ARVO 2015 Annual Meeting Abstracts

471 High Resolution Photoreceptor Imaging

Wednesday, May 06, 2015 3:45 PM–5:30 PM
Exhibit Hall  Poster Session

Program #/Board # Range: 4920–4944/B0048–B0072
Organizing Section: Visual Psychophysics / Physiological Optics
Contributing Section(s): Multidisciplinary Ophthalmic Imaging, Physiology/Pharmacology, Retinal Cell Biology, Retina

Program Number: 4920 Poster Board Number: B0048
Presentation Time: 3:45 PM–5:30 PM

The relationship between visual acuity, perifoveal achromatic-, L- and M-cone acuity and retinal structure as imaged with OCT

Elisabeth Brattie Finstad, Siri Bjørnetun Jacobsen, Jon B. Gjelle, Stuart J. Gilson, Rigmor C. Baraas. Department of Optometry and Visual Science, Faculty of Health Sciences, Buskerud and Vestfold University College, Gjøvik, Norway.

Purpose: A negative correlation between best-corrected visual acuity (BCVA) and photoreceptor and retinal pigment epithelium aggregate (PR+RPE) thickness has been reported in high myopes. We investigated BCVA, perifoveal achromatic-, L- and M-cone acuity and retinal structure in healthy young male Norwegians.

Methods: Twenty-eight healthy males aged 20-38 yrs, with normal logMAR letter acuity and no observed ocular abnormalities, were included in the study. Color vision was examined with a battery of standard tests. Perifoveal achromatic and isolated L- and M-cone spatial acuity was measured in the dominant eye with a Sloan E letter of 90% achromatic or 23% cone contrast, respectively. The Sloan E was presented at 5 deg eccentricity and fixation was verified by an eye-tracker. Observers were corrected to best logMAR letter acuity and viewed the stimuli monocularly from a distance of 2.3 m. The central 30 deg of the dominant eye was imaged with the Heidelberg Spectralis OCT. Retinal layers were analyzed by calculating longitudinal reflectivity profiles.

Results: Axial lengths ranged from 22.60–25.18 mm and spherical equivalent refraction from -4.50–2.43D. Foveal thickness and PR+RPE thickness ranged from 187–241 μm and 63–80 μm, respectively. Perifoveal retinal thickness ranged from 287–335 μm and perifoveal PR+RPE thickness from 63–80 μm. No correlation was found between BCVA (logMAR -0.16–0.04) and PR+RPE (r=-0.305, p=0.11) or foveal thickness (r=-0.237, p=0.226). Perifoveal achromatic, L- and M-cone logMAR acuity ranged from 0.28–0.53, 0.46–0.80 and 0.54–1.25, respectively. Observers with red-green color-vision deficiencies (n=4, 13.3 %) had achromatic acuity within the normal range and performed as expected according to type and degree of deficiency. No correlation was found between either perifoveal achromatic, L- or M-cone acuity and retinal thickness (r=-0.20, p=0.31; r=-0.141, p=0.48; r=-0.07, p=0.74) or thickness of the PR+RPE (r=-0.28, p=0.15; r=-0.11, p=0.60; r=0.28, p=0.14) at 5 deg temporal eccentricity.

Conclusions: The correlation between BCVA and foveal PR+RPE thickness in high myopes could not be replicated in low hyperopes to moderate myopes. The lack of correlation between different retinal thicknesses and perifoveal measures of achromatic and isolated cone acuity support this finding.

Commercial Relationships: Elisabeth Brattie Finstad, None; Siri Bjørnetun Jacobsen, None; Jon B. Gjelle, None; Stuart J. Gilson, None; Rigmor C. Baraas, None

Program Number: 4921 Poster Board Number: B0049
Presentation Time: 3:45 PM–5:30 PM

Repeatability and inter-observer variability of in vivo retinal cone imaging using a modified Heidelberg Retinal Angiography (HRA2) in normal subjects

Marketa Cilkova1,2, Juliane Matlach1, Reena Chopra1, Andy Rider1, Nilpa Shah3, Padraig Mulholland1, Steven C. Dakin1, Adnan Tufail1, Roger S. Anderson1,4. 1Institute of Ophthalmology, University College London, London, United Kingdom; 2Moorefields Eye Hospital, NIHR Biomedical Research Centre, London, United Kingdom; 3Optometry and Vision Science, University of Auckland, Auckland, New Zealand; 4Vision Science, University of Ulster, Coleraine, United Kingdom.

Purpose: To determine whether the modified narrow-angle Heidelberg Retina Angiograph (HRA2) is repeatable and reliable for acquiring good quality in vivo cone images, and the future potential to develop such an instrument for routine high resolution imaging in primary and secondary care.

Methods: 3 x 3 degrees retinal images were acquired at an eccentricity of six degrees from the fovea, in a group of thirty healthy subjects (21 – 65 years of age), using the 680nm laser arm of the modified Heidelberg Retinal Angiograph (HRA2). Two different operators performed the cone imaging and one operator repeated the measurements over two separate sessions. The images were analysed and cone counts generated by using customized software.

Results: The mean cone count was 4797 cones per scan area (7054 cones/mm²) ranging from 4258 to 5125 cones per scanned area. The cone counts acquired by the two operators were on average different by 12 cones (<1%) within the scan window with a coefficient of repeatability of 212 cones (1.96 x SD). Cone counts from images acquired by the same observer on two different occasions ranged from 4258 to 5120 with mean bias of 46 cones and coefficient of repeatability of 284 cones per scan area.

Conclusions: The narrow-scan HRA2 showed very good inter- and intra-examiner repeatability in comparison with alternative adaptive optics cone imaging devices. However, our data were acquired in a younger population (<66 years) and therefore more data are needed to determine the level of repeatability in an older population. The modified HRA2 could be used in patients with diseases affecting retinal cone density to determine how density relates to visual function at the early stages of the disease. The method has a potential for diagnosis and monitoring of retinal disease in a clinical setting.

Commercial Relationships: Marketa Cilkova, None; Juliane Matlach, None; Reena Chopra, None; Andy Rider, None; Nilpa Shah, None
High Resolution Imaging of Parafoveal Cones in Different Stages of Diabetic Retinopathy using Adaptive Optics Fundus Camera

Mohamed K. Soliman, Mohammad A. Sadiq, Aniruddha Agarwal, Salman Sarwar, Mostafa S. Hanout, Frank E. Graf, Robin High, Diana V. Do, Quan Dong Nguyen, Yasir J. Sepah. Ophthalmology and Visual Sciences, University of Nebraska Medical Center, Omaha, NE; Ophthalmology, Assiut University, Assiut, Egypt; College of Public Health, University of Nebraska Medical Center, Omaha, NE.

Purpose: Neurodegeneration has been proposed as one of the possible pathophysiologic mechanisms underlying diabetic retinopathy (DR). In this prospective cohort study, we investigated parafoveal cone density in different stages of DR using adaptive optics (AO) retinal imaging.

Methods: An AO retinal camera (rtx1; Imagine Eyes, Orsay, France) was used to acquire images of parafoveal cones from patients with diagnosis of diabetes mellitus with or without retinopathy and from healthy controls with no known systemic or ocular disease. Eyes with myopia (> 10 diopters), media opacity, current macular edema, or any coexistent retinal diseases other than DR were excluded. The final image produced by the AO camera is equivalent to \( 1.2 \times 1.2 \text{ mm} \) (4° × 4°) based on axial length (AL) of the eye. The density of the parafoveal cones was calculated in 100 × 100 \( \mu \text{m} \) square located at 300 and 500 \( \mu \text{m} \) from the foveal center (a minimum of 250 \( \mu \text{m} \) from the foveal center is recommended for feasible cone assessment) along nasal, temporal, superior and inferior quadrants. The measurements (cones/mm\(^2\)) were done using automated AOdetect Ver. 0.1. Software provided by the manufacturer. AL were measured using non-contact biometry (IOL Master®; Carl Zeiss Meditech, Germany). Correlation between diabetes control (Hb1Ac) and severity of DR with cone density was analyzed using Spearman correlation test.

Results: Ten subjects (10 eyes) with no known ocular or systemic diseases and 14 (17 eyes) with diabetes were included (Table). Among those with diabetes, 4 patients (5 eyes) did not have retinopathy, 3 (4 eyes) had mild NPDR, 5 (6 eyes) had moderate NPDR and 2 (2 eyes) had severe NPDR. The mean Hb1Ac among patients with diabetes was 8.5 ± 2. A significant difference (P < 0.001) in cone density was found between healthy controls and moderate/severe NPDR group (figure); however, no statistical significance was found between controls and no retinopathy or mild NPDR groups. An inverse relationship between cone density and Hb1Ac was observed in all parafoveal regions; however, no relationship could be correlated for the duration of diabetes.

Conclusions: Patients with diabetes may have loss of photoreceptors with increasing severity of DR. AO provides assessment of photoreceptors, which may allow better understanding of the disease pathology.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetes (No DR 30%, Mild 23%, Moderate* 35%, severe 12%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>50 (40)</td>
<td>51 (38)</td>
</tr>
<tr>
<td>Gender (M/female)</td>
<td>80</td>
<td>53.3</td>
</tr>
<tr>
<td>Axial length</td>
<td>23.28 (± 0.9)</td>
<td>24.05 (±0.6)</td>
</tr>
<tr>
<td>Duration of Diabetes (years)</td>
<td>N/A</td>
<td>14 (8)</td>
</tr>
</tbody>
</table>

* NPDR

©2015, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at publs@arvo.org.
ARVO 2015 Annual Meeting Abstracts

analysis achieved 94% classification accuracy for DM1 patients based on the complementary use of the AO-based imaging biomarkers.

Conclusions: The complementary use of cone metrics shows great potential to detect early pathologic changes of the cone mosaic in patients affected by diabetes mellitus.

Commercial Relationships: Marco Lombardo, None; Maria Cristina Parravano, None; Daniela Giannini, None; Sebastiano Serrao, None; Lucia Ziccardi, None; Giuseppe Lombardo, None

Support: PON01_00110; Italian Ministry of Health 5x1000 funding

Program Number: 4924 Poster Board Number: B0052
Presentation Time: 3:45 PM–5:30 PM
Multimodal investigation of the pathologic changes of the retinal microstructure in patients with type 1 diabetes mellitus using adaptive optics

Maria Cristina Parravano1, Lucia Ziccardi1, Giuseppe Lombardo2, Monica Varano1, Paola Giorno1, Marco Lombardo1. 1Ophthalmology, Fondazione GB Bietti-IRCCS, Rome, Italy; 2IPCF - Unit Support of Cosenza, CNR, Cosenza, Italy; 3Statistics, University of Rome La Sapienza, Rome, Italy.

Purpose: to explore the correlation between metrics of the cone mosaic imaged by an adaptive optics (AO) flood illumination retinal camera and retinal thickness in cross-section SD-OCT images in type 1 diabetes mellitus (DM1).

Methods: AO retinal images of the photoreceptor mosaic were acquired in 16 DM1 patients; 16 healthy subjects were recruited as controls. Cone density, spacing of cones and packing arrangements of cones, estimated using Voronoi diagrams, were calculated within 1.5 degree eccentricity from the fovea along the vertical and horizontal meridians. From spacing of cones and Voronoi diagrams, two novel descriptors of the cone mosaic, the Linear Dispersion index (LDi) and the Heterogeneity Packing index (Pi), were calculated respectively. The subfoveal retinal thickness (SRT) was estimated in retinal cross-sections in SD-OCT images. Linear correlation analysis was performed to determine the relationships between cone metrics and SRT.

Results: Patients had diagnosis of DM1 from 9 to 21 years earlier. Eight patients were diagnosed with no signs of diabetic retinopathy (noDR) and 8 with mild signs of DR (NPDR). The SRT was statistically significant thicker in DM1 eyes than controls (194±18 μm in NPDR, 199±31 μm in noDR and 153±36 in controls, p<0.05). Cone density showed no significant correlation with SRT (r=−0.30, p=0.09); the LDi approached statistical significance (r=0.35; p=0.05) and the HPI showed a high statistically significant correlation with SRT (r=−0.45, p=0.009).

Conclusions: Cone density alone is not descriptive of the pathologic changes of the cones. The novel metrics based on spacing and packing arrangements of cones showed to correlate with the pathologic changes of foveal retinal thickness in patients with type 1 diabetes mellitus.

Commercial Relationships: Maria Cristina Parravano, None; Lucia Ziccardi, None; Giuseppe Lombardo, None; Daniela Giannini, None; Monica Varano, None; Paola Giorno, None; Marco Lombardo, None

Program Number: 4925 Poster Board Number: B0053
Presentation Time: 3:45 PM–5:30 PM
Analysis of the Photoreceptor Mosaic Within, On and Outside the Borders of Hyperautofluorescent Rings in Retinitis Pigmentosa Using Adaptive Optics Scanning Light Ophthalmoscopy

Emily S. Smith1, Toco Y. Chui2, Ching-Lung Chen3,4, Joseph Carroll5, Alfredo Dubra1, Robert F. Cooper6, Richard B. Rosen7, Donald Hood1,2, Vivienne C. Greenstein3. 1Department of Psychology, Columbia University, New York, NY; 2Ophthalmology, New York Eye and Ear Infirmary of Mount Sinai, New York, NY; 3Ophthalmology, Columbia University, New York, NY; 4Ophthalmology, Chang Gung Memorial Hospital, Chiayi, Taiwan; 5Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 6Biomedical Engineering, Marquette University, Milwaukee, WI.

Purpose: To compare the appearance, density, and spatial organization of photoreceptor cells (PRCs) in patients with retinitis pigmentosa (RP) to measures of visual function and retinal structure within, on and outside the borders of the hyperautofluorescent (hyperAF) ring.

Methods: A custom research adaptive optics scanning light ophthalmoscope (AO-SLO) [1] was used to image the PRC mosaic in 8 eyes of 8 RP patients with visual acuities 20/20-20/30, and hyperAF rings and in 5 healthy eyes. Cone photoreceptor densities and mean nearest neighbor distances were estimated along the horizontal meridian (fovea ± 10°) using custom semi-automated cone counting software.[2] In addition, 10-2 visual fields (Carl Zeiss Meditec Inc.) and dark-adapted cone and rod mediated visual sensitivities were measured (Haag-Streit AG). Fundus autofluorescence and spectral domain-optical coherence tomography (OCT) line scans through the fovea were also obtained (Spectralis HRA+OCT Heidelberg Engineering GmbH). Total receptor layer thickness (R+: Bruch’s membrane to the border between the inner nuclear layer and outer plexiform layer) was measured and compared to values for 30 age-similar normals.

Results: Within the hyperAF ring, the ellipsoid zone band was preserved in all eyes. However, R+ thickness was significantly decreased and cone mediated visual sensitivities ranged from 0 to -8dB of normal. The AO-SLO images showed dark regions, and cone densities were decreased for 3 eyes that had significantly decreased cone sensitivities and R+ thickness values from the fovea to the inner ring border. On the ring border, for all 8 eyes, the clarity of the mosaic decreased, the number and extent of dark regions increased and PRCs appeared misshapen/fragmented. Outside the border, where sensitivities and R+ thickness values were markedly decreased, cones could not be reliably identified, although the size and shape of the cells were consistent with retinal pigment epithelium cells.

Conclusions: In patients with RP, both decreased cone density and cone structure abnormalities were observed, even within the hyperAF ring (i.e. in “relatively healthy” parafoveal regions). 1. Sulai, Dubra. Biomed Opt Express. 2012, 3:1647-61. 2. Garrieo et al. Optom Vis Sci. 2012, 89: 632-43.

Commercial Relationships: Emily S. Smith, None; Toco Y. Chui, None; Ching-Lung Chen, None; Joseph Carroll, None; Alfredo Dubra, US Patent No: 8,226,236 (P); Robert F. Cooper, None; Richard B. Rosen, Advanced Cellular Technologies (C), Allergan (C), Carl Zeiss Meditec (C), Clarity (C), OD-OS (C), Opticology (I), Optovue (C); Donald Hood, TOPCON (F); Vivienne C. Greenstein, None

©2015, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at pubs@arvo.org.
Cone spacing measures over 12 months in eyes with retinitis pigmentosa

Angela N. Baldwin1, Shane Griffin1, Nicolas Sippi-Swezy1, Panagiota Loumou1, Jaskiran Mann1, Ravi Keshavamurthy1, Travis Porco1, 2, Austin Roorda1, Jacque L. Duncan1. 1Ophthalmology, University of California San Francisco, San Francisco, CA; 2Ophthalmology, University of California San Francisco, Francis I. Proctor Foundation, San Francisco, CA; 3School of Optometry and Vision Science Graduate Group, University of California Berkeley, Berkeley, CA.

Purpose: To determine the inter-visit, inter-grader and inter-ocular variability of Adaptive Optics Scanning Laser Ophthalmoscopy (AOSLO)-derived cone spacing measures in eyes with retinal degenerations (RD) followed for 12 months.

Methods: Six patients with inherited RD including retinitis pigmentosa and choroideremia underwent AOSLO imaging at 3 visits (2 baseline visits separated by no more than 1 month and one visit 12 months after the first visit). Cone spacing was measured in multiple regions in each image by 2 independent graders. Variability of cone spacing measures between visits and between graders was determined. Standard macular measures, including visual acuity, foveal sensitivity, Goldmann perimeter, static perimeter in the central 20 degrees, and spectral-domain optical coherence tomography (SD-OCT)-derived outer segment length and inner/outer segment junction or Ellipsoid Zone (EZ) band width were obtained.

Results: Overall, cone spacing was measured in 309 regions. Agreement between graders for cone spacing measures at each location and each visit was acceptable (intraclass correlation (ICC)=0.75, 95% CI 0.61-0.87); mean difference between graders was 0.08 arcminutes (95% CI 0.07-0.1). Cone spacing measures were similar at the two baseline visits; median absolute difference was 0.044 arcmin (95% CI= 0.041-0.057). Cone spacing measures at 12 months compared to spacing measures at baseline increased by 0.027 arcminutes (95% CI 0.02-0.04). There were no significant changes in standard clinical measures of disease progression including visual acuity, foveal threshold, area of I4e and V4e kinetic isopters, or Ellipsoid Zone (EZ) band width in the central 20 degrees, EZ band width or outer segment thickness in the central 3 degrees.

Conclusions: Cone spacing measures using AOSLO showed substantial inter-grader agreement. Measures were similar between baseline visits separated by less than 1 month. Cone spacing increased over 12 months in eyes with inherited RD, although standard clinical measures showed no significant change during this period. This information establishes the repeatability for current and future clinical trials involving AOSLO imaging of eyes with inherited RD.

Commercial Relationships: Angela N. Baldwin, NIH K-12 Career Development Grant (F); Shane Griffin, None; Nicolas Sippi-Swezy, None; Panagiota Loumou, None; Jaskiran Mann, None; Ravi Keshavamurthy, None; Travis Porco, None; Austin Roorda, Foundation Fighting Blindness (F), NIH EY014375 (F), US 6,890,076 (P), US 7,118,216 (P); Jacque L. Duncan, FDA R01-41001 (F), Foundation Fighting Blindness (F), Hope for Vision (F), NIH Grant EY002162 (F), Research to Prevent Blindness (F), That Man May See Inc. (F), The Benard A. Newcomb Macular Degeneration Fund (F)

Support: National Institutes of Health (Grants: EY019861, R24EY019861, 8DP1EY02317), Foundation Fighting Blindness, Choroideremia Research Foundation, Research to Prevent Blindness, the F. M. Kirby Foundation, the Paul and Evanna Mackall Foundation Trust, Lois Pope Life Foundation, Canon Inc, Optos PLC

Clinical Trial: NCT01866371
Reliability of cone density measurements on adaptive optics images in Stargardt disease

Melissa Kasilian1, Michael G. Ring1, Rupert W. Strauss2, Moataz M. Razeen3,4, Tunde Peto1, Catey Bunce2, Joseph Carroll3, 5, Adam M. Dubis2, 6, Michel Michaelides2, 6.
1Reading Centre, Moorfields Eye Hospital, London, United Kingdom; 2Moorfields Eye Hospital, London, United Kingdom; 3Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 4Biophysics, Medical College of Wisconsin, Milwaukee, WI; 5Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI; 6Genetics, University College London, London, United Kingdom.

Purpose: Quantification of the photoreceptor mosaic in normal subjects has been done using manual and automated methods, however in patients with retinal degeneration, cone cell identification becomes increasingly challenging. It is critically important to determine how reliable cone counting measurements are and what factors affect reliability. This knowledge can then be applied to natural history studies and clinical trials that intend to use it to monitor progression and efficacy respectively.

Methods: Twelve patients (8-37 years) with molecularly confirmed Stargardt Disease (STGD) were imaged using a custom built adaptive optics scanning light ophthalmoscope (AOSLO) designed to simultaneously acquire images in confocal and split detection imaging modes. Cone photoreceptors were identified by two graders (G1 and G2) in the confocal and split detection images acquired at 100 parfoveal regions of interest (ROI). All 200 images were graded twice by each observer. Reliability of cone cell identification was compared between trials, between observers and between imaging modes. Statistical significance for all tests was assessed at the 5% level.

Results: The between trial average variance was 11% for G1 and 19% for G2 (p 0.04, paired t-test). The average variance in cones identified between observers was 15%, which did not result in a statistically significant difference in number of cones identified (p=0.06, t-test). There was a significant difference in mean cones identified (p <0.0001, paired t-test) between imaging modes. For both graders, the between trial average variance was 18% for confocal images compared to 3% for split detection images.

Conclusions: Split detection greatly facilitated more reliable cone photoreceptor identification and will be the mode of choice for natural history studies and clinical trials using AOSLO. There was significant variance between the graders, which may in part relate to the fact that this group is the first one to use non-medically trained graders in a reading-center setting to analyse images. Understanding the factors behind these differences, establishing detailed training and standardised protocols, will lead to increased measurement reliability in order to take the first step towards a reading center-based format of AOSLO analysis for large multi-centre trials.

Commercial Relationships: Melissa Kasilian, None; Michael G. Ring, None; Rupert W. Strauss, None; Moataz M. Razeen, None; Alfredo Dubra, US Patent 8,226,236. (P); Tunde Peto, None; Catey Bunce, None; Joseph Carroll, None; Adam M. Dubis, None; Michel Michaelides, None

Support: National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology; National Institutes of Health Grants R01EY017607, P30EY001931, and C06RR016511; Fight For Sight; Moorfields Eye Hospital Special Trustees; the Foundation Fighting Blindness; Retinitis Pigmentosa Fighting Blindness; and an unrestricted departmental grant (Medical College of Wisconsin) from Research to Prevent Blindness. Also supported by a multiuser equipment grant from The Wellcome Trust (099173/Z/12/Z); a Burroughs Wellcome Fund Career Award at the Scientific Interface and a Career Development Award from Research to Prevent Blindness (AD); and a Foundation Fighting Blindness Career Development Award (MM). Supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health (Grant UL1TR000055).

Longitudinal adaptive optics imaging reveals regional variation in cone and rod loss in Stargardt disease

Maxwell A. Reback1, Hongxin Song2, Lisa R. Latchney1, Mina M. Chung1, 3.
1School of Medicine and Dentistry, University of Rochester, Rochester, NY; 2Center for Visual Science, University of Rochester, Rochester, NY; 3Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 4Biophysics, Medical College of Wisconsin, Milwaukee, WI; 5Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI; 6Genetics, University College London, London, United Kingdom.

Purpose: Stargardt disease (SD) is defined clinically by lipofuscin deposition and atrophy of the retinal pigment epithelium (RPE), but the causative ABCA4 gene encodes a protein uniquely expressed in cone and rod outer segments. Segments of the macula at risk of RPE changes include areas with significantly increased rod and cone photoreceptor outer segment density and atrophy. Using adaptive optics scanning light ophthalmoscope (AOSLO), we investigated the sequence of changes in the photoreceptor mosaic in the macular atrophy phenotype of SD.

Methods: Two brothers with genetically confirmed SD underwent comprehensive eye exams and AOSLO imaging 3 times in a 28-month period. Reflectance images of the cone and rod photoreceptors were obtained from the central fovea to 10 degrees inferior. Photoreceptors were counted in sampling windows at 100μm intervals of 200x200μm for cones and 50x50μm for rods, using custom semi-automated software. Photoreceptor density and spacing were measured and compared across imaging sessions using one-way ANOVA.

Results: Over 28 months, visual acuity declined from 20/30 to 20/150 with an expanding bull’s eye lesion in the younger, more mildly affected brother; the older brother maintained 20/150 visual acuity with a central scotoma and expanding macular RPE atrophy. In the younger brother, AOSLO showed a 30% decline in peak foveal cone density after 8 months, and complete loss of foveal cones at 28 months; the older brother had no detectable foveal cones at baseline. In the peripheral macula, cone and rod spacing was greater than normal in both patients. Rod spacing increased significantly in the younger brother after 28 months (p<0.01) and remained unchanged in the older brother. There was no change in peripheral cone spacing in either patient over 28 months. The ratio of cone to rod spacing was greater than normal over all eccentricities tested, with greater divergence closer to the foveal center.

Conclusions: AOSLO demonstrates early foveal cone loss and increasing rod spacing in the peripheral macula in SD. Cones and rods are reduced in the peripheral macula in areas that appear normal by conventional imaging methods. The ratio of cone to rod spacing is increased, particularly near the foveal center. These findings suggest that central cone loss may be an early pathogenetic step in
SD. AOSLO provides the capability to track individual cone and rod changes in SD longitudinally.

**Commercial Relationships:** Maxwell A. Reback, None; Hongsik Song, None; Lisa R. Latchney, None; Mina M. Chung, None

**Support:** NEI EY021786, EY014375, EY001319, Research to Prevent Blindness Unrestricted Grant, Edward N. & Della L. Thome Memorial Foundation to Mina Chung

**Program Number:** 4930 Poster Board Number: B0058

**Presentation Time:** 3:45 PM–5:30 PM

**Factors Affecting Cone Photoreceptor Identification in RPGR-Associated Retinopathy**

Michael G. Ring 1, 2, Melissa Kasili 1, 2, James Tee 1, 4, Alfredo Dubra 1, 4, Tunde Peto 1, Catey Bunce 1, Ana Quartiilho 1, Joseph Carroll 1, 4, Michel Michaelides 1, 2, Adam M. Dubis 1, 2, 4, Moorfields, London, United Kingdom; 1Inst Ophthalmology - Genetics, UCL, London, United Kingdom; 2Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 3Biophysics, Medical College of Wisconsin, Milwaukee, WI; 4Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI; 4Moorfields Eye Hospital, London, United Kingdom; 2Institute of Ophthalmology, University College London, London, United Kingdom.

**Purpose:** Cone photoreceptor quantification has been carried out in normal subjects using either manual or semi automated methods. Natural history studies and clinical trials of subjects with degenerative retinal diseases need to have a reliable and objective method of assessing the cone mosaic if this technique is to be incorporated into trial protocols. We present data on cone counting reliability in subjects with RPGR-associated retinopathy.

**Methods:** Fourteen subjects (8-51 years) with molecularly confirmed RPGR-associated retinopathy were imaged using a custom-built adaptive optics scanning light ophthalmoscope (AOSLO) designed to simultaneously acquire images in confocal and split detection imaging modes. Cone photoreceptors were manually identified by two graders (G1 and G2) in the confocal and split detection images acquired at 42 parafoveal regions of interest. All 84 images were graded twice by each observer. Reliability of cone cell identification was compared between trials, between observers and between imaging modes. Statistical significance for all tests was assessed at the 5% level.

**Results:** Mean variance between trials was 9% for G1 and 16% for G2 across all images, however the mean number of photoreceptors identified was not statistically significantly different for either grader (p=0.1 for G1, p = 0.06 for G2, paired t-test). There was not a significant difference in mean variance between imaging modes (p=0.28, paired t-test). There was no statistically significant difference in mean variance between trials for confocal (5 ± 34%) or split detection (2 ± 6%).

**Conclusions:** While there was no statistical difference in mean variance between split detection and confocal images, the standard deviation of the variance was significantly different. Therefore split detection greatly facilitated the reliability of photoreceptor identification. The significant difference between graders will require further investigation to identify the underlying contributing factors, with refinement of training and certification methods likely to also help address this difference and thereby allow this image analysis modality to enter the reading center-based format for future large multi-center trials.

**Commercial Relationships:** Michael G. Ring, None; Melissa Kasili, None; James Tee, None; Alfredo Dubra, US 8,226,236 (P); Tunde Peto, None; Catey Bunce, None; Ana Quartiilho, None; Joseph Carroll, None; Michel Michaelides, None; Adam M. Dubis, None

**Support:** National Institute for Health Research Biomedical Research Centre at Moorfields Eye Hospital National Health Service Foundation Trust and UCL Institute of Ophthalmology; National Institutes of Health Grants R01EY017607, P30EY001931, and C06RR016511; Fight For Sight; Moorfields Eye Hospital Special Trustees; the Foundation Fighting Blindness; Retinitis Pigmentosa Fighting Blindness; and an unrestricted departmental grant (Medical College of Wisconsin) from Research to Prevent Blindness. Also supported by a multiuser equipment grant from The Wellcome Trust (099173/Z/12/Z); a Burroughs Wellcome Fund Career Award at the Scientific Interface and a Career Development Award from Research to Prevent Blindness (AD); and a Foundation Fighting Blindness Career Development Award (MM). Supported in part by the National Center for Advancing Translational Sciences, National Institutes of Health (Grant UL1TR000055).

**Program Number:** 4931 Poster Board Number: B0059

**Presentation Time:** 3:45 PM–5:30 PM

**Adaptive optics imaging of putative cone inner segments within geographic atrophy lesions**

Ethan A. Rosset 1, Kenichi Saito 2, Charles E. Granger 3, Koji Nozato 1, Qiang Yang 1, Tomoaki Kawakami 1, Jie Zhang 1, William Fischer 1, David R. Williams 1, 4, Mina M. Chung 1, Center for Visual Science, University of Rochester, Rochester, NY; 2Canon U.S.A., Inc., Melville, NY; 3The Institute of Optics, University of Rochester, Rochester, NY; 4Canon, Inc., Tokyo, Japan; 5Flaum Eye Institute, University of Rochester Medical Center, Rochester, NY.

**Purpose:** The time course of cone loss relative to the formation of geographic atrophy (GA) lesions in age-related macular degeneration is not well characterized. Confocal adaptive optics scanning light ophthalmoscopy (AOSLO) images of GA lesions contain hyper-reflective cone-like features, but such images are difficult to interpret in advanced disease states such as GA. Non-confocal split-detector imaging in AOSLO has been shown to reveal structures consistent with cone inner segments (Scoles et al. 2014). Here we use two non-confocal AOSLO methods to determine whether putative inner segments exist within GA lesions and if they correlate with the cone-like structures seen in confocal AOSLO images.

**Methods:** Six patients were imaged, five with near-infrared (NIR) light in a compact commercial AOSLO prototype modified for split-detector imaging. The central portion of the PSF was directed to one detector for confocal imaging and a knife edge prism split the remaining light into two additional detectors. Five patients were imaged with visible light in a research AOSLO with a novel non-confocal method based on offset aperture (Chui et al. 2012) and split-detector methods. At each location, images were sequentially obtained with a ~10 Airy disk aperture offset by ~6 Airy disks to 8 equally spaced radial positions. Images were co-registered with simultaneously acquired NIR images. Registered images from each position were averaged and combined to enhance contrast. Identical locations were imaged in both instruments in some eyes permitting method comparison.

**Results:** Structures consistent in appearance to putative cone inner segments were observed within some small GA lesions. Cone-like features in confocal AOSLO images did not always co-localize with putative cone inner segments, suggesting that some hyper-reflective features in confocal AOSLO arise from light scattered by other structures. Conversely, some areas where cones were not visible in confocal AOSLO contained putative cone inner segment structures in non-confocal images.

**Conclusions:** Some cones survive in small GA lesions. Images similar to those obtained with the split-detector method can be obtained using a single detector. Cone survival in small GA lesions

©2015, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at pubs@arvo.org.
recommend the use of AO-SO to evaluate the retinal structure of eyes with AZOOR during the recovery phase.

**Commercial Relationships:** Hisashi Fukuyama, None; Takashi Fujikado, Topcon Corporation (F); Suguru Miyagawa, Topcon Corporation (E); Kazuo Kitamura, Topcon Corporation (E); Hiroyuki Kanda, None; Takeshi Morimoto, None; Kohji Nishida, None

**Program Number:** 4933 **Poster Board Number:** B0061

**Presentation Time:** 3:45 PM–5:30 PM

**Extrafoveal Cone Packing Density and Geometry in Retinopathy of Prematurity (ROP)**

Ramkumar Ramamirtham1, Garima Soni1,2, James D. Akula1, Emily A. Swanson1, Tara L. Favazza1, Mircea Mujat1, R D. Ferguson1, Toco Y. Chui1, Anne Moskowitz1,2, Anne B. Fulton1,2,3,4

**Purpose:** To study cone packing density and geometry using an adaptive optics scanning laser ophthalmoscope (AOSLO) in eyes with history of ROP and age matched control subjects.

**Methods:** Subjects with a history of severe, treated ROP (n=3, SROP), with a history of mild ROP that resolved without treatment (n=3, MROP), and term born controls (n=6, CT) were studied. The subjects were aged 15–23 (median: 19.8) years at test. Two to four confocal and offset-pinhole AOSLO videos (64 frames) subtending 1°×1° visual angle were obtained from each subject at retinal eccentricities 4.5°, 9°, 13.5° and 18° (Chui et al, Biomed Opt Exp, 2012) by directing the subjects’ gaze using a fixation target. Offline, non-rigid registration was used to obtain a final image for analysis. Cones were counted using a custom MATLAB program and cone geometry was assessed by fit of Voronoi polygons. Cone density (cones/mm²) was estimated after correction of the image magnification based on axial lengths (ROP = 22.5 ± 1.11 mm vs. CT = 23.6 ± 1.10 mm). Effects of group and eccentricity were detected by repeated-measures ANOVA.

**Results:** Cone density decreased with increasing retinal eccentricity (4.5° to 13.5°) in all groups. Cone density was markedly lower in SROP than in CT and MROP eyes (p<0.01); however, image quality was also lower in SROP subjects. The Voronoi polygons were significantly more irregular in SROP than in MROP and CT eyes (p<0.05).

**Conclusions:** SROP (but not MROP) subjects displayed reduced number and organization of cone photoreceptors. This may indicate loss of cone photoreceptors, consistent with recognized visual deficits in SROP subjects. Alternatively, low cone counts maybe due to the relatively poor quality of images obtained from SROP eyes.

**Commercial Relationships:** Ramkumar Ramamirtham, None; Garima Soni, None; James D. Akula, None; Emily A. Swanson, None; Tara L. Favazza, None; Mircea Mujat, PSI Corp (E); PSI Corp (E); R D. Ferguson, PSI Corp (E); Toco Y. Chui, None; Anne Moskowitz, None; Anne B. Fulton, None

**Support:** EY10597

©2015, Copyright by the Association for Research in Vision and Ophthalmology, Inc., all rights reserved. Go to iovs.org to access the version of record. For permission to reproduce any abstract, contact the ARVO Office at pubs@arvo.org.

ARVO 2015 Annual Meeting Abstracts
The optical Stiles-Crawford effect strongly affects photoreceptor imaging in diseased retina

Michel Paques1, Chaïhra Miloudi1, Laurent Mugnier2, José Sahel6, Isabelle Bloch5, Florence Rossant6, Sarah Mrejen1. 1Clinical Investigation Center 1423, Quinze-Vingts Hospital, Paris, France; 2DOTA, ONERA, Chatillon, France; 3Telecom ParisTech, Institut Mines-Telecom, Paris, France; 4LISITE, ISEP, Issy-les-Moulineaux, France.

**Purpose:** The optical Stiles-Crawford effect (oSCE) describes the directional reflectance of cone photoreceptors. To which extent does it affect cone photoreceptor imaging in diseased eyes is poorly documented. Here we investigated by adaptive optics (AO) en face imaging and optical coherence tomography (OCT) the directional reflectance of photoreceptors in patients recovering from macular edema.

**Methods:** AO fundus images were obtained through dilated pupils with a commercially available flood imaging AO camera (rtx1™ camera; Imagine Eyes, Orsay, France) in normal eyes (n=6) and eyes recovering from macular edema (n=6). Cone density counts were compared in sets of three images with three different entrance pupils: one through the central cornea (termed here coaxial image) and two after laterally shifting the entry pupil by 2.3° in both directions. Fusion maps were constructed after realignment of the three maps. The optical characteristics of control and diseased retina were analyzed in homologous areas. In parallel, OCT scans taken through these different entry pupils were aligned and fused.

**Results:** In patients, coaxial AO images and OCT scans both showed patchy defects of photoreceptor structures. Comparison of AO images at different entrance pupils showed that, in diseased areas, 65% (range, 47-79%) of cones were detected only through one of the 3 entry pupil versus 34% (range, 23-41%) in controls. Fusion maps increased the amount of detected cones by a mean of 69% (range, 26-113%) versus 24% (range, 5-56%) in controls. Final cone counts on fusion maps ranged from 31% to 56% of controls. On OCT scans of the same areas, the interdigitation line showed the most important directional reflectance; fusion of multiangle OCT scans markedly reduced the extent of outer layer destructuration.

**Conclusions:** Following retinal edema, photoreceptors show striking modifications of their optical properties which may lead to an overestimate of destructuration of the outer retina. Integration of AO images as well as OCT scans taken at different entry pupils improves the characterization of photoreceptor density and structure. These changes may participate to visual impairment. Further investigations are needed to determine if this is due to increased oSCE and/or to cone misalignment, the evolution of these changes over time and their presence in other retinal diseases.

**Commercial Relationships:** Michel Paques, ImagineEye (C), ImagineEye (C), UPMC (P), UPMC (P); Chaïhra Miloudi, None; Laurent Mugnier, None; José Sahel, ImagineEye (S); Isabelle Bloch, None; Florence Rossant, None; Sarah Mrejen, UPMC (P).

**Support:** ANR-09-TECS-009 and ANR-12-TECS-0015-03

**Clinical Trial:** NCT01546181

**Program Number:** 4934

**Poster Board Number:** B0062

**Presentation Time:** 3:45 PM–5:30 PM

**Program Number:** 4935

**Poster Board Number:** B0063

**Presentation Time:** 3:45 PM–5:30 PM

**Perifoveal correlations between cone mosaic, achromatic acuity and L-cone acuity**

Siri Bjørnetun Jacobsen, Jon B. Gjelle, Elisabeth Brattie Finstad, Stuart J. Gilson, Rigmor C. Baraas. 1Biomedical Engineering, FH Aachen University of Applied Sciences, Juelich, Germany; 2Institut de la Vision, INSERM, CIC 1243, Paris, France; 3Eye Clinic, Munich University of Technology, Munich, Germany.

**Purpose:** To investigate perifoveal measures of achromatic acuity and L-cone acuity and its association with cone density in healthy young men using experimental psychophysics and high-resolution retinal imaging.

**Methods:** Twenty-one healthy males aged 21–31 years, with normal logMAR letter acuity and no observed ocular abnormalities, were included in the study. Color vision was examined with a battery of standard tests. Achromatic and isolated L-cone spatial acuity was measured in the dominant eye with a Sloan E letter of 90% achromatic decrement contrast or 23% L-cone increment contrast, respectively. The Sloan E was presented at 5 deg temporal eccentricity and fixation was verified by an eye-tracker. Observers were corrected to best logMAR letter acuity and viewed the stimuli monocularly from a distance of 2.3 m. Average luminance of the stimuli was 10 cd/m². Para- and perifoveal areas within the central 12 deg of the dominant eye were imaged with the Kongsberg Adaptive Optics Ophthalmoscope II. The subject’s eye was dilated and accommodation suspended with Cyclopentolate 1% prior to imaging. Cone density and nearest-neighbor distance (NND) analysis was performed using custom software.

**Results:** LogMAR acuity for achromatic and L- cone experiments ranged from 0.330.53 and 0.460.80, respectively. All observers had cone densities within the normal range. There was no correlation between achromatic logMAR and cone density or achromatic logMAR and mean NND at 5 deg. There was, however, a significant correlation between higher cone density and better L-cone logMAR (r=-0.58, p<0.01) and lower mean NND and better L-cone logMAR (r=-0.51, p<0.05).

**Conclusions:** The results indicate that L-cone acuity, but not achromatic acuity, may be a useful clinical measure to determine changes in the perifoveal cone mosaic.

**Commercial Relationships:** Siri Bjørnetun Jacobsen, None; Jon B. Gjelle, None; Elisabeth Brattie Finstad, None; Stuart J. Gilson, None; Rigmor C. Baraas, None.

**Program Number:** 4936

**Poster Board Number:** B0064

**Presentation Time:** 3:45 PM–5:30 PM

**Automatic cones counting in adaptive optics images in healthy subjects**

Konstantin E. Kotliar1, Peter Linder1, Lislotte A. Sigha Yongua1, Ines Lanz1, Jose A. Sahel1, Ieva Sliesoraityte1. 1Biomedical Engineering, FH Aachen University of Applied Sciences, Juelich, Germany; 2Institut de la Vision, INSERM, CIC 1243, Paris, France; 3Eye Clinic, Munich University of Technology, Munich, Germany.

**Purpose:** Reliable automatic cone detection and cone mosaic assessment using adaptive optics (AO) is of the key importance in tracking of macular pathologies, especially ones related to the inherited retinal degenerations (e.g. Usher syndrome). The automatic cone detection (especially fovea centered) in AO is an unsolved issue mainly due to the dense cones concentration both in healthy retina and in retinal pathologies. An automatic cone quantification tool was developed with the aim to provide quick and reliable cone counting in fovea centered AO images. The tool was first tested in healthy subjects. In addition cone mosaic profile was compared between healthy subjects and patients with Usher syndrome.

**Methods:** A flood-illumination AO retinal camera (rtx1, Imagine Eyes, France) was used to acquire fovea centered images of the cone mosaic. The cone mosaic in 10 images of 8°x8° (1500x1500 pixel) corresponding to the macular area of 1181x1181 µm were analyzed in healthy subjects. The automatic tool in LabVIEW visual programming language (National Instruments, USA) with sequenced image filtration was employed to obtain clearly discerned cell mosaic. The reliability of the automatic tool was compared to an expert.
based manual counting using a set of 30 images with 120x120 pixel (95x95μm), which were randomly selected from the initial large images. The repeatability of manual and automatic counting was tested in 3 randomly selected images. In addition, healthy subjects’ cone mosaic profile was compared with ones having genetically confirmed Usher syndrome.

**Results:** The mean amount of cones per 1 mm² was 23120(21760 – 23530) [median(1st quartile – 3rd quartile)]. There was no significant difference in cones count comparing automatic counting: 248(213 – 282) cells/picture, and manual counting: 248(214–273) cells/picture (p = 0.8, Wilcoxon test). The repeatability amounted to 100% (automatic counting) and to 98% (manual counting). Comparing healthy subjects’ cone mosaic profile with ones having Usher syndrome significant decrease of 30% in cones quantity per area was shown.

**Conclusions:** Our developed automatic cone detection tool seems to be reliable for automatic cone quantification and mosaic assessment in the normal fovea using AO images. In addition, the automatic tool could be potentially used for tracking macular pathologies, especially ones related to inherited retinal degenerations.

**Commercial Relationships:** Konstantin E. Kotliar, None; Peter Linder, None; Lislotte A. Sigga Yongua, None; Ines Lanzi, None; Jose A. Sahel, None; Ieva Silesoraityte, None

**Support:** ERAREIN58: Eur-USH

**Clinical Trial:** NCT01954953

**Program Number:** 4937 Poster Board Number: B0065

**Presentation Time:** 3:45 PM–5:30 PM

**Reliability and Repeatability of a Quality Grading System for Para-foveal Cone Photoreceptor Adaptive Optics Images**

Nick M. Muthiah1, Fred K. Chen2, Joe Zhong3, Zoe McClelland4, Ferenc Sallo5, Tunde Peto6, Pete Coffey6, Lyndon da Cruz7.

1NIHR Biomedical Rschr Ctr for Ophthalmology, Moorfields Eye Hospital & UCL IOO, London, United Kingdom; 2UCL Institute of Ophthalmology, London, United Kingdom; 3Moorfields Eye Hospital, London, United Kingdom; 4Centre for Ophthalmology and Visual Science, University of Western Australia, Perth, WA, Australia.

**Purpose:** To investigate the reliability and repeatability of an image quality – grading system for cone photoreceptors imaged on an adaptive optics (AO) camera.

**Methods:** 50 healthy subjects, aged 20-35 years old, with no eye diseases were recruited prospectively. The left eyes of the participants were imaged using an AO camera (rtx1, Imagine Eyes, Orsay, France) at 5 degrees of retinal eccentricity, twice. The 2 images acquired on each subject were anonymized and randomized and the resulting 100 independent unpaired images were graded. A set of AO images validated by two independent experts in AO images and confirmed by a master grader was used as the basis of the 3-level grading system. Two masked independent observers (A and B) graded all the 100 images twice. Grading concordance was assessed by percentage of perfect agreement and kappa statistics.

**Results:** Exact intra-grader agreement on the quality of images for A and B were 90% and 79% respectively, (unweighted k statistics 0.85 and 0.68 respectively, P<0.001). Exact inter-grader agreement between A and B’s, 1st grade was 73% and 2nd grade in 76% (unweighted k statistics 0.59 and 0.63 respectively, P’s < 0.001).

**Conclusions:** The high intra and – inter grader agreement of observer A and B’s 1st and 2nd grades from this study demonstrates a high degree of reproducibility of the grading scores. This AO image grading system can be used as a reliable indicator for assessing the quality of images acquired on this AO camera prior to assessing changes in cone counts.

**Commercial Relationships:** Nick M. Muthiah, None; Fred K. Chen, None; Joe Zhong, None; Zoe McClelland, None; Ferenc Sallo, None; Tunde Peto, None; Pete Coffey, None; Lyndon da Cruz, None

**Program Number:** 4938 Poster Board Number: B0066

**Presentation Time:** 3:45 PM–5:30 PM

**Evaluating Descriptive Metrics of the Human Photoreceptor Mosaic**

Robert F. Cooper1, Melissa A. Wilk2, Alfredo Dubra3, Joseph Carroll1, 3, 1Biomedical Engineering, Marquette University, Wauwatosa, WI; 2Cell Biology, Neurology and Anatomy, Medical College of Wisconsin, Milwaukee, WI; 3Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 3Biophysics, Medical College of Wisconsin, Milwaukee, WI.

**Purpose:** Vital to expanding the clinical utility of adaptive optics (AO) ophthalmic imaging is the development of robust geometrical metrics for describing the photoreceptor mosaic. This requires a firm theoretical understanding of the strengths and limitations of various metrics as well as empirical data on the sensitivity of individual metrics and the relationships between metrics. Here we explore the relationship between individual metrics as a potential space to identify abnormalities across subjects. In particular, we focus on the effects of diffuse and focal photoreceptor loss.

**Methods:** Between 10 and 90 regions of interest were extracted from 14 normal foveal and split-detector AO scanning light ophthalmoscope photoreceptor montages, and cone locations were identified using a previously described algorithm. Density, nearest neighbor distance (NND), inter-cell distance (ICD), furthest distance (FD), percent six-cell neighbors, nearest neighbor regularity (NRR), number of neighbors regularity (NoNR), and Voronoi cell area regularity (VCAR) were extracted from coordinates with bound Voronoi regions. Confidence intervals (CIs) with 95% significance were calculated for each of the metric relationships. Between 5-80% of cones were removed to simulate diffuse and focal cell loss.

**Results:** NND, ICD, and FD fell outside their CIs at 50, 80 and 30% diffuse cell loss, respectively. Percent six-cell neighbors, NRR, NoNR, and VCAR fell outside their CIs at 10, 30, 60, and 10% cone loss, respectively. All regularity metrics were more sensitive to cell loss at lower densities. VCAR was most sensitive to focal cell loss, with its values falling outside the CI at 5% loss. ICD was sensitive to focal changes in high-density mosaics above 15% focal loss, whereas FD fell outside its CI at 31% focal loss in only low-density mosaics. NND, NoNR, NNRI, and percent six-cell neighbors were insensitive to any focal cell loss.

**Conclusions:** Spacing metrics are insensitive to cell undersampling, which enables estimating mosaic spacing in cases where every cell cannot be reliably identified. However, these same metrics are unable to detect early pathology. Conversely, regularity metrics are highly sensitive to both focal and diffuse cell loss, but require accurate cell identification. Thus, both types of metrics may be needed in combination to provide complete and accurate assessments of mosaic integrity.

**Commercial Relationships:** Robert F. Cooper, None; Melissa A. Wilk, None; Alfredo Dubra, US Patent 8,226,236 (P); Joseph Carroll, None

**Support:** T32EY014537, R01EY017607, P30EY001931, Research to Prevent Blindness, Departmental and Career Award (Dubra)
Purpose: The objective of this project is to develop, test and validate software allowing to estimate photoreceptor density derived from the inner segment ellipsoid (ISe) band reflectivity on en-face OCT.

Methods: Eight eyes displaying various degrees of outer retinal impairment (five eyes with resolved central serous choriorioretinopathy, one with hydroxychloroquine toxicity, and two healthy eyes) were imaged using a spectral-domain OCT (Spectralis, Heidelberg, Heidelberg Engineering, Germany). En-face OCT was generated from horizontal transverse scans, 11µ apart, with a resolution of 384A scan/Bscan. Segmentation of the Ise band was automatically performed with the Heidelberg Explorer plug-in provided by the manufacturer. The relevance of the “PRI” segmentation line was checked by two readers. At the same time, a software running on Matlab, (Matlab, Mathworks Inc., Natick, USA) that transforms the grey level into cone density was developed. Its algorithm is based on a previous work comparing OCT reflectivity and adaptive optics. The program determines the cone density in a ring ranging from 1.5 to 2 degrees of eccentricity from the fovea. In order to validate the estimates, cone densities were measured in the same eyes, in a blind manner, by an independent observer, using adaptive optics (AOdetect v0.2, Imagine Eyes, Orsay, France).

Results: Thirty five points of comparison were obtained. The program estimates were closely correlated with the results of the manual counting method, i.e. adaptive optics (r=0.81, p<0.05). Bland-Altman plot displayed a moderate estimated bias of 204±92 cells/µm² (CI95%: 508-5418). The estimation was considered relevant (defined as a difference of more and less 10%) in 80% of the cases.

Conclusions: This work was supported by AFCRO grant number [130712].

Method: AO fundus images were obtained through dilated pupils with a commercially available flood imaging AO camera (rtx1™ camera; Imagine Eyes, Orsay, France; illumination wavelength 840nm) within an IRB-approved clinical study in 5 normal eyes. The routine acquisition procedure comprised a stack of 40 raw images acquired over 4.2 seconds, 2° from the fovea. For each subject, raw images were deconvolved with a myopic deconvolution method [Blanco and Mugnier, 2011] and underwent automatic cone detection [Loquin et al. 2011] over a 94x94µm area. Cone maps obtained from raw images were fused; the increment in cone density at every step was measured. The results were compared to cone maps obtained by image averaging (AO image 2.0).

Results: In three subjects, the increment in cone density reached an asymptotic plateau between the 10th and 20th map integration. In one subject the plateau was not obtained (i.e. the addition of new raw images increased the total amount of cone detected). The last subject had limited scintillation, hence there was limited increment. The relative improvement in total number of detected cone by fusing 20 raw deconvolved images compared to the number of cones detected on averaged image ranged from 5% to 71%.

Conclusions: Fusion of deconvoluted raw images improves the quality of cone detection from flood-illuminated AO images.

Purpose: To present the reproducibility and repeatability of cone detection and density measurement, comparing the automatic count to the manual count. The imaging quality and individual differences of operators are also considered using by adaptive optics scanning laser ophthalmoscopes (AOSLO) images and preprocessing with software of ARIA system, Canon Inc.

Methods: Total 22 eyes of 11 healthy subjects were included for repeatability test of AOSLO imaging system, prototype II Canon. The S1(340X340um) image was taken at each different meridian of macula of the same 1.0mm distance from fovea by the same operator in 3 different days. Another 18 eyes of 9 healthy volunteers were recruited for reproducibility test and P0 (680X680um) images were also obtained by the same method above, but performed by 3 different skilled operators following totally randomized order within 30 minutes. The 100X100um area was selected by choosing the entirely same cone mosaic location of 3 different images from different times (repeatability) and different operators (reproducibility). All the selected 100X100um images were measured automatically by ARIA and point-by-point by manual counting. Each selected image was also evaluated using the subjective score grading level from 0 to 10. Using blocking analysis of variance (ANOVA)
Conclusions: The significantly greater reflectance change where the cone density was higher (1 degree) indicates that the amount of reflectance change is related to the cone density.

Commercial Relationships: Masakazu Hirota, None; Suguru Miyagawa, Topcon (E); Hiroyuki Kanda, None; Takao Endo, None; Tibor K. Lohmann, None; Takeshi Morimoto, None; Takashi Fujikado, None.

Program Number: 4943 Poster Board Number: B0071
Presentation Time: 3:45 PM–5:30 PM
Cone spacing measures in visually normal eyes imaged at baseline and 12 months
Shane Griffin1, Angela N. Baldwin1, Nicolas Sippl-Swezey1, Jaskiran Mann1, Panagiota Loumou1, Ravi Keshavamurthy2, Travis Porco1, Austin Roorda2, Jacque L. Duncan1.
1Ophthalmology, University of California San Francisco, San Francisco, CA; 2School of Optometry and Vision Science Graduate Group, University of California Berkeley, Berkeley, CA.

Purpose: To determine the inter-visit and inter-grader variability of Adaptive Optics Scanning Laser Ophthalmoscopy (AOSLO) derived cone spacing measures in normal eyes monitored longitudinally over 12 months. Analysis of cone spacing measures in normal eyes will establish the variability of cone spacing as an outcome measure for use in clinical studies of photoreceptor change utilizing AOSLO.

Methods: Eight visually normal patients underwent AOSLO imaging at 3 visits; 2 baseline visits were separated by no more than 1 month, and one visit occurred 12 months after the baseline visits. Cone spacing was measured in each image by two independent graders. Cone spacing measures were compared between visits and graders, and then correlated with standard macular measures including visual acuity, foveal sensitivity, Goldmann perimeter, and spectral-domain optical coherence tomography (SD-OCT)-derived outer segment layer thickness.

Results: Cone spacing was measured in 424 regions within 3.5 degrees of the fovea. Agreement between graders was strong (ICC = 0.87, 95% confidence interval (CI) 0.81-0.90); mean difference between graders was 0.07 arcminutes (95% CI 0.05-0.08). Median absolute difference between cone spacing measures at each ROI between the 2 baseline visits was 0.047 arcminutes (95% CI=0.04-0.059). Cone spacing at 12 months decreased by -0.0039 arcminutes (95% CI: -0.01 to 0.0023), consistent with no change over time in this normal population. Similarly, there were no significant changes in any clinical measures of retinal degeneration.

Conclusions: A high degree of inter-visit and inter-grader agreement of AOSLO-derived cone spacing measures was observed in visually normal eyes examined twice at baseline and at 12 months. The reproducibility of cone spacing measures in normal eyes lends support to the future use of AOSLO in clinical trials studying photoreceptor change over time.

Commercial Relationships: Shane Griffin, None; Angela N. Baldwin, None; Nicolas Sippl-Swezey, None; Jaskiran Mann, None; Panagiota Loumou, None; Ravi Keshavamurthy, None; Travis Porco, None; Austin Roorda, University of Houston, US 7,118,216 (P), University of Rochester, US 6,890,076 (P); Jacqueline L. Duncan, None.


Clinical Trial: NCT00254605
Stimulus-evoked optical response of individual cone photoreceptors observed with adaptive optics scanning light ophthalmoscopy
Kenneth M. McKay1, Zachary Harvey2, Grace K. Han1, Alfredo Dubra2∗, Jessica I. Morgan1. 1Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2Ophthalmology, Medical College of Wisconsin, Milwaukee, WI; 3Scheie Eye Institute, Ophthalmology, University of Pennsylvania, Philadelphia, PA; 4Biophysics, Medical College of Wisconsin, Milwaukee, WI.

Purpose: In vivo functional assessment of the retina at the cellular level remains elusive and is increasingly important as therapies for blinding conditions are developed. Though the physiology is largely unknown, stimulus-evoked intrinsic signals have potential to assess photoreceptor function. Here, we describe a stimulus-evoked optical response, visible at the cellular level.

Methods: Neither dark adapted nor bleached parafoveal cones in 3 normal controls (2 females, ages 34 and 24 and 1 male, age 27) were imaged using a custom adaptive optics scanning light ophthalmoscope. A one-degree square field was illuminated with 8.3μW of 848nm for wavefront sensing, 75μW of 795nm ∆15nm for reflectance imaging, and a background of 344.7nW at 675nm. Half of the field was stimulated with 17.4μW of 675nm for 2s. Images were acquired at 16Hz for 5s before, during, and 20s after stimulation. Intensities of stimulated cones in the 795nm image were normalized by the average intensity of non-stimulated cones in the same image. The change in intensity ratio over time was calculated by subtracting the average pre-stimulus intensity ratio from the intensity ratio for each stimulated cone in each frame. Then, for each image, the standard deviation of the change in intensity ratios over all stimulated cones was calculated to describe the global reflectance variability across the stimulated cones.

Results: We observed a stimulus-evoked response in approximately just under half of cones in the stimulated field of all 3 subjects. Following the stimulus onset, the cone intensity ratio either oscillated between bright and dim, increased, decreased, or remained unchanged. While an individual cone’s change in intensity ratio pattern was not repeatable, each trial did produce a response. On average, the reflectance variability metric reliably increased 229ms (188, 250, 250ms) post-stimulus onset. Non-stimulated cones did not show a response.

Conclusions: We describe a cellular-level near infrared optical response to visible red light stimulation, possibly showing L cone function. Future studies will correlate this optical response to physiological function and examine the response in conditions where photoreceptor function is compromised.

Commercial Relationships: Kenneth M. McKay, None; Zachary Harvey, None; Grace K. Han, None; Alfredo Dubra, 8226236 (P), Canon, Inc. (C); Jessica I. Morgan, 8226236 (P), Canon Inc. (C), Canon Inc. (F), Optos, PLC (F), Optos, PLC (R)

Support: Foundation Fighting Blindness, Glaucoma Research Foundation Catalyst for a Cure, Research to Prevent Blindness Career Development Award, National Institutes of Health (Grant EY019861), Research to Prevent Blindness, the F. M. Kirby Foundation, the Paul and Evanina Mackall Foundation Trust, Lois Pope Life Foundation, and the Jeffrey W. Berger Medical Student Research in Ophthalmology Award.

Clinical Trial: NCT018666371