Conclusions: The sparse array of cones little affected by the bleach is consistent with the S cone mosaic in density and distribution. TPF kinetics differed from existing pigment regeneration models but were qualitatively consistent with the expected production and removal of retinoids later in the visual cycle. While TPF from other molecules qualitatively consistent with the expected production and removal of retinoids later in the visual cycle. While TPF from other molecules kinetics differed from existing pigment regeneration models but were consistent with the S cone mosaic in density and distribution. TPF during dark adaptation were different. Rod TPF during dark adaptation decreased exponentially with time constants ranging from 1.5-4 min. Cone TPF during dark adaptation showed an overall decrease, but was non-monotonic. Rod and cone TPF kinetics during light adaptation were different. Rod TPF trends strongly depended on initially available photopigment (IAP). For 0-15 % IAP (0-2 min after bleach), rod TPF decreased monotonically, and eventually plateaued. For 40-100 % IAP (>5 min after bleach), rod TPF always increased to a plateau. Cone TPF trends strongly depended on initially available photopigment (IAP). To generate dark adaptation curves, initial TPF values were plotted as a function of time in the dark after bleach. To investigate light adaptation to the TPF excitation beam, TPF was tracked over 2 min for different IAP states.

Results: During dark adaptation, rod TPF decreased exponentially with time constants ranging from 1.5-4 min. Cone TPF during dark adaptation showed an overall decrease, but was non-monotonic. Rod and cone TPF kinetics during light adaptation were different. Rod TPF trends strongly depended on initially available photopigment (IAP). For 0-15 % IAP (0-2 min after bleach), rod TPF decreased monotonically, and eventually plateaued. For 40-100 % IAP (>5 min after bleach), rod TPF always increased to a plateau. Cone TPF trends during light adaptation were consistent across all IAP conditions. Cone TPF increased to a peak within 5-15 s and then declined to a plateau. Approximately 5-8 % of the cones forming a fairly regular, sparse mosaic showed little or no increase in TPF following the bleach.

Conclusions: The sparse array of cones little affected by the bleach is consistent with the S cone mosaic in density and distribution. TPF kinetics differed from existing pigment regeneration models but were qualitatively consistent with the expected production and removal of retinoids later in the visual cycle. While TPF from other molecules cannot be excluded, these measurements are probably dominated by the kinetics of retinoids during light and dark adaptation, providing a way to quantify stages of the rod photoreceptor cycle that have been otherwise inaccessible in the living primate eye.

Commercial Relationships: Robin Sharma, Polgenix Inc (F), University of Rochester (P); Christina Schwarz, Polgenix Inc. (F); Grażyna Palczewska, Polgenix Inc. (E); Krzysztof Palczewski, Polgenix Inc. (C), US patent 8,346,345, and 7,706,863 (P); David R. Williams, Canon Inc. (F), Canon Inc. (R), Polgenix Inc. (F), University of Rochester (P); Jennifer J. Hunter, Polgenix Inc (F), University of Rochester (P)

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Presentation Time: 12:00 PM–1:45 PM
In-vivo Two-Photon imaging of Fluorescence Angiography(TPFA) and Two-Photon Autofluorescence(TPAF) of rabbit and rat retina with femtosecond Scanning Laser Ophthalmoscope(ISLO)

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Purpose: The aim of this study was to investigate the fundamental capability of TPFA imaging. A femtosecond laser (780nm, 200fs, 80MHz) was used in fluorescence angiography for fundus imaging in living animal for the first time.

Methods: Six of the male New Zealand Albino rabbits, weighing 3.0–3.5 kg and eight of the albino rats were included in the study. Each animal was used for retinal imaging two-photon fluorescence angiography and retinal toxicity evaluation. The fluorescein was injected through a catheter. Fluorescein dye was injected into the brachial vein of an anesthetized animal. The prototype in two photon mode set at 120 mW or 160 mW scanned the eye at a 15 degree or 30 degree scanning angle. A second albino rabbit’s eye was exposed to the two photon laser for 100 seconds in three spots across the retina at power output levels of 160 W, 156 W, and 158 W. A positive control was performed on this rabbit at a power output level of 180 W for 10 minutes. 6 um thick sagittal sections of the rabbits’ eyes were cut and then stained using the TUNEL system (Promega Corporation). They were then analyzed for apoptotic cells under a fluorescence microscope at 40X.

Results: In vivo Two-Photon retinal angiography was recorded for different power levels of the laser and scanned at 15 degree and 30 degrees scans (the lowest power level for the laser wavelength of 780nm is 80mW). Microvascular blood flow was recorded and the back of the retina was lightened up as well. The infrared wavelength (780 nm) of the two photon prototype eliminated the risk of having blue light toxicity. Thermal damage of the retina tissue due to the exposure of the laser was not found. In vivo Two photon autofluorescence images and videos were obtained with this animal model, and toxicity analysis by histology.

Conclusions: TPFA imaging reveals the possibility of a new imaging modality for retina angiography that could potentially be used as a new technique for diagnoses of AMD. The maximum permissible exposure (MPE) of this study is carefully considered according to the ANSI standard (2007). The two photon laser may potentially be used to monitor all the way from the early stages of AMD, by detecting increased lipofuscin fluorescence, until the late stages of AMD, by taking fluorescence angiography images, which can be evaluated for neovascularization.
In vivo two-photon fluorescence imaging of primate inner retina
Jennifer J. Hunter1, 2, Robin Sharma3, 2, Grazyna Palczewska1, Krzysztof Palczewski1, David R. Williams3, 2. 1Flaum Eye Institute, University of Rochester, Rochester, NY; 2Center for Visual Science, University of Rochester, Rochester, NY; 3The Institute of Optics, University of Rochester, Rochester, NY; 4Polgenix, Inc., Cleveland, OH; 5Department of Pharmacology, Case Western Reserve University, Cleveland, OH.

Purpose: Adaptive optics retinal imaging in vivo has enabled imaging of vasculature and nerve fiber bundles in the inner retina, but the contrast from other structures is weak. Most cells contain fluorophores that could provide sufficient contrast, but they cannot be accessed through conventional means. We used two-photon fluorescence (TPF) to image autofluorescent structures in the inner retina of living primates.

Methods: A two-photon adaptive optics (AO) scanning light ophthalmoscope was used to repeatedly image TPF in 3 macaques (730 nm excitation, 400-550 nm emission). TPF signals from inner retinal layers were recorded for up to 10 minutes and then averaged. Near infrared reflectance images were simultaneously acquired for dual registration. AO was used to focus through different layers in the retina. For histological comparison, TPF microscopy was conducted in fixed, flat-mounted retina in one of these macaques.

Results: When focused at the nerve fiber layer, a weak TPF was captured from fiber bundles. Greater TPF was observed from streak-like structures that were co-localized with dark gaps between the axon bundles in reflectance. These streaks could represent Müller cell processes known to occupy the spaces between nerve fiber bundles, although the fluorophores remain unknown.

In a narrow focus window just beneath these streaks, a mosaic of dark, circular features was observed. This mosaic resembles ex vivo images of the ganglion cell layer. Analysis of Fourier spectra revealed local maxima at similar spatial frequencies for the in vivo and ex vivo images from the same retinal eccentricities implying that the density of the dark features is the same as that of ganglion cell bodies.

TPF also emanated from within the walls of vessels in the inner retina. When the beam was focused on the superficial vessel walls, TPF from the surface was captured. For deeper focus values, TPF from a cross-section of the vessel walls appeared as two bright bands. Larger vessels near the optic disk were more well-defined than smaller ones away from the disk. Fluorescence from vessel walls is likely due to proteins such as collagen and elastin.

Conclusions: We have demonstrated non-invasive two-photon imaging of structures within the inner retina of the living primate eye. This visualization of otherwise unseen morphological features in the inner retina could be a useful tool to investigate their structural integrity in normal and diseased eyes.

Commercial Relationships: Jennifer J. Hunter, Polgenix Inc. (F), University of Rochester (P); Robin Sharma, Polgenix Inc. (F), University of Rochester (P); Grazyna Palczewska, Polgenix Inc. (E); Krzysztof Palczewski, Polgenix Inc. (C), US patent 8,346,345 and 7,706,863 (P); David R. Williams, Canon Inc. (F), Canon Inc. (R), Polgenix Inc. (F), University of Rochester (P)

Support: NIH R01 EY022371, NIH R44 AG043645, NIH P30 EY001319, Research to Prevent Blindness, NIH K23 EY016700, NIH BRP EY014375
Safe real-time imaging of human retinal pigment epithelial cells in the living eye

Qi Qiang, Hongxin Song, Charles E. Granger, Koji Nozato, Kenichi Saito, Jie Zhang, Lisa R. Latchney, Mina M. Chung, David R. Williams, Ethan A. Rossi.

Purpose: Although it has been shown that RPE cell can be imaged with fluorescence AOSLO (FAOSLO) in normal humans [1] and in AMD [2], high light levels, focusing challenges, and post-processing time have limited clinical application of these methods. We have improved FAOSLO to permit routine imaging of the RPE mosaic and montaging of multiple RPE images in a clinical setting.

Methods: We improved RPE imaging efficiency by precisely controlling light exposure with a shutter, restricting visible light delivery to the retina only during data acquisition. This improvement was implemented in a FAOSLO with optical eye tracking and digital image registration, where a fast tip/tilt mirror compensates for eye motion, allowing precise temporal control of the fluorescence excitation light. Real-time co-registration and integration of the fluorescence images, using eye motion signals derived from simultaneously acquired near infrared reflectance channel, allows the RPE image to be visualized immediately. A high-fidelity algorithm runs in real-time to filter out motion artifacts from images after optical eye tracking and digital registration. We demonstrate these methods in both normal and diseased eyes, including AMD and Stargardt’s eyes.

Results: Focus and detector optimization can be accomplished with <10 seconds of total cumulative light exposure, a reduction in total time of ~85%. This improvement was implemented using light levels that were ~40% lower than we used previously. This savings in light budget allowed for multiple RPE images to be obtained from adjacent retinal areas with sufficient overlap to produce RPE image montages. In normal eyes a contiguous mosaic was visible and in diseased eyes RPE mosaic disruption was visible. Real-time eye signal integration of the auto-fluorescence channel allowed RPE images to be displayed to the experimenter instantaneously.

Conclusions: Efficient control of visible light exposure coupled with real-time fluorescence registration and signal integration allows immediate examination of the RPE mosaic on a microscopic scale across large retinal areas in the living eye. These techniques could have broad application in high-resolution retinal imaging.


Commercial Relationships: Qi Qiang, Canon Inc. (F), Canon Inc. (F), Canon USA Inc. (P), Canon USA Inc. (P), Montana State University (P), Montana State University (P), Polgenix Inc. (F), Polgenix Inc. (F), University of Rochester (P), University of Rochester (P); Hongxin Song, None; Charles E. Granger, Canon Inc. (F); Koji Nozato, Canon USA Inc. (E); Kenichi Saito, Canon USA Inc. (E); Jie Zhang, Canon Inc. (F), Canon USA Inc. (P), University of Rochester (P); Lisa R. Latchney, None; Mina M. Chung, None; David R. Williams, Canon Inc. (F), Canon Inc. (R), Polgenix Inc. (F), University of Rochester (P); Ethan A. Rossi, None

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The Thickness of the Outer Nuclear and Henle Fiber Layers in Patients with Photoreceptor Abnormalities Measured Using Optical Coherence Tomography

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Purpose: To determine the feasibility of obtaining separate measures of outer nuclear layer (ONL) and Henle fiber layer (HFL) thickness from spectral domain optical coherence tomography (SD-OCT) of healthy controls and patients with photoreceptor abnormalities.

Methods: Using horizontal midline SD-OCT scans and computer-aided manual segmentation, the thicknesses of the ONL (external limiting membrane to distal border of the fibers of Henle) and the HFL (distal border of the inner nuclear layer to the distal border of the fibers of Henle) were measured. The B-scans were modified for best visualization of the ONL/HFL boundary by altering their brightness and contrast. ONL and HFL thicknesses in 30 healthy eyes (age: 35.6±16.6 yrs.) were compared to histological data from Curcio et al.[2] (18 eyes, age: 67.6±16.0 yrs.). The histological data were displayed without (purple line, Fig. 1) and with adjustment for shrinkage (green line, Fig. 1, dashed lines are max. and min. estimates). ONL and HFL measurements were also made for patients with cone dystrophy (CD: 15 eyes, 31.8±18.6 yrs), achromatopsia (A: 14 eyes; 33.2±16.8 yrs), and retinitis pigmentosa (RP: 8 eyes; 35.3±19.5 yrs). The RP patients had foveal sensitivities within normal limits, and the A and CD patients had reduced or non-recordable cone function on full-field electroretinography.

Results: The ONL and HFL thicknesses for controls decreased with eccentricity in a similar way to the histological data (Fig. 1). In general, the ONL and HFL thicknesses (solid black lines, Fig. 1) fell within the maximum and minimum adjusted histological thicknesses (green dashed lines, Fig. 1). However, the average ONL (45.4±10.3um) and HFL (47.5±13.5um) thicknesses were greater than those for histology (46.7 and 42.6um respectively), perhaps due to differences in age, criteria for boundary locations, and/or differential shrinkage of tissue. As expected for eyes with CD and A, the ONL thickness was thinner than normal, while for RP eyes it was only abnormal outside the central 1mm (Fig. 2).

of radiation, and an ultrafast camera for a highly parallelized acquisition.

**Methods:** The retina of healthy volunteers was illuminated with wavelengths between 816 and 867 nm by the extended beam of a tunable laser (Broad sweeper, Superlum). Retinal irradiance was below the maximum permissible exposure (MPE). Light backscattered from the retina was imaged onto an ultrafast CMOS camera (SA-Z, Photron), where it interfered with an extended reference beam. From a series of interference images at different wavelengths, volumetric OCT images of the retina were reconstructed.

**Results:** We demonstrate in vivo retinal imaging at 9.9 billion voxels per second (40 million A-scans/s with 256 axial pixels). Sacrificing depth resolution by reducing the number of axial pixels, the A-scan rate was increased to more than 1 billion A-scans per second. FF-SS-OCT allowed imaging of all important retinal structures with good quality at unprecedented imaging speed (see fig. 1). Fast volumetric imaging at up to 3000 volumes/s was used to visualize small capillaries and to analyze the pulsation of retinal arteries and veins (see fig. 2). Imaging time for an area of 4 mm x 2 mm (896 x 368 A-scans) was only 316 μs. The high volume rate and the inherent phase stability enabled quantitative measurement of the change of retinal thickness due to blood pulsation with approx. 10 nm precision. A delay of the venous pulsation with respect to the arteries was observed (approx. 11 ms). The amplitudes of higher frequency components of the venous pulsation were considerably attenuated.

**Conclusions:** FF-SS-OCT provides fast volumetric imaging of the retina with good image quality. The capillary network can be analyzed with high spatial and temporal resolution. Analysis of retinal pulsation may provide information on pathological changes of vessels and capillaries.
Visualization of structural and functional changes of in vivo rat retina during photocoagulation using correlation mapping OCT

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Purpose: Sub-threshold photocoagulation can reduce excessive laser damage. However, it is difficult to assess the effectiveness and damage during laser treatment without a monitoring technique. Here we describe a method to measure depth-resolved structural and functional changes of retinal tissue during laser irradiation using the correlation map of optical coherence tomography (OCT) signals.

Methods: Spectral-domain OCT with a newly developed image processing method so called correlation-mapping is used for monitoring, which is operated at A-scan rate of 53 kHz and B-scan rate of 50 Hz. A 532-nm coagulation laser is used for photocoagulation. The OCT illumination beam and coagulation laser are coaxially aligned, and a sequence of B-scans is taken at the same region of the retina during laser treatment. In addition to the B-scan structural image, we compute the correlation between successive B-scans to detect temporal changes of the tissue. The motion artifacts between B-scans are numerically corrected with sub-pixel resolution. Then, the motion-corrected correlation map is generated to detect de-correlated area where the alteration of micro-structure induced by photocoagulation occurs and the random motion of the scatterers in blood vessels occurs. The feasibility of the method is examined by measuring three retinal regions of 3 healthy Brown Norway rats before, during and after the laser irradiation. Coagulation laser powers are adjusted to 0.9 W, 0.4 W and 0.35 W, respectively, and the exposure time is 0.2 s.

Results: The decorrelation induced by the coagulation laser was observed in one eye (0.9 W) of three eyes. Figures show the representative OCT B-scan images (left column) and correlation maps (right column) before (first row), during (second row) and after (third row) laser irradiation. The OCT B-scan image shows the disruption of photoreceptors after the laser irradiation as indicated by a red arrow. The correlation maps show the de-correlated area at RPE complex during laser irradiation as indicated by a red circle and the stoppage of choroidal blood flow after the laser irradiation as indicated by a green circle. These findings were not found in the eyes with lower coagulation power.

Conclusions: We have successfully visualized the structural and functional changes of in vivo rat retina induced by the photocoagulation using the correlation map of OCT signals.

Commercial Relationships: Kazuhiro Kurokawa, NIDEK Co., Ltd (F); Shuichi Makita, NIDEK Co., Ltd (F); Yasuhiro Furuuchi, NIDEK Co., Ltd (E); Masaaki Hanebuchi, NIDEK Co., Ltd (E); Yoshiaki Yasuno, NIDEK Co., Ltd (F)

Support: Wallace H Coulter Foundation Translational Research Award (Title: Low Cost Retinal Imager for Early Detection of Diabetic Retinopathy)
Purpose: Eye motion is a major impediment to clinical deployment of small field of view imaging systems such as the AOSLO. To obtain high signal-to-noise ratio (SNR) images, multiple images must be registered and averaged. Reference frame based image registration is usually employed for this purpose, but this fails when the eye moves outside the field of view of the reference frame. In diseased eyes with poor vision, eye motion is increased and this problem is exacerbated, greatly reducing the efficiency of imaging. We recently showed that eye motion could be efficiently optically stabilized in a large research instrument using an expensive tip/tilt mirror (Yang et al., 2014). Here we show that our approach can be implemented in a much more compact instrument using low-cost, higher speed, one-dimensional galvanometric scanners, permitting real-time optical stabilization and digital registration.

Methods: We modified a compact AOSLO (21”x29”) prototype by adding an additional galvanometric scanner to provide optical stabilization in the fast scanning direction. The traditional ‘slow’ scanner provided optical stabilization in the orthogonal direction. A real-time image based registration algorithm calculated eye motion at ~500Hz. Mirror angle was updated using closed-loop control at ~500Hz. Residual eye motion was eliminated with real-time digital registration. Performance was evaluated by calculating the RMS residual image motion for each 5 sec-long movie using offline software that calculated the displacement between the reference frame and each subsequent frame with a cross-correlation algorithm.

Results: We verified our method by imaging a model eye, 5 normal eyes and 2 diseased eyes. Model eye testing shows that this system can track 1.6x faster movement than the previous system. Before optical stabilization, eye motion was 12–22 μm in normal eyes and 14–31 μm in diseased eyes. After optical stabilization, it decreased to 0.9–2.8μm in normal and 1.3–3.1 μm in diseased eyes. Digital registration compensated for residual motion with RMS error of less than 1 pixel resolution.

Conclusions: Optical stabilization was implemented in a compact AOSLO prototype by adding just one additional galvanometric scanning mirror. Sub-pixel accuracy can be achieved after real-time digital registration, permitting image averaging during acquisition and eliminating post-processing time.

Commercial Relationships: Koji Nozato, CANON INC. (F), CANON INC. (P), CANON U.S.A., INC. (E), University of Rochester (P); Qiang Yang, CANON INC. (F), CANON INC. (P), Montana State University (P), Polgenix Inc. (F), University of Rochester (P); Kenichi Saito, CANON INC. (F), CANON INC. (P), CANON U.S.A., INC. (E), University of Rochester (P); Kei Suzuki, CANON INC. (E), CANON INC. (P); Jie Zhang, CANON INC. (F), CANON INC. (P), University of Rochester (P); Lisa R. Latchney, CANON INC. (F), David R. Williams, CANON INC. (F), Polgenix Inc. (F), University of Rochester (P); Ethan A. Rossi, University of Rochester (P)

Support: P30 EY001319 and BRP EY014375
Two-photon noninvasive imaging of murine retina in vivo

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Commercial Relationships: Chen D. Lu, None; Woo Jhon Choi, None; Zhao Wang, None; Martin F. Kraus, Optovue, Inc (P); Joachim Hornegger, Optovue, Inc (P); Jay S. Duker, Carl Zeiss Meditech, Inc (F), Optovue, Inc (F); James G. Fujimoto, Carl Zeiss Meditech, Inc (F), Optovue, Inc (F)

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Program Number: 5979 Poster Board Number: A0150

Presentation Time: 12:00 PM–1:45 PM

(A) IS/OS ellipsoid to top of RPE thickness difference between time point and immediately after flash

(B) Outer segment thickness difference between time point and immediately after flash

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**Purpose:** Two-photon excited fluorescence (TPF) imaging of retina of living animals can help investigating mechanisms of retinal diseases and the development of ophthalmic therapies. Previously reported systems for two-photon imaging in living animal eye required application of a custom contact lens. Here we report the optical design of a periscope for coupling a light beam into the mouse eye and capturing the emitted TPF that provides subcellular images of the retinal pigmented epithelium (RPE) without a custom contact lens.

**Methods:** The optical design of this periscope maximizes on mouse eye numerical aperture and provides an interface with the TPF imaging system without the need for a custom contact lens. Images of RPE and retina were obtained from albino Rpe65\(^{-/-}\) and wild type (WT) mice with a TPF system equipped with the new periscope, a laser delivering 75 fs pulses, a dispersion compensation unit and sensor-less adaptive optics. Before imaging mice were anesthetized by injection of a solution consisting of ketamine and xylazine in distilled water. All animal procedures and experiments were approved by the Institutional Animal Care and Use Committee at Case Western Reserve University and conformed to recommendations of both the American Veterinary Medical Association Panel on Euthanasia and the Association for Research in Vision and Ophthalmology.

**Results:** The periscope imaged the RPE with 730 nm excitation light. RPE images obtained with the periscope revealed crisp and clear structure of RPE mosaic - Fig. 1. The minimal mean power of the beam required to obtain a TPF image was 5mW. The images have wider field of view, better defined RPE mosaic and the fluorescence spectrum exhibited less noise than those previously reported with a contact lens.

**Conclusions:** This newly designed periscope is capable of obtaining high quality TPF images of the RPE and retina in live mice without using custom contact lenses. This new periscope provides an interface with a commercial microscope system and could easily be modified to image the retina and RPE in the eyes of different animal species.
Using cross-correlation of images, motion due to heart beat was corrected. More than 200 lesions having sufficient image quality with respect to noise and motion artifacts were evaluated. Tissue scattering and axial movement of the neuronal retina was automatically determined from the images sequence and 26 different parameters were calculated which were then correlated with the ophthalmoscopic visibility of the lesions. Sensitivity, specificity and receiver operating characteristics (ROC) were calculated.

**Results:** During coagulation an increase of scattering was observed near the retinal pigment epithelium. It predicted the ophthalmoscopic visibility of lesions with 80% sensitivity and 78% specificity. Area under ROC curve (AUC) was 0.78, Youden index 0.58. Axial tissue motion predicted lesions only with a Youden index of 0.45; sensitivity and specificity were 63% and 80%, respectively. The AUC was 0.74. Considerably better results were obtained when the local tissue expansion was calculated. This parameter is sensitive to local changes of the tissue morphology and corrects for global tissue motion. AUC reached 0.82 with 71% sensitivity, 91% specificity and a Youden index of 0.62.

**Conclusions:** In conclusion, high-speed OCT recording during photoagulation was able to predict the visibility of the lesion with good reliability. It does provide information on tissue effects during photoagulation and may be used for an online dosimetry.

**Commercial Relationships:** Gereon Huttmann, DE 10 2010 018 679.1 (P); Zeiss Meditec (F); Stefan O. Koinzer, None; Helke Müller, None; Iris Ellerkamp, None; Alex Baade, None; Moritz Moltmann, None; Dirk Theisen-Kunde, None; Birgit Lange, None; Ralf Brinkmann, PC DE 10 2010 018 679.1 (P); Reginald Birngruber, PC DE 10 2010 018 679.1 (P)

**Support:** BMBF 3GWO043C (iCube)

**Program Number:** 5981 **Poster Board Number:** A0152

**Presentation Time:** 12:00 PM–1:45 PM

**Adaptive Optics Control Algorithm to Detect the Pupil and its Boundary in real time Using Shack Hartmann Images**

**Alberto De Castro, Xiaofeng Qi, Lucie Sawides, Stephen A. Burns.**

**School of Optometry, Indiana University, Bloomington, IN.**

**Purpose:** To automate pupil detection and tracking in an Adaptive Optics (AO) system using Shack Hartmann (SH) data. To automatically choose pupil position and shape so as to maintain high quality AO control.

**Methods:** A spot quality metric was defined for each SH lenslet. Spots with low quality metrics occur either because the eye’s pupil is not in that location or because there is an obstruction or a corneal or lenticular problem. For this study we defined the metric for each SH spot as the ratio between the intensity of a region around the spot peak equal to 30% of the CCD space, and the total intensity. We then automatically detect the boundary of the pupil. Two approaches to minimize the influence of the defective spots in the final shape of the Deformable Mirror were studied. 1) The wave front at the locations of missing spots was assumed to require no correction. 2) The missing spots were eliminated from the influence function matrix by removing the corresponding rows.

The exit pupil of an AOSLO was magnified to subtend 10.5 mm at the eye. Two subjects were imaged with dilated pupil sizes of 7 and 6 mm diameter. Image quality based on either mean intensity or spatial frequency content of the images was calculated. Results obtained when sampling the whole exit pupil using the new algorithms were compared to results from our standard approach, which requires sampling only the subject’s pupil and keeping it centered in the system.

**Results:** The quality of the images captured when the defective spots are removed in real time from the influence matrix was 99% of the intensity using the standard algorithm. The area under the FFT of images was 97%. If “missing” spots were not removed but set to zero deviation (the ideal spot position) images were darker (89%) and had lower frequency content (92%). As reported previously the images improved when the spots on the boundary of the pupil were marked as defective in either algorithm (intensity 89 to 99% and 99 to 102%, frequency content 92 to 97% and 97 to 99% respectively).

**Conclusions:** It is possible to relax the constraints on head positioning in Adaptive Optics imaging by using an adaptive algorithm. There is little impact on image quality and the algorithm chosen can operate in real time (30 Hz AO loop).

Automatic detection of the pupil can provide images comparable to those obtained when the system is sampling only the pupil of the imaged eye.

**Commercial Relationships:** Alberto De Castro, None; Xiaofeng Qi, None; Lucie Sawides, None; Stephen A. Burns, None

**Support:** EY04395; EYO19008-01A1

**Program Number:** 5982 **Poster Board Number:** A0153

**Presentation Time:** 12:00 PM–1:45 PM

**Evaluation of Motion Artifact Associated with Use of a Custom, Handheld Imaging Device for Non-Invasive Retinal Blood Flow (RBF) Measurements in Telehealth Settings**

**M Jason Brooke1, Tze-Yuan Cheng1, Karan Raje1, Antonella Mangraviti1, Betty Tyler2, Karansingh Thakur1, Nitish V. Thakor1, Abhishek Rege1, Peter L. Gehlbach2, Ingrid E. Zimmer-Galler1.**

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**Purpose:** To evaluate motion artifact during the use of a custom, non-mydriatic, handheld imaging device to non-invasively capture vessel-specific RBF using laser speckle contrast imaging (LSCI).

**Methods:** Five adult Wistar rats were anesthetized and laid on their side for retinal imaging. The custom device was held by the operator while stabilizing an elbow on the table during imaging. In each eye, two separate sets of 40 consecutive images of the same region using red laser (650 nm) illumination were acquired in rapid succession (at 100 frames/sec) and analyzed to calculate the translational shift across the image stack. Three points corresponding to prominent vascular features (branch points and vessel edges or termini) were selected on each image. For each point, the standard deviation from the mean of the magnitude of the motion across the image stack were calculated and compared to identify intra- and inter-session variability in the raw laser speckle images. An LSCI image was developed from each stack; three prominent features were compared to further evaluate inter-session translational motion.

**Results:** Intra- and inter-session variability due to motion artifact during handheld retinal imaging was low. Fig. 1A shows an image...
obtained from a raw laser speckle image stack. Each cluster (red, yellow, and pink) represents the three feature points across the full image stack, with the median (green) shown. Fig. 1B shows the amount of motion artifact based on the mean and standard deviation of the distance from the median value for each feature point for each set of image stacks across all imaging sessions. In 60% of the imaging sessions, 90% of the motion artifact was less than 8 pixels, with an overall motion artifact (mean of medians) of 5.43 ± 2.03 pixels. Motion artifact in the LSCI images (Fig. 2A), determined by the distance from the median of the mean difference between feature points, was 3.63 ± 2.21 pixels.

**Conclusions:** Mechanisms that compensate for translational motion of up to ±10 pixels will adequately address motion artifact and enable the custom, non-mydriatic, handheld device to obtain high-fidelity LSCI images for RBF measurements. Teleretinal screening of RBF has promise for the early diagnosis and longitudinal management of various ocular and systemic diseases.

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**What would be the best suited correction strategy in adaptive optics for retina imaging?**

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**Purpose:** We investigate the correction strategy in an adaptive optics system for retina imaging.

To reduce the cost and complexity of current adaptive optics systems, it is necessary to assess which specifications can be loosened without performance losses. To do so, one has first to study the perturbations to be corrected, and then derive the most suited correction scheme. We focus here on the dynamic aspect. In particular, as ocular movements are among the main contributors to ocular dynamic aberrations, we explore the potential of pupil stabilization in AO-assisted systems dedicated to retina imaging.

**Methods:** We examined dynamic aberrations simultaneously with pupil movements on a 50-eye non pathological population with a high resolution custom-built biometer. The biometer consists of a Shack-Hartmann wavefront sensor synchronized with a pupil camera and is running at 236Hz.

In parallel, we set up an adaptive optics retina imaging system featuring an 88 actuator deformable mirror with pupil stabilization and running at more than 80Hz, in order to quantify the influence of pupil stabilization as well as the impact of the frame rate on the AO correction performances.

**Results:** We highlighted a correlation between micro-saccades and the level of aberrations. Yet, we showed that correcting dynamic aberrations from pupil shifts does not fully correct for the aberrations implicated with pupil shifts: other sources of ocular movements induced aberrations exist apart from horizontal and vertical eye rotations (for instance, lens wobbling).

Undergoing tests on our AO-assisted retina imaging system will enable to conclude on the efficiency of the pupil stabilization to compensate for dynamic aberrations for various deformable mirror speeds.

**Conclusions:** We demonstrated on several subjects that a significant part of the aberration dynamics cannot be explained by the combination of static aberrations and pupil motion. We will discuss at ARVO the impact of frame rate and pupil stabilization on AO performance.
AO-OCT imaging with the Compact Adaptive Optics Retinal Imager

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Purpose: To compare adaptive optics (AO) line-scanning ophthalmoscopic (AO-LSO) and AO-OCT images obtainable in normal subjects with diffraction-limited AO correction to those obtained in subjects with poor AO correction/media or with retinal disease using the Compact Adaptive Optics Retinal Imager (CAORI). Preliminary clinical measurements have commenced at Boston Children’s Hospital. We describe some early imaging results in from a study in progress, including in a subject with Stargardt’s disease.

Methods: The AO-LSO has a nominal 3°×5° area mode and the AO-OCT channel has 3° B-scan and raster modes and serves as the AO beacon. We have compared retinal AOLSO and AO-OCT images, at 780nm and 850nm respectively, within 3 degrees of the fovea in 3 normal subjects (males, ages 25 to 40) and one Stargardt’s patient (female, 17 years of age).

Results: Retinal disease can often compromise the contrast and quality of both AO-SLO and AO-LSO images of the photoreceptor mosaic. However, a simultaneously acquired, complementary AO-OCT modality can augment photoreceptor imaging, with indications of whether viable cones exist (or at least exhibit normal reflectance properties) beneath poor media and/or with limited AO-correction. AO-OCT and AO-LSO both show very clear photoreceptor mosaics in normal subjects with near diffraction-limited correction in healthy retinas in AO-OCT B-scans at eccentricities beyond >1°, with typical beaded string appearance of reflections from the IS/OS junction. In the Stargardt’s subject, the AO-LSO has very poor contrast with no evidence of photoreceptors beyond the fovea centralis. AO-OCT shows these layers very clearly, but the appearance of the (still visible) IS/OS junction is much altered, with no discernable signs of wave-guided reflections typical of normal cones. The entire inner retina has flocculated debris and appears hyper-reflective with a dense RPE. This accounts for rapid AO-LSO cone image degradation outside the fovea.

Conclusions: Conclusion: OCT effectively gates out multiply-scattered photons which accounts for it superior contrast in disease retinas; in general, we expect AO-OCT to look better in diseased retinas than AOLSO. CAORI’s AO-OCT could be an important high resolution back-up modality in the clinic for imaging photoreceptors and other layers when media are poor.