Axial eye motion can be compensated locally by cross-correlated sequential B-scans in the volumetric data set. This is analogous to the method of cross-correlating sequential axial scans, which has been used to compensate eye motion in commercial instruments [4]. This correlation approach works well locally, but does not preserve the larger scale topography of the volumetric 3D-OCT data set along the “slow” vertical axis. Another approach is to use realtime active compensation with a voice coil driven mirror in the reference arm and eye position feedback from a corneal reflection sensor [40].

The axial eye motion can be globally corrected by registering the three dimensional data to additional registration scans acquired along the vertical axis direction [41]. In this research, for each three dimensional data set, three vertical registration scans (one at each edge and one at the center of the volume) are acquired immediately before the horizontal volumetric raster scan acquisition, as shown in Fig. 7(a). Because each B-scan is acquired in 2.0 ms, eye motion during an individual B-scan is negligible. Thus, by registering each horizontal B-scan in the volume to the vertical registration scans, eye motion in the z direction can be measured and compensated to yield a motion corrected volumetric data set. With three orthogonal registration scans, each B-scan from the raster crosses through the registration scan along 3 lines of intersection. First, local averaging with neighboring axial scans in the volume (nine axial scans representing the 3 × 3 pixel region surrounding the axial line of intersection) and spatially low pass filtering the result was performed to generate a noise reduced depth intensity profile, shown in Fig. 7(b). This depth intensity profile was compared to the spatially low pass filtered and locally averaged (3 × 1 pixel region) profile stored in the registration scan at the same location, and an exhaustive line search performed to find the position shift that resulted in the best correlation using the sum of squared differences (SSD) as the optimization metric. Figure 7(f) shows the measured axial motion profile, which is the average of the 3 registration profiles. Figure 7(e) shows the resulting motion corrected volumetric data along a slice in the direction of the slow axis scan. Comparing Fig. 7(e) to Fig. 7(d), which shows the registration scan, it can be seen that the motion is well corrected from the similarity of these two images. Figures 7(g) and 7(h) show the three dimensional data set before and after motion correction respectively. The resulting volume is now motion corrected in the z direction to generate a data set representing the true topography of the retina. These techniques promise to be important for applications which require precise measurement of three dimensional features, such as imaging the optic nerve head for glaucoma diagnosis and monitoring.

4.3. Repeated volume imaging of cone photoreceptors

Rapid repeated imaging of small volumes is enabled by the high speed acquisition of design configuration C. The ability to perform rapid volumetric imaging is important for imaging very small features, where motion artifacts can produce image distortion and loss of continuity of data. Data from 25 sequentially acquired volumes repeated over the same region of the retina is shown in Fig. 8. Using the 2.3 mm beam diameter configuration of design C for improved transverse resolution, each 128 × 100 × 400 voxel volume is acquired in 0.08 sec, with the entire sequence acquired in 2.0 sec. Figure 8(a) shows a cross sectional image along the slow axis direction of the 25 volumes. Zooming in on the 10th volume in the sequence, it can be seen in Fig. 8(b) and Fig. 8(c), which show a slow axis cross sectional image and fast axis cross sectional image with a log_{10} grey scale, that each volume is acquired with minimal motion artifact. Indeed, looking carefully, one can see what appear to be individual photoreceptors in
both the slow and fast axis images. A segmentation algorithm was applied to identify the starting depth of the photoreceptor layer by thresholding and edge detection within each B-scan. The photoreceptor layer was then isolated by multiplying each axial scan by a truncated Gaussian window profile, as shown in Fig. 8(b). The *en face* image shown in Fig. 8(d) was obtained by summing the resulting linearly scaled and Gaussian windowed intensity data along the axial direction for each transverse point in the image. Cone photoreceptors can be seen in this data set that was acquired at approximately 5 degrees peripheral to the fovea. Linearly scaled cross sectional images with their corresponding location in the *en face* image are shown in Fig. 8(e) and show clear vertical alignment between the photoreceptor inner segment/outer segment junction and end tips of the photoreceptor outer segments. Cones in this region of the eye are larger (6-7 μm) than near the fovea and the dimensions and spacing in the *en face* image agrees with histology [42], *in vivo* OCT observation with [43] and without adaptive optics [44], and with an adaptive optics scanning laser ophthalmoscope [45]. Photoreceptors in other regions of the retina are smaller and more closely spaced, which would require higher transverse image resolutions and adaptive optics for visualization. However, high speed imaging would still be important for visualizing photoreceptors because their small size makes them highly susceptible to motion induced and possibly noncontinuous image distortion from eye motion.

The effects of eye motion can be compensated to provide stabilized viewing with active eye tracking mechanisms [46]. Other approaches use registration and image warping between consecutive overlapping images [47] and can produce realtime and predictive corrections that allow for projecting light stimuli with accuracies less than the distance between cones at the fovea [48], circumventing the need for active eye tracking. In this research, the *en face* images between each volume were registered to the previous frame in a post-processing operation by using the cone pattern as a reference and searching for the x and y shifts that provide the highest correlation between scans. In the resulting movie (Fig. 8 Media 1), it is clear that the cone pattern is a result of the features in the retina and not a speckle artifact. Axial motion can be extracted from the identified location of the photoreceptor layer in each B-scan and an average position calculated for each volume. Figure 9(a) shows a plot of the x, y, and z motion as a function of time and Fig. 9(b) shows a 3 dimensional plot of the same data, indicating the trajectory of eye and head motion that occurred during the acquisition. The fast acquisition times enable measurement and correction of eye motion up to the frequency bandwidth supported by the volumetric imaging rate. This provides a powerful tool for visualizing and registering small features in volumetric data sets. Finally, it should also be noted that motion could be measured using as few as two non-parallel scans, rather than a full volumetric raster scan. This would dramatically increase the bandwidth for measuring and correcting eye motion.

### 4.4. Imaging of capillary blood flow

In addition to enabling visualization of very small features without motion artifacts, ultrahigh speed imaging also allows the visualization of volumetric features as a function of time, also referred to as four dimensional imaging (4D-OCT). Section 4.3 described how the photoreceptor layer could be isolated from a sequence of rapidly acquired, repeated volume acquisitions. A similar approach can be applied to visualize the capillary network surrounding the inner nuclear layer (INL) by isolating a volume at an offset from the identified starting boundary of the photoreceptor layer. A truncated Gaussian window function weights the depth information before summing along the axial direction to form the *en face* image. Similar *en face* methods have been used to visualize volumetric data sets [19,30,49]. Figures 10(a) and 10(b) show cross sectional images from volumes obtained with design configuration C with a 2.3 mm beam diameter at approximately 4 and 11 degrees peripheral to the fovea, respectively. Diagrams in these figures indicate the region that was isolated with the Gaussian window for repeated volumetric acquisition. The Gaussian window was centered at an offset elevation of
167 \mu m above the inner boundary of the identified RPE layer in Fig. 10(a) and 193 \mu m above the inner boundary of the identified RPE layer in Fig. 10(b). Figures 10(c) and 10(d) show the resulting fundus image created by summing the linearly scaled intensity information along the full depth of the volume (left tile), an en face image created by summing the linearly scaled and Gaussian windowed intensity data (center tile), and the maximum value (maximum projection) of the linearly scaled and Gaussian windowed intensity data (right tile). The capillary network is revealed in the local depth summation (center tile) and maximum projection (right tile) images. The additional temporal information offered by the high frame rate movies (Fig. 10 Media 2 and Media 3) aids in identifying the topology of the capillary network and may show blood flow. The advantages of “motion contrast” for revealing small vessels, as well as differential imaging from subtraction of sequential images for quantifying blood flow, has previously been shown with high frame rate fundus photography [50]. Direct observation and measurement of leukocytes in the parafoveal capillaries has been shown with an adaptive optics scanning laser ophthalmoscope [51]. When imaging with OCT, the visualization techniques presented in this paper could lead to new contrast-agent-free methods of quantifying blood flow in these capillaries, which cannot be measured with Doppler OCT because of their perpendicular orientation to the optical beam.

Time resolved volumetric imaging has also been applied to measure functional changes in the retina in response to light stimulation [52]. Ultrahigh speed volumetric imaging (4D-OCT) enables measurement and compensation of motion as well as the use of signal averaging methods, which enhance sensitivity to small changes. Beyond ophthalmology, time resolved volumetric imaging would also have many diverse applications, such as imaging brain activation, cardiac function in developmental biology, and non-destructive material process monitoring.

5. Conclusions

We have developed, characterized, and demonstrated several configurations of a spectral / Fourier domain OCT imaging system that use a high speed CMOS line scan camera to facilitate ultrahigh speed image acquisition. Configuration (A) uses a high spectral resolution spectrometer with 4096 pixels to achieve reduced sensitivity roll-off for improved imaging over long depths, while still imaging faster than any commercially available ophthalmic OCT system. Design configuration (B) simultaneously demonstrates higher speed, higher resolution, and a larger number of camera pixels in the spectrometer than any commercially available ophthalmic OCT system currently available. Configuration (C) images at 250,000 axial scans per second, which is an order of magnitude faster than commercial systems, while producing good quality images. The highest speed configuration (D) runs at 312,500 axial scans per second, which we believe is the fastest speed for ophthalmic OCT imaging reported to date. We use the high speed configuration (C) to perform densely sampled volumetric data acquisition. OCT fundus images of the fovea and disk show minimal motion artifacts in the transverse plane at these high speeds. To compensate for motion in the axial direction, we axially register the B-scans acquired during the raster scanning to orthogonally oriented registration scans acquired immediately before the volumetric acquisition. The resulting motion corrected volumetric data sets represent the true retinal topography spanning large regions. The ability to visualize topography without motion artifacts will be especially important for clinical applications such as imaging the optic disc in glaucoma. Data sets from repeated measurements can be more precisely registered to each other and could be used to detect and quantify small morphological changes occurring from visit to visit, aiding in longitudinal studies.

We also demonstrate rapid repeated imaging of small volumes which enables visualization of small retinal features without motion induced image distortion and stabilized imaging without

*Opt Express*. Author manuscript; available in PMC 2009 September 14.
eye tracking. Cone photoreceptors in some areas of the retina can be seen in en face images without the use of adaptive optics or head stabilizing with a bite bar. By registering the resulting en face and volumetric images, motion trajectories can be determined and analyzed to characterize the eye’s movements during the longer duration repeated acquisition as an alternative to active eye tracking during imaging. This technique was also demonstrated for imaging the capillary network surrounding the INL. Movies acquired at extremely high speed enable better visualization and identification of the capillary structure due to the additional temporal information and suggest the potential for visualizing blood flow. High speed repeated volumetric imaging will enable time resolved measurement of dynamic processes (4D-OCT), as well as a variety of signal averaging techniques which can improve sensitivity to small changes.

Sensitivities of the ultrahigh speed configurations presented in this paper are lower than is common for ophthalmic imaging today, where sensitivities are typically 95 dB and higher. Since the incident beam is scanned at high speeds over the surface of the retina during these ultrahigh speed acquisitions, retinal exposures could be increased in order to increase the signal levels. However, interlocks must be incorporated to ensure that retinal exposures remain within safe limits in case of electrical or mechanical failures. In applications other than ophthalmology, exposure levels and sensitivities could be increased significantly.

The results of this study suggest that high speed CMOS cameras can achieve a significant improvement in performance for ophthalmic imaging. This promises to have a powerful impact in clinical applications, improving early diagnosis, reproducibility of quantitative measurements and enabling more sensitive assessment of disease progression or response to therapy. The ability to acquire volumetric data without motion artifacts, as well as to perform repeated volumetric imaging to measure dynamic functional processes promises to be a powerful tool for ophthalmic research as well as many other fields.

Acknowledgments

This research is sponsored in part by the National Institutes of Health R01-EY11289-21, R01-EY13178-07, R01-CA75289-11, National Science Foundation BES-0522845; Air Force Office of Scientific Research contract FA9550-07-1-0014 and Medical Free Electron Laser Program contract FA9550-07-1-0101.

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Fig. 1. OCT ophthalmic imaging apparatus.
Fig. 2.
(a) Spectrum as measured by the CMOS camera for each configuration. (b) Axial point spread function before spectral shaping. (c) Axial point spread function after spectral shaping of design configurations A and B. Spectrums and point spread functions are all normalized to 1.
Fig. 3.
(a) Sensitivity roll-off vs. imaging depth. (b) Axial resolution vs. imaging depth.
Fig. 4.
OCT cross sectional images of the fovea with 500 axial scans per B-scan and 2000 axial scans per B-scan for each configuration. All scale bars represent 100 μm.
Fig. 5.
OCT cross sectional images of the optic disk with 500 axial scans per B-scan and 2000 axial scans per B-scan for each configuration. All scale bars represent 100 μm.
Fig. 6.
Dense 3D volumetric data sets acquired at 250,000 axial scans per second. Select cross sectional images of (a) the fovea and (b) optic disk volumes. OCT fundus images of (c) fovea 3D data set and (d) optic disk 3D data set. All scale bars represent 500 μm.
Fig. 7.
Motion corrected volumetric acquisition.
Fig. 8.
Rapid repeated volumetric imaging of cone photoreceptors (Media 1).
Fig. 9.
Ocular motion extracted from repeated volume imaging of cones: (a) x, y, and z motion vs. sequence number (time) and (b) spatial motion trajectory plotted in 3D space.
Fig. 10.
Imaging of capillary blood flow. (a) and (b) Cross sectional image and associated Gaussian window for regions 1 and 2. (c) and (d) Transverse images of regions 1 and 2. The additional temporal information obtained with the rapid sequential volumetric imaging aids in identifying the capillary network (Region 1: Media 2. Region 2: Media 3.).
### Table 1

System Design Configurations and Performance Measures

| Design Configuration | A       | B       | C       | D       |
|----------------------|---------|---------|---------|---------|
| Camera pixels        | 4096    | 2528    | 800     | 576     |
| Camera line rate (lines per second) | 70,000  | 106,382 | 250,000 | 312,500 |
| Camera line period   | 14.2 μs | 9.4 μs  | 4.0 μs  | 3.2 μs  |
| Camera exposure time | 13.0 μs | 8.2 μs  | 2.8 μs  | 2.0 μs  |
| Camera starting pixel| 1       | 801     | 1633    | 1761    |
| Light source         | 2 × SLD | 3 × SLD | Ti-Sapph| Ti-Sapph|
| Light source FWHM    | 144 nm  | 181 nm  | 33 nm   | 27 nm   |
| Fiber optic coupler ratio | 50/50  | 50/50   | 90/10   | 90/10   |
| Spect. collimating lens focal length | 70 mm   | 50 mm   | 50 mm   | 50 mm   |
| Spect. focusing lens focal length | 160 mm  | 80 mm   | 80 mm   | 80 mm   |
| Calculated depth range in air | 4.4 mm  | 2.1 mm  | 2.0 mm  | 2.0 mm  |
| Calculated depth range in tissue | 3.3 mm  | 1.6 mm  | 1.5 mm  | 1.5 mm  |
| Measured beam diameter on cornea | 1.6 mm  | 1.6 mm  | 1.6/2.3 mm | 2.3 mm |
| Performance Measure  |         |         |         |         |
| Measured sensitivity | 94 (93) dB | 92 (91) dB | 91 dB   | 89 dB   |
| Measured 10 dB roll-off depth | 2.36 mm | 1.13 mm | 1.43 mm | 1.26 mm |
| Measured axial resolution in air | 3.6 (4.0)μm | 2.8 (3.3)μm | 11.4μm | 11.6μm |
| Estimated axial resolution in tissue | 2.7 (3.0)μm | 2.1 (2.5)μm | 8.6μm | 8.7μm |

* ( ) indicates sensitivity or axial resolution after spectral shaping.