Trans-retinal cellular imaging with multimodal adaptive optics

ZHUOLIN LIU,1,* JOHNNY TAM,2 OSAMAH SAEEDI,3 AND DANIEL X. HAMMER1

1Center for Devices and Radiological Health (CDRH), U.S. Food and Drug Administration, 10903 New Hampshire Ave, Silver Spring, MD 20993, USA
2National Eye Institute, National Institutes of Health, Bethesda, MD, USA
3Department of Ophthalmology and Visual Sciences, University of Maryland Medical Center, 419 W. Redwood St., Baltimore, MD 21201, USA
*zhuolin.liu@fda.hhs.gov

Abstract: Adaptive optics (AO), when coupled to different imaging modalities, has enabled resolution of various cell types across the entire retinal depth in the living human eye. Extraction of information from retinal cells is optimal when their optical properties, structure, and physiology are matched to the unique capabilities of each imaging modality. Despite the earlier success of multimodal AO (mAO) approaches, the full capabilities of the individual imaging modalities were often diminished rather than enhanced when integrated into multimodal platforms. Furthermore, many mAO designs added unnecessary complexity, making clinical translation difficult. In this study, we present a novel mAO system that combines two complementary approaches, scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT), in one instrument using a simplified optical design, flexible alternation of scanning modes, and independent focus control. The mAO system imaging performance was demonstrated by visualization of cells in their mosaic arrangement across the full depth of the retina in three human subjects, including microglia, nerve fiber bundles, retinal ganglion cells and axons, and capillaries in the inner retina and foveal cones, peripheral rods, and retinal pigment epithelial cells in the outer retina. Multimodal AO is a powerful tool to capture the most complete picture of retinal health.

References and links
1. J. Liang, D. R. Williams, and D. T. Miller, “Supernormal vision and high-resolution retinal imaging through adaptive optics,” J. Opt. Soc. Am. A 14(11), 2884–2892 (1997).
2. D. R. Williams, “Imaging single cells in the living retina,” Vision Res. 51(13), 1379–1396 (2011).
3. R. S. Joomal, O. P. Kocaoglu, R. J. Zawadzki, Z. Liu, D. T. Miller, and J. S. Werner, “A review of adaptive optics optical coherence tomography: Technical advances, scientific applications, and the future,” Invest. Ophthalmol. Vis. Sci. 57(9), OCT51–OCT68 (2016).
4. M. Pircher and R. J. Zawadzki, “Review of adaptive optics OCT (AO-OCT): Principles and applications for retinal imaging [Invited],” Biomed. Opt. Express 8(5), 2536–2562 (2017).
5. J. I. Morgan, A. Dubra, R. Wolfe, W. H. Merigan, and D. R. Williams, “In vivo autofluorescence imaging of the human and macaque retinal pigment epithelial cell mosaic,” Invest. Ophthalmol. Vis. Sci. 50(3), 1350–1359 (2009).
6. D. Scoles, Y. N. Sulai, and A. Dubra, “In vivo dark-field imaging of the retinal pigment epithelium cell mosaic,” Biomed. Opt. Express 4(9), 1710–1723 (2013).
7. Z. Liu, O. P. Kocaoglu, and D. T. Miller, “3D imaging of retinal pigment epithelial cells in the living human retina,” Invest. Ophthalmol. Vis. Sci. 57(9), OCT533–OCT543 (2016).
8. J. Tam, J. Liu, A. Dubra, and R. Fariss, “In vivo imaging of the human retinal pigment epithelial mosaic using adaptive optics enhanced indocyanine green ophthalmoscopy,” Invest. Ophthalmol. Vis. Sci. 57(10), 4376–4384 (2016).
9. T. Liu, H. Jung, J. Liu, M. Droethboom, and J. Tam, “Noninvasive near infrared autofluorescence imaging of retinal pigment epithelial cells in the human retina using adaptive optics,” Biomed. Opt. Express 8(10), 4348–4360 (2017).
10. A. Dubra, Y. Sulai, J. L. Norris, R. F. Cooper, A. M. Dubis, D. R. Williams, and J. Carroll, “Noninvasive imaging of the human rod photoreceptor mosaic using a confocal adaptive optics scanning ophthalmoscope,” Biomed. Opt. Express 2(7), 1864–1876 (2011).
11. D. Merino, J. L. Duncan, P. Tiruvveedhula, and A. Roorda, “Observation of cone and rod photoreceptors in normal subjects and patients using a new generation adaptive optics scanning laser ophthalmoscope,” Biomed. Opt. Express 2(8), 2189–2201 (2011).
12. E. M. Wells-Gray, S. S. Choi, A. Bries, and N. Doble, “Variation in rod and cone density from the fovea to the mid-periphery in healthy human retinas using adaptive optics scanning laser ophthalmoscopy,” Eye (Lond.) 30(8), 1135–1143 (2016).
13. F. Felberer, J. S. Kroismayer, B. Baumann, S. Zetter, U. Schmidt-Erfurth, C. K. Hitzenberger, and M. Pircher, “Adaptive optics SLO/OCT for 3D imaging of human photoreceptors in vivo,” Biomed. Opt. Express 5(2), 439–456 (2014).
14. D. Scoles, Y. N. Sulai, C. S. Langlo, G. A. Fishman, C. A. Curcio, J. Carroll, and A. Dubra, “In vivo imaging of human cone photoreceptor inner segments,” Invest. Ophthalmol. Vis. Sci. 55(7), 4244–4251 (2014).
15. D. Scoles, B. P. Higgins, R. F. Cooper, A. M. Dubis, P. Summerfelt, D. V. Weinberg, J. E. Kim, K. E. Stepien, J. Carroll, and A. Dubra, “Microscopic inner retinal hyper-reflective phenotypes in retinal and neurologic disease,” Invest. Ophthalmol. Vis. Sci. 55(7), 4015–4029 (2014).
16. K. Kurokawa, Z. Liu, and D. T. Miller, “Adaptive optics optical coherence tomography angiography for morphometric analysis of choriocapillaris [Invited],” Biomed. Opt. Express 8(3), 1803–1822 (2017).
17. M. Salas, M. Augustin, L. Ginner, A. Kumar, B. Baumann, R. Leitgeb, W. Drexlner, S. Prager, J. Hafner, U. Schmidt-Erfurth, and M. Pircher, “Visualization of micro-capillaries using optical coherence tomography angiography with and without adaptive optics,” Biomed. Opt. Express 8(1), 207–222 (2017).
18. D. T. Miller, O. P. Kocaoglu, Q. Wang, and S. Lee, “Adaptive optics and the eye (super resolution OCT),” Eye (Lond.) 28(3), 321–330 (2011).
19. J. Tam, J. A. Martin, and A. Roorda, “Noninvasive visualization and analysis of parafoveal capillaries in humans,” Invest. Ophthalmol. Vis. Sci. 51(3), 1691–1698 (2010).
20. T. Y. Chui, D. A. Vannasdale, and S. A. Burns, “The use of forward scatter to improve retinal vascular imaging with an adaptive optics scanning laser ophthalmoscope,” Biomed. Opt. Express 3(10), 2537–2549 (2012).
21. E. A. Rossi, C. E. Granger, R. Sharma, Q. Yang, K. Saito, C. Schwarz, S. Walters, K. Nozato, J. Zhang, T. Kawakami, W. Fischer, L. R. Latchney, J. J. Hunter, M. M. Chung, and D. R. Williams, “Imaging individual neurons in the retinal ganglion cell layer of the living eye,” Proc. Natl. Acad. Sci. U.S.A. 114(3), 586–591 (2017).
22. Z. Liu, K. Kurokawa, F. Zhang, J. J. Lee, and D. T. Miller, “Imaging and quantifying ganglion cells and other transparent neurons in the living human retina,” Proc. Natl. Acad. Sci. U.S.A. 114(48), 12803–12808 (2017).
23. O. P. Kocaoglu, B. Cense, R. S. Jonnal, Q. Wang, S. Lee, W. Gao, and D. T. Miller, “Imaging retinal nerve fiber bundles using optical coherence tomography with adaptive optics,” Vision Res. 51(16), 1835–1844 (2011).
24. G. Huang, T. J. Gast, and S. A. Burns, “In vivo adaptive optics imaging of the temporal raphe and its relationship to the optic disc and fovea in the human retina,” Invest. Ophthalmol. Vis. Sci. 55(9), 5952–5961 (2014).
25. Y. Jia, S. Lee, M. J. Ju, M. Heisler, W. Ding, R. J. Zawadzki, S. Bonora, and M. V. Sarunic, “Lens-based wavefront sensorless adaptive optics swept source oct,” Sci. Rep. 6(1), 1–14 (2016).
26. D. X. Hammer, R. D. Ferguson, C. E. Bigelow, N. V. Iftimia, T. E. Ustun, and S. A. Burns, “Adaptive optics scanning laser ophthalmoscope for stabilized retinal imaging,” Opt. Express 14(8), 3354–3367 (2006).
27. 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 ophthalmoscope,” J. Opt. Soc. Am. A 24(5), 1313–1326 (2007).
28. J. Zhang, Q. Yang, K. Saito, K. Nozato, D. R. Williams, and E. A. Rossi, “An adaptive optics imaging system designed for clinical use,” Biomed. Opt. Express 6(6), 2120–2137 (2015).
29. L. W. Sun, R. D. Johnson, V. Williams, P. Summerfelt, A. Dubra, D. V. Weinberg, K. E. Stepien, G. A. Fishman, and J. Carroll, “Multimodal imaging of photoreceptor structure in choroideremia,” PLoS One 11(12), e0175266 (2016).
30. R. J. Zawadzki, S. M. Jones, S. Pilli, S. Balderas-Mata, D. Y. Kim, S. S. Olivier, and J. S. Werner, “Integrated adaptive optics optical coherence tomography and adaptive optics scanning laser ophthalmoscope system for simultaneous cellular resolution in vivo retinal imaging,” Biomed. Opt. Express 2(6), 1674–1686 (2011).
31. D. X. Hammer, R. D. Ferguson, M. Mujat, A. Patel, E. Plumb, N. Iftimia, T. Y. Chui, J. D. Akula, and A. B. Fulton, “Multimodal adaptive optics retinal imager: Design and performance,” J. Opt. Soc. Am. A 24(12), 2598–2607 (2012).
32. A. Meadway, C. A. Girkin, and Y. Zhang, “A dual-modal retinal imaging system with adaptive optics,” Opt. Express 21(24), 29792–29807 (2013).
33. M. Mujat, A. Patel, N. Iftimia, and D. Ferguson, “Compact adaptive optics line scanning retinal imager; closer to the clinic,” Proc. SPIE 8930, 89301B (2014).
34. M. Salas, W. Drexlner, X. Leyeveq, B. Lamory, M. Ritter, S. Prager, J. Hafner, U. Schmidt-Erfurth, and M. Pircher, “Multi-modal adaptive optics system including fundus photography and optical coherence tomography for the clinical setting,” Biomed. Opt. Express 7(5), 1783–1796 (2016).
35. D. Merino, C. Dainty, A. Bradu, and A. G. Podoleanu, “Adaptive optics enhanced simultaneous en-face optical coherence tomography and scanning laser ophthalmoscopy,” Opt. Express 14(8), 3345–3353 (2006).
Outcome assessment owing to tremendous technological advances over the last two decades.

1. Introduction

High-resolution retinal imaging has gained increased use for disease diagnosis and treatment outcome assessment owing to tremendous technological advances over the last two decades. Pivotal in these advances is adaptive optics (AO), which when combined with various ophthalmic imaging modalities, can probe the living retina at the cellular level. Since its first demonstration for ophthalmic imaging [1], AO has been successfully integrated into many imaging modalities, including non-confocal (i.e., flood illumination and detection) fundus photography, confocal scanning laser ophthalmoscopy (SLO), optical coherence tomography (OCT), and one- and two-photon fluorescence imaging, enabling microscopic views of single retinal cells in the living eye [2–4]. To date, many cells and cellular structures across the

36. M. Pircher, R. J. Zawadzki, J. W. Evans, J. S. Werner, and C. K. Hitzenberger, “Simultaneous imaging of human cone mosaic with adaptive optics enhanced scanning laser ophthalmoscopy and high-speed transversal scanning optical coherence tomography,” Opt. Lett. 33(1), 22–24 (2008).
37. A. Dubra and Y. Sulai, “Reflective afocal broadband adaptive optics scanning ophthalmoscope,” Biomed. Opt. Express 2(6), 1757–1768 (2011).
38. E. M. Wells-Gray, S. S. Choi, R. J. Zawadzki, S. C. Finn, C. Greiner, J. S. Werner, and N. Doble, “Volumetric imaging of rod and cone photoreceptor structure with a combined adaptive optics-optical coherence tomography-sclanning laser ophthalmoscope,” J. Biomed. Opt. 23(03), 1–15 (2018).
39. D. A. Atchison and G. Smith, “Chromatic dispersions of the ocular media of human eyes,” J. Opt. Soc. Am. A 22(1), 29–37 (2005).
40. A. Agrawal, T. J. Pfefer, P. D. Woolliams, P. H. Tomlins, and G. Nehmetallah, “Methods to assess sensitivity of optical coherence tomography systems,” Biomed. Opt. Express 8(2), 902–917 (2017).
41. ANSI, Z136.1 – 2014. American National Standard for Safe Use of Lasers. Orlando, FL: Laser Institute of America; (2014).
42. Z. Liu, K. Kurokawa, F. Zhang, and D. T. Miller, “Characterizing motility dynamics in human rpe cells,” Ophthalmic Technologies XXVII 10045, 1004515 (2017).
43. A. Dubra and Z. Harvey, Registration of 2D Images From Fast Scanning Ophthalmic Instruments (Springer, Berlin, Heidelberg. 2010).
44. S. A. Agemy, N. K. Scripsema, C. M. Shah, T. Chui, P. M. Garcia, J. G. Lee, R. C. Gentile, Y. S. Hisao, Q. Zhou, T. Ko, and R. B. Rosen, “Retinal vascular perfusion density mapping using optical coherence tomography angiography in normals and diabetic retinopathy patients,” Retina 35(11), 2353–2363 (2015).
45. C. A. Curcio, K. R. Sloan, R. E. Kalina, and A. E. Hendrickson, “Human photoreceptor topography,” J. Comp. Neurol. 292(4), 497–523 (1990).
46. M. Alpern, C. C. Ching, and K. Kitahara, “The directional sensitivity of retinal rods,” J. Physiol. 343(1), 577–592 (1983).
47. G. Staurenghi, S. Sadda, U. Chakravarty, and R. F. Spaid; International Nomenclature for Optical Coherence Tomography, “Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the in*oct consensus,” Ophthalmology 121(8), 1572–1578 (2014).
48. C. A. Curcio and K. A. Allen, “Topography of ganglion cells in human retina,” J. Comp. Neurol. 300(1), 5–25 (1990).
49. T. Laforest, D. Carpentras, M. Künzi, L. Kowalczuk, F. Behar-Cohen, and C. Moser, “A new microscopy for imaging retinal cells,” arXiv: 1712.08472 (2017).
50. T. Y. Chui, T. J. Gast, and S. A. Burns, “Imaging of vascular wall fine structure in the human retina using adaptive optics scanning laser ophthalmoscopy,” Invest. Ophthalmol. Vis. Sci. 54(10), 7115–7124 (2013).
51. F. LaRocca, D. Nankivil, T. DuBose, C. A. Toth, S. Farsiu, and J. A. Izatt, “In vivo cellular-resolution retinal imaging in infants and children using an ultracompact handheld probe,” Nat. Photonics 10(9), 580–584 (2016).
52. E. A. Rossi, P. Rangel-Fonseca, K. Parkinsons, W. Fischer, L. R. Latchney, M. A. Folwell, D. R. Williams, A. Dubra, and M. M. Chung, “In vivo imaging of retinal pigment epithelium cells in age related macular degeneration,” Biomed. Opt. Express 4(11), 2527–2539 (2013).
53. A. Roorda, Y. Zhang, and J. L. Duncan, “High-resolution in vivo imaging of the RPE mosaic in eyes with retinal disease,” Invest. Ophthalmol. Vis. Sci. 48(5), 2297–2303 (2007).
54. S. G. Adie, B. W. Graf, A. Ahmad, P. S. Carney, and S. A. Boppart, “Computational adaptive optics for broadband optical interferometric tomography of biological tissue,” Proc. Natl. Acad. Sci. U.S.A. 109(19), 7175–7180 (2012).
55. D. Hillmann, H. Spahr, C. Pflügler, H. Sudkamp, G. Franke, and G. Hüttermann, “In vivo optical imaging of physiological responses to photostimulation in human photoreceptors,” Proc. Natl. Acad. Sci. U.S.A. 113(46), 13138–13143 (2016).
56. F. A. South, K. Kurokawa, Z. Liu, Y.-Z. Liu, D. T. Miller, and S. A. Boppart, “Combined hardware and computational optical wavefront correction,” Biomed. Opt. Express 9(6), 2562–2574 (2018).
57. I. Gorczynska, J. V. Migacz, R. Jonnal, R. J. Zawadzki, R. Poddar, and J. S. Werner, “Imaging of the human choroid with a 1.7 MHz a-scan rate FDML swept source OCT system,” Proc. SPIE 10045, 1004510 (2017).
Entire retinal depth have been resolved using different imaging modalities, including retinal pigment epithelium (RPE) [5–9], cones and rods [10–13], cone inner segments [14], Henle’s fiber bundles [15, 16], retinal capillaries [17, 18] and vessel walls [19, 20], retinal ganglion cells (RGC) [21, 22], nerve fiber bundles (NFB) [23–25], and microglia [22]. Each of these AO imaging modalities has unique advantages for resolving particular cell types or retinal features [18], and therefore there has been increased interest in the use of multimodal AO (mAO) systems for a more complete picture of retinal health.

Some of the first attempts to develop mAO instruments resulted in easier scan navigation or stabilization by adding a non-AO wide-field imaging or tracking device to existing AO systems [26–28]. While those approaches improved clinical utility, they did not extend the AO performance achieved using a single AO imaging modality. Many studies used multiple stand-alone imagers, for example, adaptive optics scanning laser ophthalmoscopy (AOSLO) and conventional OCT [29], the former to provide high transverse resolution (2-3 µm) and the latter to provide high axial resolution (3-4 µm). However, because the images were acquired from two separate systems, full interpretation required additional post-processing tasks to register the SLO and OCT images. Moreover, the lateral resolution of the OCT images, acquired without AO, was sub-optimal, and the imaging session duration was necessarily long. To address these problems several investigators integrated different imaging modalities into a single AO beam path [12, 30–32], including prototype devices intended for clinical use [33, 34]. Early attempts to combine AOSLO with adaptive optics optical coherence tomography (AOOCT) typically slowed the OCT B-scan rate to the SLO frame rate, resulting in under-utilization of the OCT mode. Others optimized transverse imaging speed for both modalities but necessarily sacrificed OCT volume rate and axial resolvability due to axial motion artifact [35, 36], the latter issue overcome by use of depth tracking schemes [13]. Those solutions diminished the full capability of the two imaging modalities, and often added unnecessary system complexity with implementation.

In this study, we present a novel design for a mAO retinal imaging system (henceforth FDA mAO), which combines two complementary approaches (AOSLO and AOOCT) in one imager through a simplified optical design with minimized system aberration for the investigation of cells and cellular structures in the living human retina. The system provides optimal imaging performance for each modality and flexible alternation between the two imaging modes with independent focus control. The FDA mAO system performance is demonstrated by imaging cells across the entire retinal depth on three healthy subjects. The multimodal approach provides a platform to study retinal physiological and structural properties in both healthy and pathological eyes, paving the way to assess new therapeutic treatment outcomes.

2. Material and methods

2.1 Description of the FDA multimodal AO system

The FDA mAO system, which combines AOSLO and AOOCT channels in a single instrument, was designed with Zemax optical design software (Zemax LLC, Washington, USA). The system provides optimized optical performance and flexibility for joint or independent operation of the two imaging modes. Figure 1 shows a schematic of the FDA mAO system. The primary beam path (and OCT sample arm) consists of four pairs of afocal telescopes. The telescopes are configured for out-of-plane operation to compensate system astigmatism [27, 37], which arises from off-axis use of spherical mirrors (SM) and is known to degrade closed-loop AO performance. The afocal telescopes conjugate the pupil of the eye with the system active components, including the Shack-Hartmann Wavefront Sensor (SHWS) lenslet array, the deformable mirror (DM, ALPAO, France), the resonant scanner (EOPC, New York NY USA), and the galvanometer scanners (Thorlabs, Newton NJ USA). SM focal length is an attribute that represents a trade-off in the system design: long focal length SMs with small rotational angles are favorable for minimizing off-axis astigmatism.
[37], while shorter focal length SMs create a more compact instrument and greater OCT stability from a shorter reference arm optical path length. Here we implemented a relatively compact design where the SMs, labeled from 1 to 8, have focal lengths ($f$) from 150 mm to 375 mm with incident beam angles ($I_x, I_y$) of 2.2° to 5°. The total length of the beam path is 4.15 m. The system is designed to produce a 6.7-mm diameter beam at the eye and a 3.6° × 3.6° (1.08 × 1.08 mm²) maximum retinal field of view (FOV). Additional system details are provided in the figure caption and Table 1. The compact optical setup (sample arm) has a footprint of 38 in. (wide) × 20 in. (depth) (0.95 m × 0.50 m).

Fig. 1. Schematic of the FDA mAO retinal imaging system (flattened for clarity). AL: adaptive lens, APD: avalanche photodiode, D1-3: dichroic beamsplitters, DG: diffraction grating, DM: deformable mirror, GS: G: galvanometer scanners, I: iris, P: pinhole, PBS: pellicle beamsplitter, RS: resonant scanner, SHWS: Shack-Hartmann Wavefront Sensor, SM1-8: spherical mirrors, TS: translation stage.

The AOOCT imaging beam ($\lambda_c = 830$ nm, $\Delta\lambda = 60$ nm) is produced with a superluminescent diode (SLD, D-840-HP-1, Superlum, Ireland), which also serves as the SHWS beacon. The AOSLO imaging beam ($\lambda_c = 756$ nm, $\Delta\lambda = 20$ nm) is also produced with an SLD (Exalos, Schlieren Switzerland). The AOSLO and AOOCT imaging beams are combined and split using two custom-designed high-performance, high-efficiency (transmission >98%) dichroic beam splitters D1 and D2 (Semrock, Rochester NY USA). The novel optical configuration and symmetric placement of D2, GS, and RS splits the beams to their respective scanners to match channel optical path length, provide collinear and coincident travel, and minimize non-common path aberration differences between channels. The AOSLO and AOOCT imaging beams are estimated to have theoretical transverse confocal resolution of 1.7 and 1.8 μm, respectively, in an eye with a 6.7 mm pupil. The AOOCT axial resolution in tissue ($n = 1.38$) is estimated by the bandwidth to be 3.7 μm. Collinear alignment was achieved by carefully minimizing the beam offset (centroids of the beams) between the two imaging channels at both pupil and image planes using a pupil-retina camera. This alignment procedure was conducted at two planes where the beams are split and recombined, between D1 and SM1 and between D2 and SM3. The AOSLO light back-
scattered from the eye is split with a 70/30 beamsplitter (BS) with 70% directed toward an avalanche photodiode (APD, Hamamatsu Photonics K.K., Japan). A pellicle beamsplitter (PBS) directs 92% of the AOCT light to a high-speed, high-performance spectrometer (Cobra-S 800, Wasatch Photonics Inc., Durham NC USA), and 8% to the SHWS. A telescope (75 and 45 mm focal length achromats) demagnifies the WS beam from 10 mm to 6 mm to fit the SH camera chip (UNIQ Vision Inc., Santa Clara CA USA), and an iris is placed in the focal plane of the telescope to reject corneal reflections. Wavefront measurements are obtained with a SHWS (40 × 40 lenslet array, 250-μm pitch). An adaptive lens (AL, Optotune, Edmund Optics, Barrington NJ USA) is placed in the SLO path to provide independent AOSLO focus control (see Section 2.2 for details). An organic light-emitting diode (OLED) microdisplay (DSVGA, eMagine, NY) is used for fixation. The microdisplay image (cross target on a black background) is projected onto the retina with a Badal lens relay. The microdisplay and one lens of the relay are mounted on a computer-controlled translation stage, which is adjusted to compensate for the subject’s refractive error (range: +5 to −10D).

Table 1. FDA multi-modal AO sample arm

| Optical element | f (mm) | \( I_x \) (deg) | \( I_y \) (deg) | D (mm) |
|----------------|--------|----------------|----------------|--------|
| Imaging Beam   | 10.0   |                |                |        |
| SM1            | 375    | 2.20           | 0.00           |        |
| SM2            | 150    | −5.00          | 0.00           |        |
| HS (Gx & RSh)  | 13.00  | 0.00           | 4.0            |        |
| SM3            | 200    | −5.00          | 0.00           |        |
| SM4            | 200    | 0.00           | −4.98          |        |
| Gv             | −7.50  | 8.30           | 4.0            |        |
| SM5            | 150    | 0.00           | −3.45          |        |
| SM6            | 250    | −4.44          | 0.00           |        |
| DM             | 6.80   | −4.00          | 6.7            |        |
| SM7            | 375    | 0.00           | 4.07           |        |
| SM8            | 375    | 4.07           | 0.00           |        |
| Eye pupil plane|        |                |                | 6.7    |

\( f = \text{focal length}, \ I_x, \ I_y = \text{beam angles}, \ D = \text{beam diameter} \)

2.2 System control and electronics

FDA mAO system control is accomplished with a single host personal computer (PC), running two programs: AO control software and image acquisition software. The AO control software collect and displays the SHWS camera images, calculates wavefront spot centroids and slopes, performs AO closed-loop control, and controls the DM and AL. It also calculates the Zernike coefficients and wavefront aberration for real-time display and provides autofocus and preset focus settings for the DM and AL. The image acquisition software collects and displays in real-time the OCT and SLO images, sets the field size via scanner (galvanometer and resonant scanner) control, and operates the fixation target (FT). The system PC uses three framegrabbbers (PCIe-1430 and PCIe-1433, National Instruments Inc., Austin TX USA and Solios eA/XA, Matrox Electronic Systems Ltd, Dorval, Quebec, Canada) to collect the SLO, OCT, and WS images and two data acquisition cards (PCIe-6363 and USB-6259, National Instruments Inc.) to process galvanometer and resonant scanner position and drive waveform signals. The DM and FT stage communicate with the host PC via USB.

Custom control, image and signal processing, user interface, and analysis software for the FDA mAO system was written in LabVIEW (National Instruments Inc., Austin TX USA), MATLAB (Mathworks Inc., Natick MA USA), and C/C++. Three programs were developed to use the video card graphical processing unit (GPU, GeForce GTX-760, NVIDIA, Santa Clara CA USA) via the Compute Unified Device Architecture (CUDA) parallel programming platform for OCT image processing, WS spot centroiding, and SLO image de-warping, all
performed in real-time. The system is designed to operate in ‘slow scan’ or ‘fast scan’ modes by a single selection in the user interface. The WS camera operates at 10 Hz, and real-time GPU-based spot centroiding allows a closed-loop dynamic ocular aberration correction bandwidth of several Hz.

2.2.1 Simultaneous AOSLO/OCT imaging: slow scan mode

The ‘slow scan’ mode operates similarly to previously published multimodal AO systems [30, 31, 38], in which the SLO is the primary imaging modality. Simultaneous SLO/OCT imaging is achieved with each OCT B-Scan synchronized to every SLO raster scan, as depicted in Fig. 2. Because the SLO RS rate is fixed, all timing and image frame rates are derived from the resonant scanner frequency. The SLO RS used in the FDA mAO system has a resonant frequency of 13.5 kHz, the SLO frame rate operates at 27 Hz (500 × 500 pixels), and the OCT A-line acquisition speed is matched to the RS frequency. Because the SLO image y-axis and OCT B-scan (1024×500 pixels) are produced using the same scanner (Gv), the beams are always precisely registered, within the limit of the optical alignment (i.e., collinearity of SLO and OCT beams) and transverse chromatic aberration. Furthermore, OCT volumetric scans can be created by sweeping the second galvanometer (Gh) across a retinal patch, the size of which can either be matched to the SLO image size or different. Due to the slow OCT scan speed (set and limited by the SLO resonant scanner frequency), eye motion results in gaps in the OCT en face images [38]. To fill the gaps requires acquisition of multiple OCT volumes.

Although the SLO and OCT channels are locked in terms of acquisition timing and vertical position, independent SLO focus control is accomplished with a tunable adaptive lens (AL), placed immediately in front of the SLO collimator (see Fig. 1). The AL focus range is −1.5 to 3.5D, which is sufficient to traverse the entire retinal thickness. The independent focus control feature permits navigation and frame rate display of structure of interests with SLO. It also allows correction of the longitudinal chromatic aberration (LCA) between 760 nm (SLO) and 830 nm (OCT) imaging wavelengths and the LCA variation across population [39].

2.2.2 Volumetric AOOCT imaging: fast scan mode

The ‘fast scan’ mode takes full advantage of high-speed OCT imaging capabilities by maximizing the OCT A-line acquisition speed up to 210 kHz for the FDA mAO system. The fast acquisition speed is achieved by reading out the central 1024 pixels of the spectrometer in 8-tap, 10-bit camera mode (limited by the NI framegrabber). The measured spectrometer sensitivity and roll-off were 83 dB and −9.9 dB/mm (−7.7 dB/mm up to 1 mm depth), following the method reported by Agrawal [40]. The SLO channel is not active in ‘fast scan’ mode. For the applications shown below in Section 3, OCT volumes of 300 × 300 lateral...
pixels were collected covering a 1.5° × 1.5° FOV at a volume rate of 2.3 Hz. System focus through retinal layers was achieved with the DM.

2.3 Experimental design

FDA mAO retinal imaging performance was demonstrated in healthy human subjects. All human subject procedures were approved by the FDA Institutional Review Board and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained after potential risk explained to each subject.

Retinal locations between the fovea and 12° retinal eccentricity were recorded in the right eye of three subjects with ages from 23 to 33 years old (S1: 31, S2: 33, S3: 23 years old). All subjects had best corrected visual acuity of 20/20 or better and were free of ocular disease. The AOSLO and AOOCT beam power at the cornea were measured to be 200 and 420 μW, respectively, and were within safe limits established by American National Standards Institute (ANSI) [41] for the retinal illumination pattern and length of the experiment used. The right eye was cyclopleged and dilated with Tropicamide 0.5%. The eye and head were aligned and stabilized using a chin and head rest attached to manually-driven XYZ patient translation stage.

Two tests were conducted to assess the FDA mAO system imaging performance with dynamic AO correction. First, SLO and OCT images were collected while the system operated in ‘slow scan’ mode, primarily to characterize SLO image quality. SLO/OCT videos (100-200 SLO frames and OCT B-scans) were acquired with a FOV between 0.75° × 0.75° and 2° × 2° of the photoreceptor or retinal capillary network at multiple retinal locations with AO best focus set to the corresponding layer. Pinholes with 1 and 8 Airy disc diameter were used respectively for photoreceptor and capillary imaging. SLO videos were also collected using the two independent focus control methods (AL vs. DM). The second test was designed to assess OCT imaging quality in ‘fast scan’ mode. For this test, 40 OCT videos with 3 volumes/video (900 total B-scans) were collected at an eccentricity 7° temporal to the fovea in two subjects with the focus set to the photoreceptor-RPE complex. This test examines the ability of the FDA mAO system to resolve retinal structures with tight physical axial separation. It has been demonstrated that individual RPE cells can be resolved by reducing speckle noise from the OCT en face image [7]. To capture the temporal dynamics of RPE cell organelle motility with speckle decorrelation, the OCT volume sets were collected with a separation of at least 3 s (typically ~5 s) [42]. One best volume was selected from each of the 40 OCT videos for post processing to produce the averaged RPE image. To assess the FDA mAO system’s ability to detect weakly light scattered cells such as RGC and other fine granular structures in the inner retina, another 40 videos with 5 volumes/video (1500 total B-scans) were also acquired at 12° temporal to the fovea on two subjects with system focus set to the inner retina just below the nerve fiber layer (NFL). Volumes with blinks or significant eye motion artifact were excluded for post processing.

Depending upon the retinal target, patient imaging sessions lasted about an hour, including initial alignment and frequent rests for patient comfort. SLO image collection (e.g., photoreceptor and capillary network mosaics) took ~30 minutes to complete, while RPE and RGC imaging (collection of 40 volumes) each took ~15 minutes to complete.

2.4 Post processing and analysis

Improved image signal-to-noise ratio (SNR) can be achieved by averaging multiple images collected from the same retinal location. However, eye motion is generally several times larger than the retinal structures under investigation. Therefore, to achieve optimal results, eye motion must be corrected. We achieved registration of image sequences by applying a 2-D strip-wise registration approach [43] for SLO images, and a 3-D registration algorithm for OCT volumes [22]. Reference images/volumes were manually selected according to criteria including retinal structure sharpness, minimal eye movement artifacts, and common overlap.
with other registered images. After registration, averaged or variation intensity images (of the capillaries) were generated for further data analysis to extract morphological parameters, such as cell density, cell size, foveal avascular zone (FAZ) area, capillary density, and tortuosity. Cell density was calculated using a Voronoi analysis approach [7, 22].

3. Results

3.1 Predicted system performance

Figure 3 summarizes the predicted system optical performance for scan angles across the system’s 3.6° × 3.6° FOV without AO correction (i.e., with flattened DM) at 830 nm. The left graph illustrates the system image quality at the eye. All rays fall inside the solid circle denoting the diffraction-limited blur size. The corresponding wavefront RMS error is below diffraction criteria (λ/14) across the entire FOV. The right graph shows the predicted beam displacement of the system at two critical pupil conjugate planes: the DM and the eye pupil. The beam displacement (0.023 ± 0.016 mm) is at least 10 × smaller than the SH lenslet pitch (0.28 mm) at these two planes. The fact the beam displacement at the eye plane is similar to the upstream DM plane indicates minimal aberration accumulation in the optics between the two planes.

![Fig. 3. Predicted system optical performance. PSF spot diagrams at the retina (left) with flattened DM. The solid circles denote diffraction-limited blur size. Beam displacement at DM and eye pupil planes (right) for ± 1.8° vertical (V) and horizontal (H) scans. Dashed line represents lenslet pitch at DM and eye pupil planes.](image)

3.2 Microscopic imaging of inner and outer retina with the ‘slow scan’ mode

To directly validate the FDA mAO imaging system performance in ‘slow scan’ mode, where SLO is the primary imaging modality, images were acquired of inner and outer retinal structures. To assess SLO transverse resolution, foveal cones, peripheral rods and retinal capillaries were imaged. Overlapping AOSLO videos in the macula were used to generate montages of retinal capillaries (perfusion map, Fig. 4(B)) and photoreceptors (Fig. 4(C)).

Macula perfusion maps with enhanced dynamic flow contrast and suppressed static background were generated by computing the intensity variation of registered image stacks following the motion contrast enhancement approach reported by Tam et al. [19]. Macula cone images were calculated by registering and averaging image stacks. One conventional clinical view, the Spectralis SLO image (Fig. 4(A)), shows only the shadows of large blood vessels, while the AOSLO perfusion map collected from the same region provides much more detailed visualization of all vessels, even the smallest retinal capillaries. Individual erythrocytes can be identified flowing through capillaries in the recorded video (Visualization 1). Clinical evidence shows the small capillaries are more prone to be sites of disease initiation and development compared to the larger vessels [44].
Fig. 4. Cellular structures of the center macula using AOSLO. (A) Red square in subject S2 denotes location imaged with AOSLO. (B) Montage of the macula vessels by 3x3 overlapping 2° FOV AOSLO videos reveals both the big blood vessels and smallest capillaries. (*) denotes the center of FAZ. Scale bar in (B) also applies to (C). The photoreceptor mosaic in (C) was generated by shifting the system focus to the photoreceptor layer, and a 1 airy disc pinhole was used for confocal imaging. Cones at foveal center labeled as yellow box in (C) is showed in zoomed in view in (D). Simultaneous collected AOOCT single B-Scan shows distinct retinal layers in (E). The OCT image is displayed in logarithmic scale. Keys: ILM: inner limiting membrane; NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: outer nuclear layer; ELM: external limiting membrane; ISOS: inner segment/outer segment junction; COST: cone outer segment tip; and RPE: retinal pigment epithelium.

The perfusion maps were further processed to quantify capillary morphological characteristics, including FAZ area, capillary density (vessel pixels / total pixels), and capillary tortuosity (vessel branch length / Euclidean distance). The following processing steps were applied to the perfusion maps: contrast stretch, threshold, median filter, particle filter, skeletonization, and Watershed transform in LabVIEW. The FAZ area was calculated after Watershed transform. The density was calculated after the particle filter on the thresholded perfusion maps. The tortuosity was calculated from the skeletonized image in ImageJ (‘Analyze skeleton’). Quantification of density and tortuosity was done on four
equally sized quadrants about the center of macula, which is identified as the FAZ center. For two imaged subjects, the FAZ area was measured to be 0.308 and 0.367 mm² (3.64 and 4.33 deg²), in line with previous reports for healthy eyes [19, 20]. The capillary densities for the two subjects for the entire perfusion maps were measured to be 0.295 and 0.314 and for the four quadrants were measured to be 0.268 and 0.332 (temporal: T), 0.340 and 0.304 (nasal: N), 0.220 and 0.308 (superior: S) and 0.352 and 0.308 (inferior: I). Some differences across quadrants and between subjects may be attributed to AO image quality, as it is not expected that there be significant regions of non-perfusion in healthy eyes. The differences were generally in the 10-20% range. The tortuosity (avg ± std) measured in the two subjects was 1.114 ± 0.168 (2134 branches) and 1.111 ± 0.112 (2136 branches). Only small differences were measured between the four quadrants. Some observed differences may relate to how each subject’s pupil was aligned in the system.

In the same retinal region (centered on the fovea), with system focus set to the outer retina, cone photoreceptors, including those most densely packed at the foveal center, are clearly resolved (Fig. 4(C, D)). The high resolution cross-sectional view of the retinal layers at foveal center was achieved by simultaneous AOOCT imaging, shown in Fig. 4(E). The foveal cone mosaics on an additional two subjects are shown in Fig. 5. The Voronoi maps were generated using cone cell centers that were identified semi-automatically in the AOSLO images (similar to a previously described method [7]). The measured cone densities for a region-of-interest sized 0.5° were 129, 121 and 123 × 1000 cells/mm² for the three subjects. The values are similar to those reported in post-mortem histological measurements [45]. The resolution of foveal cones may also be feasible with the AOOCT channel using dense spatial sampling and high volume rates to overcome eye motion artifact.

![Foveal cone mosaics from three subjects imaged with AOSLO. Cones are resolved across the fovea in all three subjects (top row). Cones are identified with semi-automatic software and the resultant cone locations (dots) and Voronoi maps calculated (second row).](image)

Although complete foveal cone mosaics were successfully resolved in all three subjects, demonstrating the system capability to resolve fine spatial details, the peripheral rod mosaic is more challenging to image, despite cells of similar dimension. The difficulties are imposed
not only by the constrained spatial arrangement (2-3 μm separation) of the rod photoreceptor mosaic [10, 11, 45], but also the broader acceptance angle (Stiles-Crawford) of the rod photoreceptors [46], which makes them much dimmer than the surrounding cone photoreceptors. A complete rod mosaic over a patch of retina may require collecting image sets with large temporal separation (i.e., hours between sets) to reduce reflectivity differences [10] or using shorter wavelengths for better transverse resolution [10, 12], approaches not tested in the current study (single videos collected at each retinal location using 760 nm light). Nevertheless, the granular appearance of the cone and rod photoreceptors are evident in the SLO image and OCT cross-sectional views in Fig. 6.

Fig. 6. Photoreceptor mosaics in peripheral retina using AOSLO. (A) Red square at 7.5°-10° temporal to the fovea in subject S2 denotes location imaged with AOSLO. (B) Montage of the cone and rods by 4x4 overlapping 0.75° FOV AOSLO videos. (C) Simultaneous collected AOOCT B-Scan at the same patch of retina indicated as green dashed line in (B) shows paired hyper-reflections that originate from the segments of the photoreceptors in the outer retina. Photoreceptor mosaics at single location (12° temporal retina) (D) in S1, and (E) in S3. Scale bar in (D) also applies for (E). AOSLO images are displayed with logarithmic intensity, and the AOOCT B-scan is displayed with linear scale.

In general, AO system focus control is required to target different retinal structures and layers in depth. The FDA mAO system permits flexible navigation and display of the microscopic retinal features at a specific SLO focus plane, independent from the cross-sectional OCT image obtained at a different plane. This independent focus control is achieved
by use of an adaptive lens (AL), placed immediately after the SLO beam collimator (see Fig. 1). With simultaneous focus control by the DM, the SLO and OCT channels share the same system focus. Visualization 2 shows SLO-OCT locked focus shift from outer to inner retina using the DM. In contrast, independent focus control with the AL in Visualization 3 shows SLO focus shifted from photoreceptors to NFL with OCT focus held at the outer retina. Figure 7 contains two frames from each of those two videos. The static SLO images in Fig. 7 present detailed views as the focus (A: DM, B: AL) is shifted from the outer retina (left) to the inner retina (right). Note the reflectance shifts from the photoreceptors to the nerve fibers and red blood cells flowing through vessels. The OCT images in Fig. 7(A) shows a simultaneous focus shift using the DM, while in Fig. 7(B), the AL only shifts the SLO. Besides independent focus navigation, use of the AL provides two additional advantages: first to overcome the LCA difference between the two imaging channels, and second to provide more reliable SH spots while imaging different depths, primarily offered by waveguided light of photoreceptors and multiple scattered light of RPEs, which empirically results in more stable AO performance.

However, because the AL precedes all telescopes (see Fig. 1.), any alignment imprecision (i.e., AL lateral position with respect to the OCT chief ray) may cause a deviation between SLO and OCT beams, resulting in degraded image quality and additional AL defocus. This imperfection is evident in Visualization 3, when system focus (DM focus) is set in the outer retina, and an AL focal shift from outer to inner retina causes an intensity decrease and loss of confocality compared to the Visualization 2 with DM focus control.

![AOSLO focus control by DM](image1)

![AOSLO focus control by AL](image2)

Fig. 7. Simultaneous imaging with independent focus control of the FDA mAO system (Visualization 2 and Visualization 3) on subject S2 at 4° inferior and temporal to the fovea shows cellular details across the thickness of retina. (A) Simultaneous AOSLO and AOCT focus controlled by DM, and (B) independent AOSLO focus control by AL. White arrows indicate the estimated focus plane in depth.

### 3.3 Microscopic imaging of human inner and outer retina with ‘fast scan’ mode

The ‘fast scan’ mode is designed to take full advantage of AO-OCT capabilities for high-speed, high-sensitivity, high isotropic resolution volumetric imaging. Unlike the highly reflective retinal structures (cones, rods, blood vessel walls, nerve fiber bundles, etc.), other retinal cells, such as RPEs and RGCs, are more difficult to visualize in the healthy living human retina [5–7, 13, 21, 22]. This challenge arises from the fundamental optical properties
and organization of retinal cell layers. Melanin and melanosomes in RPE cells make them highly scattering at near-infrared wavelengths, however their tight anatomical arrangement with overlying photoreceptors in the outer retina imposes difficulties for axial discrimination. The RGC cells, on the other hand, are mostly transparent, and have smaller complex refractive index differences compared to the adjacent retinal substrate and thus their weakly scattered light is more difficult to detect. To assess the AO OCT channel capability, we captured AO OCT videos from both the outer and inner retina of two subjects.

Figure 8 shows the AO OCT images from the photoreceptor-RPE complex. The averaged AO OCT B-scan (Fig. 8(A)) shows four distinct hyper-reflective bands in the outer retina corresponding to the inner segment/outer segment junction (IS/OS), cone outer segment tip (COST), the rod outer segment tip (ROST), and RPE layers. The latter two layers are often not separated in conventional OCT and are identified as a single band by the international Nomenclature for Optical Coherence Tomography Panel [47]. The segmented photoreceptor en face image (IS/OS + COST) in Fig. 8(B) shows cone mosaics that are similar to those collected with AOSLO at perifoveal locations (see for example, Fig. 4(C)). Eye motion is more pronounced in the OCT en face image than the SLO frame due to the slower volume rate of 2.3 Hz (vs. SLO frame rate of 27 Hz). The underlying RPE cells exhibit a different spatial arrangement as evidenced by the clear separation in the 2-D power spectra in Fig. 8(E). The cell densities for two subjects were calculated after performing manual cell identification (Fig. 8(D)) and were 12,208 and 9,939 cells/mm² for cones, and 4262 and 5121 cells/mm² for RPE cells, yielding a cone-to-RPE ratio of 2.84 and 1.94, consistent with previous literature reports [7, 9, 45]. Although the axial resolution allows segmentation of the rod signal (i.e., the ROST band), visualization of individual rod photoreceptors was not achieved due to the coarse pixel sampling (1.5 μm/pixel) chosen to tradeoff eye motion.

![Figure 8](image)

Fig. 8. AO OCT cross-sectional and en face images extracted from the photoreceptor-RPE complex in S1 at 7° temporal retina. Total 40 volumes are averaged. (A) Averaged B-scan and corresponding A-scan profile reveal distinct reflectance bands corresponding to IS/OS, COST, ROST, and RPE layers. En face projection shows mosaic of (B) cones, and (C) RPEs. The cell locations were identified in (D) where yellow dots denote cone centers and cyan denotes the RPE Voronoi map. Nonlinear scanner artifact is not corrected in (D)-(E). (E) 2-D power spectra of (B) and (C) are superimposed and color coded (cones: yellow; RPE cells: cyan).

Ganglion cells are one of five primary cell types of retinal neural circuitry, but their high transparency and dense 3-D packing make them elusive to observe in the living human eye. Recently, AO OCT was used to reveal not only the RGC somas through the entire depth of ganglion cell layer (GCL), but also their 3-D packing geometry, primary subtypes, and spatial organization with respect to other retinal cell types [22]. This progress portends great promise for improved diagnosis and treatment of glaucoma, a highly prevalent family of diseases that
affects ganglion cells and their axons during disease progression. Here we demonstrate similar resolution of the RGC layer with the FDA mAO system, albeit with slightly longer imaging wavelength (830 nm compared to earlier reports at 790 nm wavelength [22]).

(A) AOOCT volume. (B) AOOCT B-Scan. (C) ILM. (D) NFL. (E) GCL. (F) IPL-INL. (G) INL-OPL. (H) Composite.

Fig. 9. Inner retina cells and structures imaged with averaged of 154 AOOCT volumes (Visualization 4). (A) Three-dimensional perspective of a registered and averaged AOOCT volume at 12° temporal to the fovea in subject S1, where green dashed line denotes corresponding cross-section shown in (B). Red arrow indicates same GCL soma in B and E. Scale bar in C also applies to D–E. (C) Star-like microglial cells sparsely cover the surface of the ILM. (D) A complex web of nerve fiber bundles of varying size project across the NFL. Some have a diameter as large as 30 μm (blue arrow). Others are as small as 3 μm (green arrow), which matches the known caliber of a single large GC axon. GCL somas appear between the overlying bundles (black arrow). (E) A mosaic of GCL somas of varying size tile the layer. Red arrow points to a large soma, thought to be a parasol RGC. Additional projection views of capillary networks are showed in (F and G) at IPL-INL and INL-OPL layer respectively. (H) Composition of (C, E-G) with segmented features of interest shows the spatial arrangement of retinal structure at different depths.
The rich tapestry of neurons, glia, and blood vessels in the inner retina were all observed in our data sets. The cross-sectional view illustrates a single neuron identified with the GCL (Fig. 9(B), red arrow). At this eccentricity (12° T) the GC somas are organized in a monolayer. *En face* projections provide a detailed view of other inner retina features of interest, including microglia cells and their processes at the ILM (Fig. 9(C)), NFBs and individual GC axons (Fig. 9(D)), and the GCL soma mosaic, where different characteristic sizes and reflectance differentiate RGC subtypes (Fig. 9(E)). The averaged AO OCT volume shows clear delineation of three layers of retinal vessels (Fig. 9(E-G)), which are often visualized by OCT angiography techniques (collection of multiple B-scans at each lateral location) for higher contrast [17]. The *en face* fly-through for this subject is shown in Visualization 4. The soma density and diameter were quantified in the GCL for two subjects and found to be 4252 and 3592 cells/mm² (with subtraction of predicted Amacrine cell population [48]) and 14.57 ± 2.95 and 14.71 ± 3.28 µm, in agreement with previously reported *in vivo* human results [22] and histological measurements [48].

4. Discussion

The promise of adaptive optics has been more than adequately attained since its first demonstration imaging cone photoreceptors more than twenty years ago [1]. With it, various researchers have resolved for the first time in the live human eye, rod photoreceptor cells [10–12], cone photoreceptor inner segments [14], the retinal pigment epithelial cell mosaic [5–8, 13, 49], NFBs [23, 24], retinal capillaries, vascular mural cells and wall structure [20, 50], and most recently, microglia [22], retinal ganglion cells [35], and the RGC mosaic [22]. Often this was accomplished with elegant new methodology, for example detection of light multiply scattered outside the confocal pinhole [6, 14, 21]. Other times, cells were detected by the compound benefit to contrast realized by careful correction of eye motion to micron and sub-micron precision along with image averaging on a massive scale [5, 8, 10]. Parallel imaging feats have also been accomplished without adaptive optics [51]. But it should be clear by now that a state-of-the-art AO imager makes these accomplishments easier to achieve as well as to expand the patient population in which they can occur.

SLO and OCT, in all of their various incarnations, remain fundamentally better suited at different imaging tasks. And this is why they are complementary when married in a multimodal instrument such as the one demonstrated herein. SLO is a confocal technique but retains immense flexibility in the scope and manner in which scattered light is blocked or detected. It is therefore better suited to detection of light from waveguiding photoreceptors or structural boundaries and interfaces where complex refractive index differentials are highest allowing multiple photon scatter. OCT is hyper-confocal and detects predominantly singly scattered photons. However, its axial resolution is uncoupled from its numerical aperture, allowing micron-scale depth sectioning by use of high bandwidth sources. In recent years, the benefit in the *en face* plane has become more profound as technological developments of new sources have enabled volumetric acquisition speeds at close to video rates (~15-30 volumes/s). These benefits have made OCT better suited to detection of light from cells tightly packed in depth and those with high transparency, particularly in the inner retina. The truth of this statement is demonstrated by the fact that both RPEs and RGCs reside directly beneath highly reflective layers, and yet they are more easily resolved with OCT using AO, robust 3-D registration, and image averaging. With intrinsic contrast, the monolayer of RGL somas are resolvable over a retinal area with little or no overlying NFL with an AOSLO [21] and RPE mosaics only with use of (auto-)fluorescence [5, 9, 52], or under restricted conditions where the overlying photoreceptors are absent due to disease [53], or in the central fovea where the signal leakage from other cells is eliminated [6]. However, in most cases, the RPE and RGC mosaics are more readily, directly, and easily visualized with AOOCT. Moreover, because OCT collects the full complex interferometric spectrum, phase-based techniques can be further exploited to improve contrast (e.g., computational AO) [54–56].
The time when OCT line rates match SLO scan rates is already upon us [57]. Despite these advancements in imaging speed, OCT remains less flexible in detection of multiply scattered light, the contrast benefits of which we are only beginning to realize. Thus for a more complete in vivo cellular survey of the retina, the two modalities function on complementary terms.

Our objective in the development of the FDA mAO system was to demonstrate that all of the retinal cells and structures imaged in live human eyes reported previously, including foveal cones, peripheral rods, RPE, RGC, microglia, capillaries, etc., could be resolved with one imager. As the results demonstrate, we have gone a long way toward completing this goal. However, there are some limitations that have yet to be overcome. While the A-line acquisition rate of 210 kHz was sufficient to collect OCT volumes at 2.3 Hz for RPE and RGC imaging, the OCT speed was still too slow that it placed constraints on the optical design and system functionality. A MHz source (e.g., FDML laser) would allow a more elegant optical design with fewer scanners (i.e., a single resonant scanner for both OCT and SLO) and also simultaneous collection of SLO frames and OCT volumes at high speeds (i.e., video rates). The current design is an improvement over previous multimodal designs [12, 30, 31] because it allows collection of high speed OCT volumes (‘fast scan mode’), but it still falls short of its full potential because it does this without acquisition of SLO images. Implementation of a multimodal AOSLO-AOOCT system whose OCT source has a A-scan rate as fast as the SLO pixel clock (~10 MHz) will allow more optimal operation.

Despite the rapid technological advancements and great clinical potential for early diagnosis and treatment outcome assessment, AO has yet to achieve full clinical translation. The establishment of a collaborative research project in AO between FDA and NIH is intended to help foster the clinical translation of this important ophthalmic imaging technology. Both the FDA and NIH are responsible in various ways (FDA as part of our regulatory science program and NIH in early stage and translational funding as well as through its intramural research program, among other efforts) to help provide greater access for patients to novel technologies that are proven safe and effective. The FDA mAO will serve as a platform for future studies to develop new methodology for improved clinical utility and to increase our understanding of visual physiology and eye disease.

**Funding**

This work was partially funded by a grant from the FDA Critical Path Initiative and the intramural research program of the National Institutes of Health, National Eye Institute. Dr. Saeedi is supported by an NIH Career Development Award (K23EY025014).

**Acknowledgments**

We thank Donald Miller (Indiana University School of Optometry) for use of OCT 3-D registration software and diagnostic hardware; Alfredo Dubra (Stanford University) for use of SLO registration software; Ethan Cohen (FDA) for use of high-speed spectrometer; and Ankit Patel, R. Daniel Ferguson, Mircea Mujat, and Nicusor Ifimia (Physical Sciences Inc.) for use of GPU centroiding software. We thank Anant Agrawal and Nikita Kedia (FDA) for technical assistance and Victor Krauthamer (FDA) for mentorship.

**Disclaimer:** The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the US Department of Health and Human Services.

**Disclosures**

The authors declare that there are no conflicts of interest related to this article.