Adaptive optics optical coherence tomography at 1 MHz

Omer P. Kocaoglu,* Timothy L. Turner, Zhuolin Liu, and Donald T. Miller
School of Optometry, Indiana University, Bloomington, IN, 47405, USA
*okocaogl@indiana.edu

Abstract: Image acquisition speed of optical coherence tomography (OCT) remains a fundamental barrier that limits its scientific and clinical utility. Here we demonstrate a novel multi-camera adaptive optics (AO-)OCT system for ophthalmologic use that operates at 1 million A-lines/s at a wavelength of 790 nm with 5.3 μm axial resolution in retinal tissue. Central to the spectral-domain design is a novel detection channel based on four high-speed spectrometers that receive light sequentially from a 1 x 4 optical switch assembly. Absence of moving parts enables ultra-fast (50 ns) and precise switching with low insertion loss (~0.18 dB per channel). This manner of control makes use of all available light in the detection channel and avoids camera dead-time, both critical for imaging at high speeds. Additional benefit in signal-to-noise accrues from the larger numerical aperture afforded by the use of AO and yields retinal images of comparable dynamic range to that of clinical OCT. We validated system performance by a series of experiments that included imaging in both model and human eyes. We demonstrated the performance of our MHz AO-OCT system to capture detailed images of individual retinal nerve fiber bundles and cone photoreceptors. This is the fastest ophthalmic OCT system we know of in the 700 to 915 nm spectral band.

OCIS codes: (110.1080) Active or adaptive optics; (170.4500) Optical coherence tomography; (120.3890) Medical optics instrumentation; (170.0110) Imaging systems; (170.4470) Ophthalmology; (330.5310) Vision - photoreceptors.

References and links
1. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. G. Stinson, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, “Optical coherence tomography,” Science 254(5035), 1178–1181 (1991).
2. A. G. Podoleanu, “Optical coherence tomography,” J. Microsc. 247(3), 209–219 (2012).
3. A. Rollins, S. Yazdanfar, M. Kulkarni, R. Ung-Arunyawee, and J. Izatt, “In vivo video rate optical coherence tomography,” Opt. Express 3(6), 219–229 (1998).
4. B. Potsaid, I. Gorczynska, V. J. Srinivasan, Y. Chen, J. Jiang, A. Cable, and J. G. Fujimoto, “Ultrahigh speed spectral / Fourier domain OCT ophthalmic imaging at 70,000 to 312,500 axial scans per second,” Opt. Express 16(19), 15149–15169 (2008).
5. S. Moon and D. Y. Kim, “Ultra-high-speed optical coherence tomography with a stretched pulse supercontinuum source,” Opt. Express 14(24), 11575–11584 (2006).
6. T. Klein, W. Wieser, L. Reznicek, A. Neubauer, A. Kampik, and R. Huber, “Multi-MHz retinal OCT,” Biomed. Opt. Express 4(10), 1890–1908 (2013).
7. I. Grulkowski, J. J. Liu, B. Potsaid, V. Jayaraman, C. D. Lu, J. Jiang, A. E. Cable, J. S. Duker, and J. G. Fujimoto, “Retinal, anterior segment and full eye imaging using ultrahigh speed swept source OCT with vertical-cavity surface emitting lasers,” Biomed. Opt. Express 3(11), 2733–2751 (2012).
8. O. P. Kocaoglu, R. D. Ferguson, R. S. Jonnal, Z. Liu, Q. Wang, D. X. Hammer, and D. T. Miller, “Adaptive optics optical coherence tomography with dynamic retinal tracking,” Biomed. Opt. Express 5(7), 2262–2284 (2014).
9. L. An, P. Li, T. T. Shen, and R. Wang, “High speed spectral domain optical coherence tomography for retinal imaging at 500,000 A-lines per second,” Biomed. Opt. Express 2(10), 2770–2783 (2011).
10. B. Cense, W. Gao, J. M. Brown, S. M. Jones, R. S. Jonnal, M. Majat, B. H. Park, J. F. de Boer, and D. T. Miller, “Retinal imaging with polarization-sensitive optical coherence tomography and adaptive optics,” Opt. Express 17(24), 21634–21651 (2009).

©2014 Optical Society of America
11. R. J. Zawadzki, B. Cense, Y. Zhang, S. S. Choi, D. T. Miller, and J. S. Werner, “Ultrahigh-resolution optical coherence tomography with monochromatic and chromatic aberration correction,” Opt. Express 16(11), 8126–8143 (2008).
12. Z. Liu, O. P. Kocaoglu, and D. T. Miller, “In-the-plane design of an off-axis ophthalmic adaptive optics system using toroidal mirrors,” Biomed. Opt. Express 4(12), 3007–3029 (2013).
13. O. P. Kocaoglu, S. Lee, R. S. Jonnal, Q. Wang, A. E. Herde, J. C. Derby, W. Gao, and D. T. Miller, “Imaging cone photoreceptors in three dimensions and in time using ultrahigh resolution optical coherence tomography with adaptive optics,” Biomed. Opt. Express 2(4), 748–763 (2011).
14. M. Mujat, B. H. Park, B. Cense, T. C. Chen, and J. F. de Boer, “Autocalibration of spectral-domain optical coherence tomography spectrometers for in vivo quantitative retinal nerve fiber layer birefringence determination,” J. Biomed. Opt. 12(4), 041205 (2007).
15. ANSI Z136, “Safe use of lasers,” Laser Institute of America (2007).
16. I. Grulkowski, M. Gora, M. Szkulmowski, I. Gorczynska, D. Szlag, S. Marcos, A. Kowalczyk, and M. Wojtkowski, “Anterior segment imaging with Spectral OCT system using a high-speed CMOS camera,” Opt. Express 17(6), 4842–4858 (2009).
17. S. A. Burns, R. Tumbar, A. E. Elsner, D. Ferguson, and D. X. Hammer, “Large-field-of-view, modular, stabilized, adaptive-optics-based scanning laser opthalmoscope,” J. Opt. Soc. Am. A 24(5), 1313–1326 (2007).
18. M. Choma, M. Sarunic, C. Yang, and J. Izatt, “Sensitivity advantage of swept source and Fourier domain optical coherence tomography,” Opt. Express 11(18), 2183–2189 (2003).
19. J. F. de Boer, B. Cense, B. H. Park, M. C. Pierce, G. J. Tearney, and B. E. Bouma, “Improved signal-to-noise ratio in spectral-domain compared with time-domain optical coherence tomography,” Opt. Lett. 28(21), 2067–2069 (2003).
20. R. Leitgeb, C. Hitzenberger, and A. Fercher, “Performance of Fourier domain vs. time domain optical coherence tomography,” Opt. Express 11(8), 889–894 (2003).

1. Introduction

Since its first report in 1991 [1], optical coherence tomography (OCT) has undergone tremendous advances in almost all aspects of its underlying technologies and methods. For ophthalmic imaging, one of the most impactful advances has been the substantial improvement in image acquisition speed. Increased speed has enabled larger fields of view (FOV) of the retina to be imaged faster and with finer spatial and temporal sampling than ever before. These have greatly expanded the scientific and clinical utility of OCT and have opened new directions into imaging both structure and function of the retina.

Podoleanu recently summarized the evolution of speed improvements in OCT acquisition from its introduction in 1991 to the state-of-the-art in 2012 [2]. As reported, increased speed has extended through time domain (TD, up to 8 KHz [3]), spectral domain (SD, up to ~313 KHz [4]), and swept source (SS, up to 5 MHz [5]) OCT systems. Today the fastest image acquisition rates for retinal imaging have been demonstrated with SS-OCT, peaking at 6.7 MHz at 1050 nm using multiple imaging beams [6, 7]. The advantages and challenges of OCT imaging at MHz speeds is discussed in detail by Klein et al. [6] for a Fourier-domain mode locked (FDML) laser-based SS-OCT system. In general, megahertz speeds have been found advantageous in reducing the unwanted effects of retinal motion, and have led to denser lateral sampling of the retina, larger FOV coverage, and improved registration and post processing of OCT images. These advantages are particularly attractive for retinal imaging using adaptive optics optical coherence tomography (AO-OCT) due to its high lateral resolution and magnification for imaging the retina at the cellular level, as we recently quantified [8]. Thus, AO-OCT can benefit greatly from increased acquisition speed to minimize eye motion artifacts, and to increase image sampling density as well as FOV. Such high speeds, however, are not without penalty-posing challenges in terms of decreased system sensitivity and increased system complexity.

While SS-OCT provides the fastest speeds at spectral bands of 1050nm and higher, SD-OCT does so in the most common band for ophthalmic imaging, 700 to 915 nm. Most notable is a 500 KHz SD-OCT system at 850 nm, demonstrated using a dual camera configuration that combined two high-speed spectrometers via a beam splitter [9].

In this study, we develop and validate a novel multi-camera AO SD-OCT system at 790 nm. The overarching strategy is to minimize retinal motion artifacts by maximizing acquisition speed, while reducing the performance of other system parameters (sensitivity,
axial resolution, and roll-off) to levels still sufficient for AO-OCT retinal imaging tasks. In doing so we advanced the multi-camera system beyond that reported by An et al. [9] on several key fronts: image acquisition speed, light efficiency, and optical resolution. Central to our design is a novel detection channel based on four high-speed spectrometers that receive light sequentially from a 1 × 4 optical switch assembly that consists of a cascade of three optical switches operating at nanosecond-level toggle periods, all tightly synchronized down to 10 ns. This manner of control makes use of all available light in the detection channel and avoids camera dead-time, both critical for imaging at high speeds. In combination with AO, not only does lateral resolution improve, but also additional signal-to-noise accrues from use of a larger pupil compared to that in OCT alone [10]. We experimentally validate the megahertz system and demonstrate performance by acquiring cellular-resolution retinal images in subjects at various imaging speeds using single, dual, and quadruple spectrometer configurations. From a practical standpoint, our system is based on widely used spectral domain technology and readily available components.

2. Materials and methods

Methods section is divided into five parts. Section 2.1 provides an overview of the Indiana MHz AO-OCT system. Novel for this study is the multi-camera, multi-optical-switch detection arm and associated custom control software. The new detection arm is presented in Section 2.2 and the necessary control and synchronization of the spectrometers and switches in Section 2.3. Experiments to validate the system are described in Section 2.4. Finally, methods to process the acquired AO-OCT images are presented in Section 2.5.

2.1. Overview of the MHz AO-OCT system

Figure 1 shows a schematic of the Indiana MHz AO-OCT system. Its four channels (source, sample, reference, and detection) are connected by a 2 × 2 780HP fiber coupler with 90/10 splitting ratio for capturing 90% of the light reflected back from the sample channel. The source arm contains a superluminescent diode laser (BLMD-S-HP3, Superlum, Ireland) with central wavelength at $\lambda_c = 790$ nm and bandwidth of $\Delta\lambda = 42$ nm. Axial resolution based on manufacturer's specifications was 4.7 $\mu$m in retinal tissue ($n = 1.38$).

The principal components of the sample arm are X and Y galvanometer scanners, a Shack-Hartman wavefront sensor (SHWS), a deformable mirror (DM97, ALPAO, France), a custom achromatizing lens designed for correcting ocular chromatic aberrations [11], and numerous relay optics, mirror-based telescopes to conjugate these active components to the eye pupil. Astigmatism generated at both retinal and pupil conjugate planes due to the off-axis use of spherical mirrors was corrected with three customized toroidal mirrors (TM1, TM2, and TM3 in Fig. 1). Details of the toroidal mirror design and AO system that dynamically measured and corrected the ocular aberrations of the subject can be found in Liu et al. [12]. Custom AO control software was developed in Matlab (Mathworks, Natick, MA) and incorporated the ALPAO Core Engine (ACE) Matlab libraries. AO performance was assessed in terms of the residual wavefront root-mean-square (RMS) error and quality of the en face retinal AO-OCT images.

The reference arm consisted of a fiber collimator as the first element and a reference mirror as the last. The mirror was positioned at the back focal plane of a focusing lens that together moved in tandem on a translation stage. The optical path length of this arm matched that of the sample arm and employed flat folding mirrors to reduce space, a water vial for coarse dispersion matching of the ocular medium (finer dispersion compensation was done numerically in post processing), a custom achromatizing lens that duplicated the one in the sample arm in order to dispersion match it, and a neutral density filter wheel for adjusting the reference arm power.
2.2. Multi-camera, multi-switch detection channel

Single spectrometer OCT systems detect the source spectrum only when their line-scan camera is exposing, not reading out. While the readout time is usually small (ours is 1.3 to 2 µs, with the Basler Sprint), it becomes appreciable at maximum imaging rates (ours at 250 KHz, which corresponds to 4 µs image interval). Loss of light during the readout can be avoided by toggling the OCT beam between two identical cameras that are synched with out-of-phase exposure and readout periods. Synching in this way also doubles the effective imaging rate (500 KHz instead of 250 KHz), an increase that is proportional to the number of cameras used. In this study, we achieved megahertz imaging speed using four high speed spectrometers based on CMOS line-scan cameras that operated at 2 µs exposures at their fastest setting. A 1 × 4 fiber-based optical switch assembly directed the light to the four spectrometers and was realized by cascading three 1 × 2 optical switches in a double-tiered fashion (Fig. 1). The switches (Nanona) were custom manufactured by Boston Applied Technologies, Inc. (Woburn, MA). With no moving parts, the switches provided ultra-fast (<50 ns) and highly repeatable switching between their two output channels along with low insertion loss (∼0.18 dB average attenuation per channel).

Fig. 1. Schematic of Indiana MHz AO-OCT system. Key: ACL, achromatizing lens; BS, beam splitter; C, collimator; CMOS, Basler Sprint camera; DG, diffraction grating; DM, deformable mirror; FT, fixation target; GS, glass slide; L, lens; OS, optical switch; PC, polarization controller; PM, planar mirror; P, pupil conjugate plane; R, retinal conjugate plane; S, scanner; SM, spherical mirror; TM, toroidal mirror; WS, wavefront sensor; WV, water vial.

Our multi-camera detection channel was designed to detect the OCT beam at all times (100% effective duty cycle) by sequentially routing the beam to the four cameras in a manner that assured it was always incident on an exposing camera. To realize this efficiency advantage and also quadruple the speed over that of any single spectrometer in the system required accounting for three exposure parameters: light exposure, camera exposure, and dark exposure. The first, light exposure, is the time period during which the optical switches route the beam to a specific camera. The second, camera exposure, is the time period during which the specific camera integrates. Finally the third, dark exposure, is the portion of the camera exposure during which no spectrum is incident on the camera, but it still integrates.
Each of these parameters is shown in the timing diagram of Fig. 2. The diagram is configured for 100% effective duty cycle at a MHz A-scan rate and includes the performance specifications of the Basler Sprint cameras. Note that the imaging speed of our system is set by the light exposure (1 µs for 1 MHz imaging speed), during which one A-line is acquired. Since the minimum permissible camera exposure of the Basler cameras is 2 µs (which does not include the 1.3 to 2 µs readout time), the optical switches must toggle collectively 2 × faster than the single camera exposure to achieve MHz imaging speed, i.e., light exposure = 1 µs and camera exposure = 2 µs. In this way, each camera collects light for 1 µs and dark exposes for the remainder of their 2 µs camera exposure. This is then followed by the camera’s readout. Thus for any 4 µs interval, four successive A-line spectra are acquired by four different cameras, each receiving 1 µs of the source. Because the optical switches are highly efficient in routing the OCT light to the four output fibers (−0.18 dB), detection efficiency surpasses high-speed single-camera approaches (because of camera readout time), and even more so for multi camera systems based on beam splitters (because of their 3 dB and 6 dB loss per channel for two- [9] and, if pursued, four-camera designs).

Fig. 2. Timing diagram showing synchronized operation of the four camera detection channel in Fig. 1. The diagram illustrates the sequence for an exemplary B-scan that sweeps over a +/−V voltage range of the galvanometer scanner during which 40 A-lines are acquired, 10 by each camera, at an acquisition rate of 1 MHz (A-lines/second). The portions of the time trace at which light, camera, and dark exposures occur are labeled.

2.3. Control software for optical switches and multi camera detection system

Synchronization of the four high-speed cameras, three optical switches, and two galvanometer scanners required precise triggering and timing. These were achieved by a custom control algorithm developed in C++. The algorithm generated a symmetric triangular waveform and a saw tooth waveform for the XY scanners to perform the fast and slow scans that were synced to the trigger signals of CMOS line-scan cameras and optical switches. Latencies were incorporated to account for each component's specific response time. The cameras and optical switches were tightly synchronized down to 10 ns. The symmetric triangular shape of the fast scan waveform better preserved (compared to a saw tooth) the linearity of the galvanometer scanner response to inputs at the B-scan rates we wanted to achieve (up to 2.5 KHz).

Spectra acquired by each camera were saved separately along with metadata, which included camera identification and acquisition time. Each saved image contained one-fourth of the A-lines acquired and was processed separately in order to account for slight differences in the spectrometers and optical switches. B-scans were constructed by interleaving the A-lines from the four image files. Volumes were created from B-scans after every other B-scan.
was flipped to compensate for the triangular fast scan waveform. Acquired volume videos were visualized without any registration for demonstration of speed benefit. Images were laterally and axially registered using previously described methods [13] for demonstrating the advantage of higher speeds for image averaging.

Separate from the rigorous reconstruction steps executed offline, the control software processed a portion of the image acquisition stream in real time for visualization purposes in order to provide feedback about the AO-OCT performance during the experiment. The image data was processed and displayed at 20 fps, and provided visualization of the raw spectra, A-scan, fast and slow B-scan, and C-scan (en face) projection of the retinal layers of interest.

2.4. Experiments

We validated system performance by several experiments. First we measured and calibrated the timing pattern that controlled the four spectrometers, three optical switches and two galvanometer scanners. Second, we optically measured the switch assembly performance via four high-speed point detectors connected temporarily to the switch output ports. Third, we measured system SNR, sensitivity, roll-off (dB/mm), axial resolution, and A-line shift using a model eye that consisted of a focusing lens, protected silver flat mirror placed at the lens’ focal plane, and neutral density filters. Signal-to-noise ratio (SNR) was calculated by taking the ratio of peak amplitude of sample mirror to the root-mean-square of the noise level. Sensitivity was measured using a set of neutral density filters in front of the sample mirror and adding its double-pass signal attenuation level to the given SNR. K-space mapping and numerical dispersion compensation was applied separately to each spectrometer, the former using a glass slide in the source arm [14]. Fourth, we acquired retinal volumes with different system configurations on two normal subjects. Table 1 summarizes the AO-OCT configurations used in the imaging experiments. To demonstrate the effect of acquisition speed on retinal images as well as the flexibility of our system to trade off speed and sensitivity, three different speeds were used (250 KHz, 500 KHz, and 1 MHz A-lines/s), realized with one, two, and four cameras. Field of view was adjusted between 1.3° and 3.6° while maintaining a lateral A-line sampling of 1 μm/px. Axial pixel sampling in depth was at 1.2 μm/px (for n = 1.38). Table 2 summarizes subject information.

Table 1. AO-OCT parameters for retinal imaging.

|                        | Speed comparison experiment | Microscopic retinal imaging experiment |
|------------------------|----------------------------|---------------------------------------|
| A-line acquisition rate | 250 KHz                   | 500 KHz                               | 1 MHz | 1 MHz | 1 MHz | 1 MHz |
| A-line sampling         | 1 μm/px                   | 1 μm/px                               | 1 μm/px | 1 μm/px | 1 μm/px | 1 μm/px |
| # of A-lines per B-scan | 400                        | 400                                   | 400     | 800   | 800   | 800 |
| # of B-scans per volume | 400                        | 400                                   | 400     | 400   | 400   | 400 |
| B-scan acquisition rate | 625 Hz                     | 1.25 KHz                              | 2.5 KHz | 1.25 KHz | 893 Hz |
| # of volumes per video  | 10                         | 10                                    | 10      | 4     | 1     |
| Volume rate (Hz)        | 1.56                       | 3.12                                  | 6.25    | 1.56  | 0.8   |
| Volume video duration   | 6.4 s                      | 3.2 s                                 | 1.6 s   | 2.6 s | 1.25 s |
| Image FOV               | 1.3° × 1.3°                | 1.3° × 1.3°                           | 1.3° × 1.3° | 2.6° × 2.6° | 3.6° × 3.6° |
| # camera pixels used    | 768                        | 768                                   | 768     | 768   | 768   | 768   |

Table 2. Subject information.

| Subject # | Age (y) | Gender | Spherical equiv. refractive error (D) | Axial length (mm) | Imaged Retinal Eccentricity (from fovea) |
|-----------|---------|--------|--------------------------------------|-------------------|-----------------------------------------|
| 1         | 32      | F      | 0                                    | 23.7              | 3° Nasal                                |
| 2         | 36      | M      | -3                                   | 26.1              | 3° Nasal                                |

The AO-OCT light incident on the subject’s cornea was 430 μW, centered at a wavelength of 790 nm and scanned over a 1.3° × 1.3°, 2.6° × 2.6° or 3.6° × 3.6° patch of retina. The light power was more than an order of magnitude below safe limits defined by ANSI [15]. All procedures on the subjects strictly adhered to the tenets of Helsinki declaration and the...
Institutional Review Board of Indiana University, and informed consent was obtained from all subjects.

3. Results

3.1. MHz AO-OCT system performance

Figure 3 shows oscilloscope recordings of the nine system control signals (four camera triggers, three optical switches, and two scanners) to achieve 1 MHz A-line rate. Also shown are measured light traces at the four output ports of the 1 × 4 optical switch assembly. Correct timing of the optical switches (OS1, OS2, and OS3) results in 1 µs duration top-hat pulses repeated at 4 µs intervals. The four detector traces (#1 to #4) in the figure confirm the pulse timing and shape, and the negligible loss between pulses. Also shown are the four camera exposures that are properly synchronized to overlap with the detector traces (color coded) in this way providing 100% fill.

Next we used the focusing lens, neutral density filters and a protected silver mirror as a target for quantifying performance of the four spectrometers. The reference arm power was maintained (with a neutral density filter wheel) at a maximum spectral power of approximately 90% of the CMOS pixel well capacity. This assured photon limited detection [16].

![MHz AO-OCT system control signals](image)

Fig. 3. Oscilloscope recordings of MHz AO-OCT control signals (scanners, optical switches, and cameras) and detected light at output ports of the 1 × 4 optical switch (OS) assembly. Horizontal time axes have tick intervals as specified. Vertical voltage axes are of varying scales, e.g., OS and camera control trigger signals are 0–5V TTL signals. CMOS cameras were run in line-trigger mode in which the rising-edge initiated camera exposure. Semi-transparent boxes mark when the camera exposure occurred. Synchronization required time offsets to be applied to the control signals for correction of hardware latencies.

Figure 4 shows the spectra and corresponding A-lines for the four spectrometers, acquired under the same conditions with the model eye. While differences are apparent in the spectra, their overall shape and width are similar, especially considering the extent of the non-common paths in the detection channel. These include different paths through the optical switches, connecting fibers, and spectrometers. We reduced these differences by fabricating optical switches and spectrometers from the same design. Yet inevitably, differences in assembly, tolerances of components, and sensitivity across pixels of the CMOS line-scan cameras occur and contribute to performance differences between the four spectrometer channels. These differences are also apparent in the reconstructed A-scans shown in the same figure. To quantify these differences, Table 3 summarizes spectrometer performance in terms of SNR, roll-off, full width at half maximum (FWHM) of the A-line peak, and center of
gravity (COG). COG is the axial center of mass of the A-scan reflectance profile and measures the extent to which each spectrometer axially shifts the A-scan content. As shown in the table, the SNR ranged from 40.0 to 42.5 dB, sensitivity from 70.0 to 72.3 dB, roll-off from 8.7 to 10.8 dB/mm, FWHM from 5.1 to 5.8 μm, and COG from 73.9 to 76.9 μm. The largest shift in COG between spectrometers was 3 μm (between #2 and #4), a factor of almost two smaller (1.8 × ) than the measured axial resolution (5.3 μm) of the system. The right most column of Table 3 shows the average and standard deviation in performance of the spectrometers and reveals spectrometer variations (std/avg) in SNR, sensitivity, and COG of 1% to 2%, FWHM of 6%, and roll-off of 10%.

Fig. 4. Plots compare raw spectra from the spectrometers (A, top) and reconstructed A-lines in normalized logarithmic (log) amplitude (A bottom, B) and normalized linear amplitude (C). Spectra were collected with 30 dB attenuation of the mirror reflection using neutral density filters. CMOS cameras #1, #2, #3, and #4 are color coded per key in spectra plot. COG and FWHM of the four spectrometers are shown in the linear plot (C).

Table 3. MHz AO-OCT system performance for the four spectrometers.

| Spectrometer #: | 1  | 2  | 3  | 4  | Avg ± Std dev |
|-----------------|----|----|----|----|---------------|
| Color in figures| green | magenta | cyan | red | -             |
| SNR (dB)        | 41.0 | 40.0 | 42.3 | 41.5 | 41.2 ± 1.0    |
| Sensitivity (dB)| 71.0 | 70.0 | 72.3 | 71.5 | 71.2 ± 1.0    |
| Roll-off (dB/mm)| 8.7  | 10.8 | 8.8  | 9.8  | 9.5 ± 1.0     |
| Axial Resolution (FWHM, μm) | 5.1 | 5.2 | 5.8 | 5.2 | 5.3 ± 0.3 |
| COG location (μm) | 74.9 | 76.9 | 76.0 | 73.9 | 75.4 ± 1.3 |

Measurements were obtained with the model eye in the sample arm and 30 dB attenuation. SNR measurements were made at the mirror peak. Axial resolution (FWHM) is adjusted for retinal tissue (n = 1.38).

3.2. Imaging the microscopic retina

Figure 5 and Media 1 illustrate the benefit of imaging at 1 MHz. The figure shows slow scan and en face projections of volumes acquired at three different A-line rates (250 KHz, 500 KHz, and 1 MHz) on subject 2. The projections are displayed without registration and cropping, and therefore show all 1.6 million A-lines acquired. To quantify the reduction in motion artifacts, we performed a running cross-correlation (between successive frames) across all frames of each projection view sequence (slow scan and en face), and compared cross-correlation displacements as well as cross-correlation coefficients as a function of imaging speed. Cross-correlation displacements and coefficients reflect the extent of inter-frame motion (motion between frames) and intra-frame motion (motion within frames), respectively. We used the slow scan projection views to determine the axial (Z) displacement,
shown in the plot, Fig. 5(C), left. We used the *en face* projections to determine lateral (XY) displacements, here represented by a radial displacement ($R = \sqrt{X^2 + Y^2}$), Fig. 5(C). As the two plots show, both axial and lateral displacements (inter-frame motion) were noticeably reduced at the fastest (1 MHz) imaging speed. On average, the axial displacement of frames for 250 KHz, 500 KHz, and 1 MHz were 23.2 ± 21.3 μm, 22.9 ± 11.3 μm, and 9.5 ± 13.1 μm, respectively. Similarly, the lateral displacement of frames for 250 KHz, 500 KHz, and 1 MHz were 17.7 ± 23.4, 15.7 ± 15.9, and 6.1 ± 7.4 μm.

The corresponding cross correlation coefficients are shown in the rightmost two plots of Fig. 5(C) and reveal that the largest correlation (least intra-frame motion) occurred at the fastest (1 MHz) imaging speed. On average, the axial correlation coefficients for 250 KHz, 500 KHz, and 1 MHz were 0.65 ± 0.06, 0.70 ± 0.09, and 0.81 ± 0.05, respectively. Similarly the lateral correlation coefficients for 250 KHz, 500 KHz, and 1 MHz were 0.42 ± 0.07, 0.49 ± 0.14, and 0.73 ± 0.09. These measurements confirm our visual observation that while motion artifacts were not fully eliminated at the 1 MHz speed, notable reduction did occur.

Figure 6 illustrates the performance of our MHz AO-OCT system to capture detailed images of retinal nerve fiber layer (RNFL) bundles and photoreceptors with fine spatial sampling (1 μm/pixel) over a relatively large 3.6° field of view. The wide-field SLO image denotes the retinal location of AO-OCT volumes acquired, one with AO focus optimized for imaging the RNFL (Fig. 6(D) and 6(F)) and the other for imaging the photoreceptor layer (Fig. 6(C) and 6(E)). The 3D volume image shown in the center of Fig. 6 merges the two volumes with different AO focus. The *en face* projections were extracted from single MHz AO-OCT volumes without cropping or registration, and along with the cross-sections demonstrate that sufficient signal-to-noise (dynamic range of 29.6 ± 0.7 dB and 30.3 ± 1.1 dB for the photoreceptor and RNFL images, respectively) is obtained to image these layers even at MHz speeds. Retinal locations of the photoreceptor (Fig. 6(E)) and RNFL bundle (Fig. 6(F)) cross sections are color-coded on the corresponding *en face* images.

Figure 7 and Media 2 shows MHz AO-OCT volume video of RNFL acquired over a 2.6° field of view centered at 3° nasal to the fovea of Subject 2. The sequence of 4 volumes was acquired in 2.6 seconds with AO focus at RNFL and with sampling of 800 A-lines/B-scan × 800 B-scans/volume corresponding to a 1 μm/pixel lateral sampling. Reduced motion artifacts facilitated improved registration of volumes to one another and better visualization of the retinal nerve fiber bundles (RNFBs) as shown in Fig. 7(B). Figure 7(C) B-scan image also displays a clear view of RNFBs in cross section.

Figure 8 and Media 3 shows MHz AO-OCT volume video of photoreceptors acquired over a 2.6° field of view centered at 3° nasal to the fovea of Subject 1. The four volume sequence was acquired in 2.6 seconds with AO focus at the photoreceptors and with sampling of 800 A-lines/B-scan × 800 B-scans/volume corresponding to a 1 μm/pixel lateral sampling. Again reduced motion artifacts facilitated improved registration, illustrated by the preservation of cone detail in the five-frame averaged image shown in Fig. 8(B). The B-scan image (Fig. 8(C)) shows details of individual photoreceptors are also present in cross section, in this case at the retinal location denoted by the red line.
Fig. 5. Faster image acquisition reduces axial and lateral retinal motion artifacts in the AO-OCT video stream. Retinal image sequence is shown as linear scale projections of the entire volumes in slow scan projection (A). Each sequence is composed of ten successive AO-OCT volumes of a 1.3° × 1.3° patch of retina at 3° nasal to the fovea in subject 2 (400 A-lines/B-scan, 400 B-scans/volume, and 10 volumes/video; totaling 1.6 million A-lines/video). A-lines were acquired using one (250 KHz), two (500 KHz), and four camera (1 MHz) configurations. En face images with characteristic motion artifacts were chosen (B) to illustrate the effects of motion at each speed. None of the en face images in the 1 MHz sequence had a noticeable motion artifact, thus we display the first image of the sequence. All of the en face images at the three speeds can be viewed in Media 1. En face views in the video display all 1.6 million A-lines acquired without cropping or registration. Thus blur along the vertical sides is more apparent at the higher speeds as faster reversal of the galvanometer scanner is needed, especially at 1 MHz. At this highest speed, the fast galvanometer scanner operated at 1.25 KHz (2.5 KHz B-scan rate). This fixed blur pattern can be corrected in post processing [17], but we decided not to in order to show the extent to which the scanner can preserve the symmetric triangular waveform at various speeds. The leftmost two plots (C) show the cross-correlation displacements between successive frames in both projection views, thus depicting inter-frame motion. The rightmost two plots (C) show the cross-correlation coefficient between successive frames, thus depicting intra-frame motion (image warp).
Fig. 6. MHz AO-OCT images of 3.6° × 3.6° patch of retina at 3° nasal to the fovea of subject 2 acquired with AO focus at photoreceptors (C, E) and at RNFL (D, F). Lateral sampling was 1120 A-lines/Bscan × 1120 B-scans/volume (1 μm/pixel). The wide field SLO image (A) shows the location of AO-OCT volumes (B). All AO-OCT images are shown on a linear amplitude scale and without correction of the scanner reversal blur, which appears at the top and bottom of the images (C, D, E, F). Note this blur is smaller than that in the MHz image of Fig. 5 due to the larger FOV. Dynamic range of volumes acquired with focus at photoreceptors and RNFL were 29.6 ± 0.7 dB and 30.3 ± 1.1 dB, respectively.

Fig. 7. MHz AO-OCT volume sequence of 2.6° × 2.6° patch of retina at 3° nasal to the fovea of subject 2 acquired with AO focus at RNFL. Lateral sampling was 800 A-lines/Bscan × 800 B-scans/volume × 4 Volumes/video (1 μm/pixel, en face). Shown are single unregistered en face frame (A), registered and averaged over 4 en face frames (B), and cross-sectional (B-scan averaged over three successive B-scans) image of RNFL bundles from the location marked by the red line (C). Media 2 combines the registered and unregistered en face videos to facilitate comparison. All AO-OCT images are shown on a linear amplitude scale and without correction of the scanner reversal blur. Dynamic range of volumes was 31.3 ± 0.6 dB.
Fig. 8. MHz AO-OCT volume sequence of 2.6° × 2.6° patch of retina at 3° nasal to the fovea of subject 1 acquired with AO focus at the photoreceptors. Lateral sampling was 800 A-lines/Bscan × 800 B-scans/volume corresponding to 1μm/pixel. (A) A single unregistered en face frame. (B) Registered and averaged over five en face frames. Also shown is an average of three contiguous B-scans cropped about the photoreceptor and RPE layers (C). B-scan location is marked by the red line. Media 3 shows the registered and unregistered en face videos side by side for comparison. All AO-OCT images are shown on a linear amplitude scale and without correction of the scanner reversal blur. Dynamic range of volumes was 29.5 ± 1.0 dB.

4. Discussion

We have investigated the performance of a novel multi-camera AO-OCT system for ophthalmologic use that operates at up to 1 million A-lines/s at a central wavelength of 790 nm. Key to the spectral-domain design is a novel detection channel based on four high-speed spectrometers that receive light sequentially from a 1 × 4 optical fiber switch assembly. The MHz system was validated by a series of experiments that included imaging in both model and real eyes. Four main points encompass these findings and are discussed in order below: (1) optimization of system performance, (2) flexibility to control number of spectrometers, (3) variability in performance across spectrometers, and (4) imaging real eyes.

4.1. Optimizing system performance

There is a fundamental tradeoff in OCT performance between image acquisition speed, sensitivity, sensitivity roll-off, and axial resolution. For SD-OCT systems, shorter exposure duration of the line-scan camera and use of fewer camera pixels increase speed, but come at the expense of reduced sensitivity and axial resolution, and steeper sensitivity roll-off. Because the best tradeoff is application specific, we chose to optimize these parameters for the application of AO-OCT imaging of the living human retina. For this, high magnification imaging with AO-OCT also magnifies eye motion and results in considerable motion artifacts at the cellular level. Our strategy therefore was to minimize these artifacts by maximizing acquisition speed while reducing the other parameters to levels still sufficient for AO-OCT tasks.
For this, we maximized the line rate of a single Basler Sprint camera at 250 KHz. Use of line trigger mode limited the number of pixels read out to 768 (Table 3). Across these pixels we constrained the spectral bandwidth of the SLD light source to 42 nm. With a somewhat short central wavelength of 790 nm, this bandwidth resulted in a theoretical axial resolution of 4.7 μm in retina, which is more than acceptable to distinguish the major retinal bands and is similar to that of clinical OCT. The relatively coarse sampling of the spectrum (0.055 nm/px) resulted in a steep sensitivity roll off (9.5 dB/mm on average, Table 3). While for many applications this is undesirable, for our application the narrow thickness of the retina (<300 μm) and the even narrower depth of focus of AO-OCT diminish much of the benefit of a shallow roll-off.

To increase speed further and without loss in performance of the other OCT parameters, we converged on a four spectrometer design that utilized a highly efficient 1x4 optical switch assembly (~0.18 dB average attenuation per channel). This provided a 1 MHz A-line rate and assured not only that the detection channel light was always directed to an exposing camera (an advantage over other multi-camera designs, as for example those based on beam splitters [9]), but it also avoided the camera readout period, which at high speeds becomes comparable to the camera exposure time (an advantage over single camera designs). Thus this latter advantage actually recovers a portion of the sensitivity lost when imaging speed is increased by converting a single-camera design into a multi-camera one.

At 1 MHz speed, light exposure (1 μs) is 40 × shorter than the 40 μs of conventional SD-OCT systems (25 KHz), which corresponds to a nominal 16 dB loss in sensitivity. Conventional SD-OCT systems have an observed sensitivity of 85-88 dB (for an exposure time of 40 μs) [18–20]. In comparison, our MHz AO-OCT system has an observed sensitivity of 71.2 dB (average system sensitivity in Table 3), which is consistent with the expected 16 dB decrease due to 40 × shorter exposure time and 2.5 dB gain due to use of more efficient collection by 90/10 fiber coupler. As a result, the average sensitivity of 71.2 dB is 14-16 dB below that of conventional systems. However a large fraction of this is recouped by the AO for retinal imaging as it collects light over a larger pupil of the eye, e.g., 6.7 mm compared to 1.25-2 mm (approximately that of clinical OCT) giving an areal increase of 11.2-28.7 times. An AO gain of approximately 8 to 12 dB for retinal imaging has been reported elsewhere [10]. Note that this light collection efficiency benefit of AO is not captured by standard sensitivity measurements made with a planar mirror, as was done here to obtain our 71.2 dB. Indeed the dynamic range of our MHz AO-OCT retinal images are comparable to that of clinical SD-OCT systems (~30 dB), a point reiterated below in Section 4.4.

4.2. Flexibility to control number of spectrometers

Fast, software-controlled switching provides flexibility and scalability to the multi-camera design. In particular, this feature extends the range over which system speed and sensitivity can be balanced to better match to imaging conditions. To demonstrate, AO-OCT retinal images were acquired in the same subject at three imaging speeds realized by single, dual, and quadruple spectrometer configurations.

Figure 5 (A, B, and accompanied video) illustrate and Fig. 5(C) quantifies the impact of retinal motion for the three configurations. The number of frames used in the study were limited, but nevertheless provided clear demonstration of the benefit of MHz rates to reduce motion artifacts compared to even ultrafast single-camera systems (250 KHz). Our cross correlation analysis of the AO-OCT volumes showed 59% and 66% decrease in axial and lateral displacement between frames, respectively, for the four-spectrometer configuration (1 MHz) compared to the single camera (250 KHz). Similarly intra-frame motion decreased as indicated by the cross-correlation coefficient, showing 25% and 74% reduction in axial and lateral motion, respectively, between the same two spectrometer configurations. Note that the number of spectrometers used in the experiment was entirely configured in the control user
interface, requiring no additional changes as for example moving mechanical components in the system.

The efficiency of the optical switch assembly (−0.18 dB average attenuation per channel) makes the multi-camera design scalable unlike multi-camera approaches based on beam splitters or similar means of redirecting the light that lose 3 dB for every doubling of speed. As an example the AO-OCT retinal images acquired with single (250 KHz), dual (500 KHz), and quadruple (1 MHz) spectrometer configurations had light exposure durations of 2.5 μs, 2 μs, and 1 μs, respectively, with the single configuration dropping 1.5 μs due to readout time. Therefore quadrupling the speed resulted in a −4.18 dB ( = \(10 \log \frac{1\mu s}{2.5\mu s}\) dB - 0.18 dB) attenuation compared to that of the single camera. In contrast, quadrupling of speed by conventional methods (e.g., beamsplitters) adds −6 dB (not −4.18 dB) due to the speed increase plus an additional −6 dB for loss at two beam splitters. The total loss of −12 dB makes practical use of such conventional methods extremely difficult for OCT retinal imaging.

4.3. Variation in performance across spectrometers

Synchronization of four spectrometers and three 1x2 optical switches is noticeably more complex than a single spectrometer system. A potentially confounding issue is the considerable non-common paths through the optical switches, connecting fibers, and spectrometers. These non-common paths coupled with inevitable variations in performance and tolerances of the componentry generate performance differences between the four channels (Table 3 and Fig. 4). We found that placement of three polarization controllers on the input ports of the optical switches (see Fig. 1) helped reduce these differences, but did not eliminate them. Regardless, variations across the four spectrometer channels were small (Table 3), being only a couple of percent for SNR, sensitivity, and COG, 6% for FWHM, and 10% for dB roll-off. The measured FWHM axial resolution across the four spectrometers was 5.3 ± 0.3 μm (range: 5.1-5.8 μm), 0.6 μm wider than the 4.7 μm theoretical prediction based on the source spectrum alone. As mentioned in the results section, the largest shift in COG between spectrometers was 3 μm, a factor of almost two smaller (1.8x) than the measured axial resolution (5.3 μm) of the system. At this level, the slight shifts between adjacent A-scans that composed B-scans are not visually apparent in the cross-sectional images and accompanied videos of Figs. 6, 7, and 8. However, these slight variations (in COG and FWHM of successive A-lines) may affect quantitative analyses of the retina at the micron level, such as those that seek to quantify the spatial relationship between neighboring structures e.g., cones, blood vessels or nerve fiber bundles. We anticipate that refined calibration of the A-scans beyond the minimal one conducted here will reduce this variability well below the 1.3 μm RMS measured (Table 3). This remains future work.

4.4. MHz retinal imaging

Figures 5 through 8 demonstrate the efficacy of MHz AO-OCT for imaging the cellular retina in normal subjects. Volume videos were acquired at rates of 0.8 to 6.25 Hz covering a field of view of 1.3°x1.3° to 3.6°x3.6°, all with a dense 1 μm/px A-line sampling. Figure 7 presents MHz results of en face and cross sectional views of the retinal nerve fiber layer. The striation pattern of individual bundles is clearly evident both in individual frames and the registered average. Likewise Fig. 8 shows similar results of cone photoreceptors obtained in single frames and the registered average. Individual cone photoreceptors are identifiable in both en face and cross section. It is important to note that MHz imaging does not eliminate eye motion artifacts as is clearly evident in the videos that accompany both figures. However, the remaining motion manifests itself more as image translation and less as localized distortion compared to that at slower imaging speeds, as apparent and quantified in Fig. 5 results. This
difference is critical as image translation is more effectively corrected in post processing than is local distortion. The effectiveness of the registration to remove these artifacts down to the micron (sub-cellular) level is evident in the figure. Regardless of motion, however, it would be near impractical to acquire an identical image on a clinical OCT system such as the Heidelberg Spectralis. AO is necessary to reliably resolve these small structures, but equally important at 40K A-lines/s (high-speed mode), the Spectralis would need 16 seconds to acquire a single volume compared to the 0.64 s for our system.

In this study we limited our imaging to RNFL bundles and photoreceptors, two of the most commonly imaged structures in the retina. Both produce some of the strongest reflections in the retina and are best viewed on a linear amplitude scale, as was done here. While the dimmer retina bands are typically difficult to visualize on such a scale, the dynamic range of our images was consistently around 30 dB (see figure captions for actual values), in line with that of clinical OCT systems and sufficient for detecting these dim bands.

5. Conclusion

Image acquisition speed of OCT remains a fundamental barrier that limits its scientific and clinical utility. Here we developed and validated a novel multi-camera based AO-OCT system that operates at 790 nm and at A-lines rates up to 1 MHz. While the 1 µs exposure duration places a premium on system sensitivity, use of a high-speed, high-efficiency detection channel in conjunction with AO yields retinal images of comparable dynamic range to that of clinical OCT and of sufficient 3D resolution to resolve cellular details.

Acknowledgment

This study was supported by National Eye Institute grants: R01-EY018339 and P30-EY019008. We thank Thomas Kemerly, Daniel Jackson, and William Monette for machining and electronic support.