Clinical Applications of Retinal Imaging with Adaptive Optics

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Abstract

The first images of the living human retina were published in 1886. In the 125 years since then, clinicians and researchers alike have relied on the continued development of new and improved ways to visualize the fundus to provide better *in vivo* views of the normal and diseased retina. Adaptive optics-based technologies offer non-invasive, cellular resolution views of the living retina, and bring with them several exciting clinical applications. Here we review some of these applications, with an emphasis on photoreceptor imaging.

Keywords

Retina, imaging, photoreceptors, retinal degeneration

Adaptive Optics Imaging—The Basics

Before getting into the clinical utility of adaptive optics imaging technology, it is prudent to first review the basic principles of imaging with adaptive optics. With conventional optical imaging, the major factor limiting the achievable resolution is the eye's monochromatic aberrations, which are due to imperfections in the optics of the eye. These wavefront aberrations can be separated mathematically into shapes described by low order polynomials (defocus and astigmatism) and higher order polynomials (e.g. coma and trefoil). Although lower order aberrations can be effectively corrected using spectacles or contact lenses, the higher order aberrations cannot over a large field of view. Their effect on visual function is not typically severe; however, higher order aberrations interfere with high-resolution retinal imaging. Ophthalmic adaptive optics systems are designed to measure and correct for these higher-order aberrations, and can provide image resolution that is limited only by the pupil diameter of the eye, the axial length of the eye, and the wavelength of light. As shown in *Figure 1*, ophthalmic adaptive optics imaging systems have three main components—a wavefront sensor (typically a Shack-Hartmann design, for measuring the eye's aberrations), a corrective element (typically a deformable mirror, for correcting the aberrations), and an imaging device (typically a charge-coupled device [CCD] or photomultiplier tube). These design principles are not absolute, and alternative approaches that do not use a wavefront sensor[1] or that use multiple corrective elements[2,3] have been demonstrated. Nevertheless, the unifying feature of adaptive optics imaging systems is mitigation of the eye's aberrations to achieve nearly diffraction-limited imaging. These imaging systems have so far taken the form of an adaptive optics fundus camera,[4,5] an adaptive optics scanning laser ophthalmoscope,[6] or an adaptive optics optical coherence tomograph (OCT).[7–9]

Current imaging systems are able to noninvasively resolve numerous structural features of the living human retina. As demonstrated by multiple groups, it is now possible to image both rod and cone photoreceptors, including foveal cones, which are the smallest photoreceptor cells in the retina (see *Figure 2*).[10–13] Much work has also been done on characterizing the normal photoreceptor mosaic,[10,11,14–18] although larger databases and convergence on image analysis metrics is needed. While much of the clinical efforts have been directed at imaging the photoreceptors, the ability to resolve other features of the retina is likely to be useful in studying diseases such as glaucoma (lamina cribrosa, nerve fiber layer, ganglion cells), age-related macular degeneration (retinal pigment epithelium [RPE]), and diabetic retinopathy (retinal vasculature). There have been a handful of reports on visualizing RPE cells in the normal human retina using intrinsic autofluorescence in the normal retina[19] or reflectance in some patients with photoreceptor degeneration.[20] In addition, many groups have developed motion-based techniques to examine the retinal vasculature, which makes it possible to noninvasively measure blood velocity and visualize the smallest foveal capillaries.[6,21–26] Efforts to image these and other retinal structures are likely to continue to increase in the coming years, and will have important clinical applications. In this article,

we focus on the current knowledge of imaging photoreceptors in retinal diseases using various adaptive optics imaging modalities.

A Brief History of Retinal Imaging with Adaptive Optics

The early scientific applications of adaptive optics imaging of the human retina focused on the organization of the trichromatic cone mosaic.[27,28] Investigations since then have explored the waveguide tuning of individual photoreceptors,[29] variability in the trichromatic cone mosaic,[30] and temporal variability in photoreceptor reflectance.[31–37] There has also been extensive application of adaptive optics to study visual system function, and these studies have been recently reviewed elsewhere.[38] In 2000, Austin Roorda reported the first application of adaptive optics to image human retinal pathology when he discussed a patient with a cone–rod dystrophy.[39] Since that time, the clinical use of adaptive optics imaging has increased (see *Figure 3*). The following highlights a few examples where adaptive optics imaging has provided advancement in our understanding of a retinal disease or where it has been used to demonstrate potential future clinical applications.

Retinal Degenerations

Retinal degenerations are an extremely heterogeneous class of retinal disease, both in their genetic basis and clinical presentation. They are typically associated with pronounced vision loss, disrupted color vision, and variable degrees of fundus abnormalities, nystagmus, and light sensitivity. There has been great interest in examining the cellular phenotype associated with these conditions in which photoreceptor structure is compromised.[14,40,41] In patients with cone–rod dystrophy or retinitis pigmentosa, disruptions in the cone mosaic have been observed using an adaptive optics fundus camera, although these were all in areas already identified as abnormal on standard clinical tests such as fundus imaging, multifocal electroretinography (mfERG), or perimetry.[14,40,41] In the future, access to cellular-resolution images should allow examination of the earlier phases of these retinal degenerative diseases before the overt disruption of structure or function that can be detected clinically.

By themselves, adaptive optics images are just pictures. When coupled with genetic information, a context is gained that enables more insightful interpretation of the features in the images. Jacque Duncan and Austin Roorda pioneered the approach of studying the cone mosaic in patients with retinal degeneration and known genetic mutations. In their first study, they examined a patient with X-linked cone–rod dystrophy (caused by a mutation in the RPGR gene) and a patient with autosomal dominant retinitis pigmentosa (caused by a mutation in the rhodopsin gene).[14] Since then, they have expanded their efforts to *peripherin/RDS*-associated retinal degeneration,[18] ABCA4 mutations in Stargardt disease,[42,43] and mitochondrial DNA mutations in neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) syndrome.[44,45] It is likely that the combination of high-resolution phenotyping with adaptive optics imaging with detailed molecular genetic analyses will be the area of largest growth in the coming years as other investigators adopt a similar approach to clinical imaging with adaptive optics imaging systems.

Inherited Color Vision Deficiencies

It is well known that inherited color vision defects affect cone photoreceptor function, but until recently it was unclear how cone photoreceptor structure

**Figure 1: Schematic of an Adaptive Optics Retinal Imaging System**
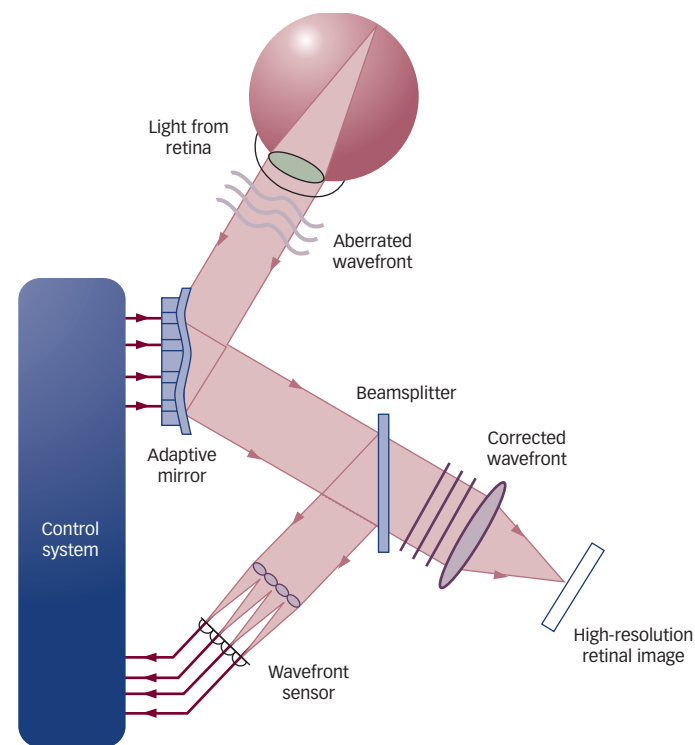
A beam of light is shined into the eye, and a small amount is reflected back out of the eye and into the optical system. Reflected light is split between a wavefront sensor, which measures the aberrations, and the imaging device. The control system sends a signal to the adaptive/deformable mirror, whose surface then changes shape to minimize the wavefront aberration. Source: modified from Carroll et al., 2005, with permission.[117]

might be compromised in these patients. Imaging studies on patients with inherited red–green and blue–yellow color vision defects have shown loss of healthy waveguiding cones, although the degree of cone loss and pattern of cone mosaic disruption varies depending on the particular genotype.[46–50] One specific mutation leading to a red–green defect is actually a deleterious combination of otherwise normal polymorphisms found in the long- (L) and middle-wavelength sensitive (M) pigments (encoded by exon 3 of the L [or M] pigment gene). In a patient whose M gene encoded this mutant pigment, we observed a patchy-appearing cone mosaic, presumably reflecting isolated dropout of the M-cone submosaic (see *Figure 4*). Besides a deutan color vision defect, this particular disruption in the cone mosaic had no effect on vision measured clinically, although a microperimeter equipped with adaptive optics was able to demonstrate that the dark areas of the image results in functional microscotomas.[51] Consistent with the idea that only cones expressing the mutant M pigment were structurally compromised, there was no progression visible over a period of nearly six years.[52] It is still not clear how common these mosaic disruptions are; however, given that tools that can accurately detect such a phenotype have only been available for a few years, we suspect that more examples will emerge as more patients are examined with adaptive optics imaging tools.

Although rare, there are more severe forms of color vision deficiency—achromatopsia and blue cone monochromacy (BCM). Patients with these diseases can have reduced/absent cone function, reduced

Figure 2: Adaptive Optics Images of the Human Photoreceptor Mosaic
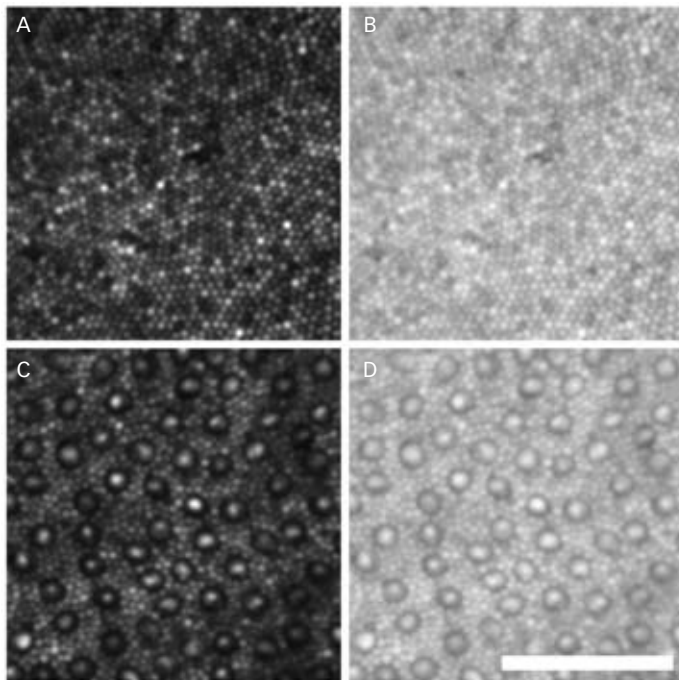
Images of the foveal center (A, B) and peripheral retina (C, D) are shown for a single subject. Images are presented on a linear (A, C) and logarithmic (B, D) scale. All the cells in the foveal images are presumed to be cones, whereas the peripheral image contains both cones and rods. The rods are the smaller structures packed between the coarser cone mosaic. The dark region in the lower right of panel C is the shadow of a blood vessel. In the peripheral image, the dark ring associated with each cone is presumably the boundary of the inner segment, and the central reflective core is from the outer segment and/or inner–outer segment junction. Scale bar is 50 μm.

Figure 3: Number of Peer-reviewed Manuscripts by Year that have Used Adaptive Optics Systems to Image the Retina in One or More Patients
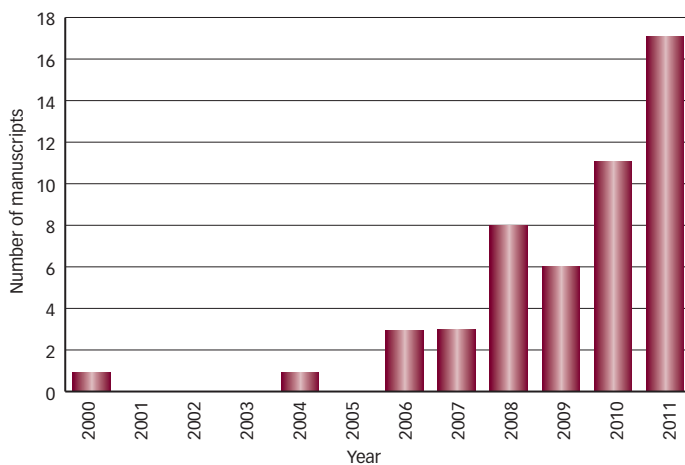
A literature search (August 18, 2011) found 50 such papers.[10,12–16,18,39–50,52,56–58,62,63,71,72,76–96,98,99]

visual acuity, nystagmus, and photophobia. The fate of the cone photoreceptors in these conditions has taken on added relevance given recent successes in gene therapy in animal models of achromatopsia.[53–55] Despite the substantial loss of cone function, imaging with adaptive optics has shown that patients with achromatopsia have retained cone structure, although to a variable degree.[12,56,57] *Figure 4B* shows an image

of the photoreceptor mosaic from a patient with achromatopsia. The large dark circular structures presumably represent intact cone inner segments, although they are reduced in number compared with normal. Some of the cones in these patients even have what appears to be a central reflective core,[12,56,57] which is consistent with the appearance of normal peripheral cones (see *Figure 2*). A study of female carriers of BCM found significant reduction in cone density with fairly continuous foveal cone packing, suggesting that cones in these patients degenerated early in retinal development.[58] More work remains to clarify the degree of cone structure, to examine how it correlates with documented genetic heterogeneity in these patients, and to assess the integrity of these cones over time in the same patients.

Albinism

Albinism is associated with disrupted melanin biosynthesis, resulting in decreased or absent pigment in the hair, skin, and/or eyes. All forms involve significant ocular manifestations, including iris transillumination, macular translucency, photosensitivity, refractive errors, astigmatism, nystagmus, and reduced acuity. Foveal manifestations include absence of a foveal avascular zone (FAZ), foveal hypoplasia, and loss of an annular reflex. The maturity of the foveal cone mosaic has been a topic of interest[59–61] because it may help shed some light on the retinal versus cortical contributions to the reduced visual function in these individuals. Marmor et al. were the first to apply adaptive optics imaging to patients with suspected albinism.[62] They found evidence that despite the absence of a fully developed foveal pit, there was still a relative increase in cone density near the fovea compared with the parafovea. McAllister et al. followed this study and showed that some patients with albinism have near-normal cone packing, whereas others had no increased packing of foveal cones (see *Figure 5*).[63] Whether this is because of different albinism subtypes, mutations, or constitutional pigment background remains to be determined, but there is clearly pronounced variation across patients. The role of this variation in visual function is also currently unexplored, although recent OCT data demonstrate that the foveal pit does not correlate with visual function but outer segment length does.[64] Future studies should include measurements of foveal morphology with OCT, cone packing with adaptive optics, as well as measurements of visual acuity.

Glaucoma

Current clinical monitoring of glaucoma progression involves monitoring nerve fiber layer thickness, cup-to-disk ratio, visual field sensitivity, and inter-ocular pressure. Adaptive optics imaging has begun to be applied to glaucoma through examination of numerous retinal features. For example, in an experimentally induced primate model of glaucoma, *in vivo* adaptive optics images revealed altered morphology of the lamina cribrosa in glaucomatous versus fellow control eyes.[65] Ivers et al. demonstrated high reproducibility of measurements of lamina pore geometry in normal monkeys and humans.[66] Such measurements may also be possible with spectral domain-OCT (SD-OCT),[49,67] which offers additional morphological measurements not accessible with *en face* adaptive optics images.[68,69] It has been suggested that cone photoreceptor structure might also be compromised in glaucoma.[70] This has recently been demonstrated in human patients using flood-illuminated adaptive optics imaging,[71,72] although this needs to be examined with higher resolution devices to confirm how universal this feature is across a larger patient population and whether rods are also involved.

The structure of the nerve fiber layer bundles can also be assessed using adaptive optics OCT[49,73,74] and adaptive optics scanning laser ophthalmoscopy.[75] This provides yet another image-based metric that could be used to clarify the etiology of glaucoma *in vivo*. Although there are only a few groups exploring glaucoma with adaptive optics technology, given the prevalence of the condition and the ambiguity regarding the affect on retinal anatomy, these efforts are likely to increase in the coming years.

Other Clinical Studies

Given the novelty of adaptive optics imaging, there has been and continues to be interest in applying it to a wide array of retinal conditions, including 'coffee-and-donut' maculopathy,[76] basal laminar drusen,[77] bilateral macular dystrophy,[78] forms of macular dystrophy,[79,80] macular telangiectasia,[13,81–83] central serous chorioretinopathy,[84] unexplained metamorphopsia,[85] epiretinal membrane,[86] cotton wool spot,[87] laser retinal injury,[88] solar retinopathy,[89] optic nerve drusen and optic neuropathies,[90,91] macular arteriovenous malformation,[92] central retinal vein occlusion,[93] acute zonal occult outer retinopathy,[12] and foveal damage as a result of habitual popper use.[94] In many cases, when a disease is imaged with adaptive optics, it represents the first time anyone has seen the disease from this perspective. An obvious question is: what is the utility of such observational case studies? However, cumulatively these observations may help us to develop an intuition for how to interpret adaptive optics images obtained in more common retinal disorders. Space limitations prevent discussion of all these studies; however, there are three that highlight some important concepts in the clinical application of adaptive optics imaging. One example comes from a patient with a history of commotio retinae after an industrial accident. A 43-year-old male described a five-year history of a stable, crescent-shaped purple scotoma nasal to central fixation in his right eye that developed after he sustained significant head and body trauma. Clinical examination revealed vision of 20/20 OU and no retinal fundus abnormalities. Fluorescein angiogram and SD-OCT were unremarkable. However, a small non-specific area of visual dysfunction near fixation in the right eye was noted on Humphrey Visual Field 10-2 testing and microperimetry. Images of the photoreceptor mosaic near the fovea obtained with an adaptive optics ophthalmoscope revealed a well-defined crescent-shaped area of photoreceptor disruption just temporal to the fovea. SD-OCT of this same area showed no outer retinal irregularities. Smaller areas of focal photoreceptor irregularities surrounding the fovea were also seen in the adaptive optics images.[95] This case exposes the potential disconnect between photoreceptor structure visualized by clinical OCT and that resolved by adaptive optics imaging, highlighting the complimentary role these imaging modalities will need to play in studying the normal and diseased retina. If adaptive optics imaging is more sensitive than SD-OCT in detecting subtle photoreceptor changes, then future potential clinical applications could include using adaptive optics as a screening tool to detect retinal photoreceptor pathology at an earlier stage than is possible with current retinal imaging modalities. There are significant logistical challenges to increasing the accessibility of this technology, and it is likely that any screening approach would have to be highly targeted to be effective. Nevertheless, it is important to at least explore this application so as to best focus future research efforts. As a proof-of-principle, Stepien et al. described a case where hydroxychloroquine retinopathy was detected with adaptive optics imaging, but was not visible on other imaging modalities.[96] A 57-year-old asymptomatic female on hydroxychloroquine

Figure 4: Disrupted Photoreceptor Mosaics in Color Vision Defects
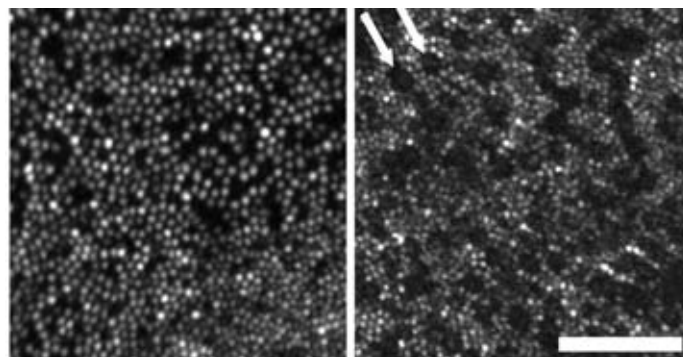

A foveal image from an individual with a red–green color vision defect (left) reveals a patchy photoreceptor mosaic owing to a reduced number of healthy waveguiding cones. A peripheral from an individual with achromatopsia (right) reveals a severely disrupted photoreceptor mosaic with reduced cone density. The larger dark circles (white arrows) indicate that numerous inner segments remain, and even a few appear to contain the central reflective core that is characteristic of normal peripheral cone structure (as seen in Figure 2*). Scale bar is 50 μm.*

for 20 years at a dose of 6.15 mg/kg/day for systemic lupus erythematous was referred for abnormal visual field testing. Clinical exam was unremarkable but SD-OCT showed areas of perifoveal outer retinal loss consistent with a beginning bull's-eye maculopathy. Adaptive optics imaging showed severely disrupted or loss of photoreceptor mosaic in areas of outer retinal loss as seen by SD-OCT. Furthermore, the adaptive optics images showed an irregular photoreceptor mosaic in areas of the retina where both visual field testing and SD-OCT imaging were normal. It is likely that these areas were already affected by hydroxychloroquine toxicity but not yet detected by current screening modalities. Patients taking medicines with possible retinal side effects represent a class of patients where adaptive optics imaging could become an effective tool for screening. A final example comes from a condition called oligocone trichromacy, a cone dysfunction syndrome characterized by reduced visual acuity, mild photophobia, reduced amplitude of the cone electroretinogram with normal rod responses, normal fundus appearance and normal/near-normal color vision.[97] It is unclear whether the reduced cone function in oligocone trichromacy is due to a reduced number of cones or whether the cones are present but not functioning normally. Given that the genetic basis of the condition is not known, information about photoreceptor structure could be of use in clarifying the etiology of the condition. Upon imaging four patients with suspected oligocone trichromacy, three out of four were found to have significant disruptions in the cone mosaic, with cone density reduced in the fovea by almost a factor of two compared with normal controls.[98] The fourth patient was found to have normal cone density together with a slightly different clinical presentation and the patient was heterozygous for a mutation in the CNGB3 gene. These data and the adaptive optics imaging results suggest that this patient does not have the same condition as the other three patients. This illustrates the potential use of adaptive optics imaging in confirming diagnoses or even in refining current clinical phenotypes to enable more accurate classification of retinal diseases.

Clinical Applications of Adaptive Optics Imaging—Moving Forward

As exciting as the past few years have been with regard to the emergence of more widespread clinical applications of adaptive optics imaging, the

Figure 5: Foveal Anatomy in Oculocutaneous Albinism
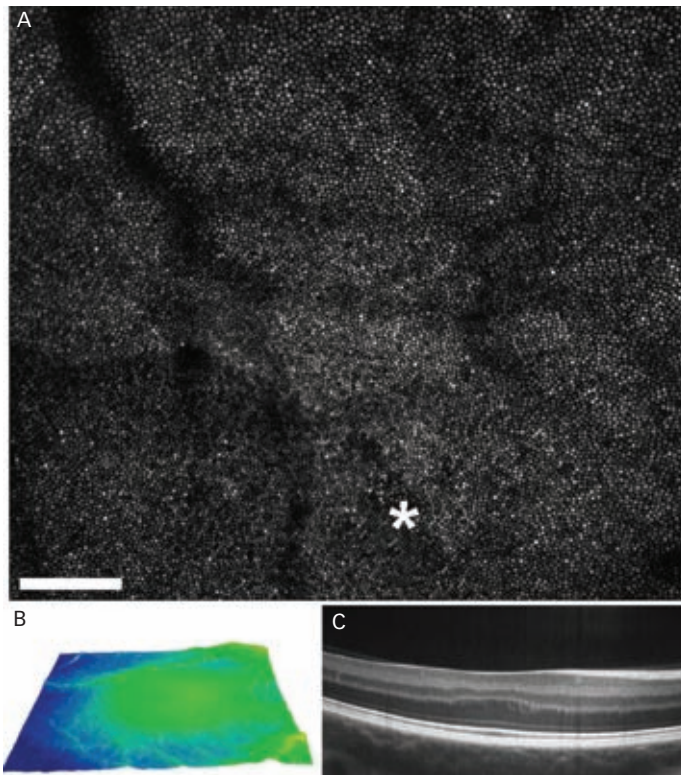
A montage of the foveal cone mosaic is shown (A), along with a topographical map of retinal thickness obtained with spectral domain-optical coherence tomography (SD-OCT) (B) and a horizontal SD-OCT scan through the center of fixation (C). Both of the OCT images reveal an absence of a normal foveal pit; however, as evident in the foveal montage, substantial cone packing can still occur. In this patient, peak foveal cone density (*) is 71,203 cones/mm^2, whereas density at ~4 degrees inferior is 14,896 cones/mm^2. Scale bar is 100 µm.

best is likely yet to come. Commercialization of robust, clinic-friendly devices is sure to expand access to cellular retinal imaging capabilities. How clinicians and research leverage this access remains to be seen, but there appears to be room for significant clinical applications. One such area where cellular imaging could make a positive impact is in the treatment of retinal diseases—for example, by identifying suitable patients for a specific therapeutic approach or by evaluating the retinal response to intervention. The potential for this latter application was recently demonstrated in a trial that aimed to use ciliary neurotrophic factor to preserve cone function in retinitis pigmentosa.[99] Efforts directed to foster communication and collaboration between clinicians and engineers should help in this regard,

and this will be particularly important as new applications of adaptive optics imaging are discovered. Particularly intriguing is the integration of adaptive optics with other imaging modalities such as photoacoustic imaging,[100] two-photon microscopy,[101] and OCT.[102,103] Other hardware improvements being brought to adaptive optics imaging systems include eye tracking and image stabilization.[104,105] Given that these devices are largely still in research laboratories, there is ample need to examine the clinical utility of these and other adaptive optics imaging modalities in the years to come. It is worth reiterating that this article focused on current and emerging applications of adaptive optics as it relates to imaging the human photoreceptor mosaic. There are, of course, numerous other applications, two of which are mentioned here. A comprehensive review on the use of adaptive optics for testing visual function has recently been published,[38] so we only touch on the topic here. Early applications of adaptive optics for testing visual function focused on demonstrating the benefits of correcting the eye's aberrations—such as improved contrast sensitivity and visual acuity.[5,106,107] However, there has been tremendous growth in this field, owing to improvements in adaptive optics technology, in particular, advances made with eye tracking and stimulus delivery.[104,108] From mapping receptive fields of geniculate neurons on a single-cell level[109] to probing chromatic sensations elicited by stimulating individual cones,[110] the scientific applications appear to be limited only by the creativity of the researchers involved. One of the more intriguing clinical applications is simultaneous imaging and stimulus delivery, which allows one to link some aspect of visual function to a specific photoreceptor or group of photoreceptors. For example, Rossi and Roorda were able to examine acuity at specific retinal locations where the cone mosaic had been visualized, allowing them to show that only at the fovea does visual acuity match the sampling limits of the cone mosaic.[111] In patients with certain retinal diseases, this approach could help clarify the functional significance of disruptions seen in adaptive optics images that currently escape definitive interpretation.

There are a growing number of applications of adaptive optics retinal imaging to different animals, including cats,[112] mice,[113] rats,[114] and non-human primates.[115] There is more freedom in these imaging experiments with regard to labeling specific cell types using contrast enhancing agents, which offers the possibility to image cell types (such as ganglion cells) that are currently not able to be imaged in the human retina.[115,116] With the diverse array of animal models of retinal disease, the ability to image retinal structure *in vivo* over time should have a significant impact on studies of disease etiology and also on the assessment of therapeutic response to experimental treatments for a given disease. ■

1. Hofer H, Sredar N, Queener H, et al., Wavefront sensorless adaptive optics ophthalmoscopy in the human eye, *Opt Express*, 2011;19:14160–71.
2. Li C, Sredar N, Ivers KM, et al., A correction algorithm to simultaneously control dual deformable mirrors in a woofer-tweeter adaptive optics system, *Opt Express*, 2010;18:16671–84.
3. Zou W, Qi X, Burns SA, Woofer-tweeter adaptive optics scanning laser ophthalmoscopic imaging based on Lagrange-multiplier damped least-squares algorithm, *Biomed Opt Express*, 2011;2:1986–2004.
4. Miller DT, Williams DR, Morris GM, et al., Images of cone photoreceptors in the living human eye, *Vision Res*, 1996;36:1067–79.
5. Liang J, Williams DR, Miller D, Supernormal vision and high-resolution retinal imaging through adaptive optics, *J Opt Soc Am A*, 1997;14:2884–92.
6. Roorda A, Romero-Borja F, Donnelly III WJ, et al., Adaptive optics scanning laser ophthalmoscopy, *Opt Express*, 2002;10:405–12.
7. Hermann B, Fernandez EJ, Unterhuber A, et al., Adaptive-optics ultrahigh-resolution optical coherence tomography, *Opt Lett*, 2004;29:2142–4.

8. Zhang Y, Rha J, Jonnal R, et al., Adaptive optics parallel spectral domain optical coherence tomography for imaging the living retina, *Opt Express*, 2005;13:4792–811.
9. Zhang Y, Cense B, Rha J, et al., High-speed volumetric imaging of cone photoreceptors with adaptive optics spectral-domain optical coherence tomography, *Opt Express*, 2006;14:4380–94.
10. Li KY, Tiruveedhula P, Roorda A, Intersubject variability of foveal cone photoreceptor density in relation to eye length, *Invest Ophthalmol Vis Sci*, 2010;51:6858–67.
11. Dubra A, Sulai Y, Norris JL, et al., Non-invasive imaging of the human rod photoreceptor mosaic using a confocal adaptive optics scanning ophthalmoscope, *Biomed Opt Express*, 2011;2:1864–76.
12. Merino D, Duncan JL, Tiruveedhula P, et al., Observation of cone and rod photoreceptors in normal subjects and patients using a new generation adaptive optics scanning laser ophthalmoscope, *Biomed Opt Express*, 2011;2:2189–201.
13. Rossi EA, Chung M, Dubra A, et al., Imaging retinal mosaics in the living eye, *Eye*, 2011;25:301–8.
14. Duncan JL, Zhang Y, Gandhi J, et al., High-resolution imaging with adaptive optics in patients with inherited retinal

degeneration, *Invest Ophthalmol Vis Sci*, 2007;48:3283–91.
15. Kitaguchi Y, Bessho K, Yamaguchi T, et al., *In vivo* measurements of cone photoreceptor spacing in myopic eyes from images obtained by an adaptive optics fundus camera, *Jpn J Ophthalmol*, 2007;51:456–61.
16. Chui TYP, Song HX, Burns SA, Individual variations in human cone photoreceptor packing density: variations with refractive error, *Invest Ophthalmol Vis Sci*, 2008;49:4679–87.
17. Chui TY, Song H, Burns SA, Adaptive-optics imaging of human cone photoreceptor distribution, *J Opt Soc Am A Opt Image Sci Vis*, 2008;25:3021–9.
18. Duncan JL, Talcott KE, Ratnam K, et al., Cone structure in retinal degeneration associated with mutations in the peripherin/RDS gene, *Invest Ophthalmol Vis Sci*, 2011;52:1557–66.
19. Morgan JI, Dubra A, Wolfe R, et al., *In vivo* autofluorescence imaging of the human and macaque retinal pigment epithelial cell mosaic, *Invest Ophthalmol Vis Sci*, 2009;50:1350–9.
20. Roorda A, Zhang Y, Duncan JL, High-resolution *in vivo* imaging of the RPE mosaic in eyes with retinal disease, *Invest Ophthalmol Vis Sci*, 2007;48:2297–303.

21. Burns SA, Zhangyi Z, Chui TYP, et al., Imaging the inner retina using adaptive optics, *Invest Ophthalmol Vis Sci*, 2008;49:E-Abstract 4512.

22. Martin JA, Roorda A, Pulsatility of parafoveal capillary leukocytes, *Exp Eye Res*, 2009;88:356–60.

23. Tam J, Martin JA, Roorda A, Non-invasive visualization and analysis of parafoveal capillaries in humans, *Invest Ophthalmol Vis Sci*, 2010;51:1691–8.

24. Zhong Z, Song H, Chui TY, et al., Noninvasive measurements and analysis of blood velocity profiles in human retinal vessels, *Invest Ophthalmol Vis Sci*, 2011;52:4151–7.

25. Tam J, Tiruveedhula P, Roorda A, Characterization of single-file flow through human retinal parafoveal capillaries using an adaptive optics scanning laser ophthalmoscope, *Biomed Opt Express*, 2011;2:781–93.

26. Popovic Z, Knutsson P, Thaung J, et al., Noninvasive imaging of human foveal capillary network using dual-conjugate adaptve optics, *Invest Ophthalmol Vis Sci*, 2011;52:2649–55.

27. Roorda A, Williams DR, The arrangement of the three cone classes in the living human eye, *Nature*, 1999;397:520–2.

28. Roorda A, Metha AB, Lennie P, Williams DR, Packing arrangement of the three cone classes in primate retina, *Vision Res*, 2001;41:1291–306.

29. Roorda A, Williams DR, Optical fiber properties of individual human cones, *J Vis*, 2002;2:404–12.

30. Hofer H, Carroll J, Neitz J, et al., Organization of the human trichromatic cone mosaic, *J Neurosci*, 2005;25:9669–79.

31. Pallikaris A, Williams DR, Hofer H, The reflectance of single cones in the living human eye, *Invest Ophthalmol Vis Sci*, 2003;44:4580–92.

32. Rha J, Jonnal RS, Thorn KE, et al., Adaptive optics flood-illumination camera for high speed retinal imaging, *Opt Express*, 2006;14:4552–69.

33. Jonnal RS, Rha J, Zhang Y, et al., In vivo functional imaging of human cone photoreceptors, *Opt Express*, 2007;14:16141–60.

34. Grieve K, Roorda A, Intrinsic signals from human cone photoreceptors, *Invest Ophthalmol Vis Sci*, 2008;49:713–9.

35. Rha J, Schroeder B, Godara P, et al., Variable optical activation of human cone photoreceptors visualized using short coherence light source, *Opt Lett*, 2009;34:3782–4.

36. Pircher M, Kroisamer JS, Felberer F, et al., Temporal changes of human cone photoreceptors observed *in vivo* with SLO/OCT, *Biomed Opt Express*, 2010;2:100–12.

37. Cooper RF, Dubis AM, Pavaskar A, et al., Spatial and temporal variation of rod photoreceptor reflectance in the human retina, *Biomed Opt Express*, 2011;2:2577–89.

38. Roorda A, Adaptive optics for studying visual function: a comprehensive review, *J Vis*, 2011;11(7):pii:6.

39. Roorda A, Adaptive optics ophthalmoscopy, *J Refract Surg*, 2000;16:S602–S7.

40. Wolfing JI, Chung M, Carroll J, et al., High-resolution retinal imaging of cone–rod dystrophy, *Ophthalmology*, 2006;113:1014–9.

41. Choi SS, Doble N, Hardy JL, et al., *In vivo* imaging of the photoreceptor mosaic in retinal dystrophies and correlations with visual function, *Invest Ophthalmol Vis Sci*, 2006;47:2080–92.

42. Chen YF, Roorda A, Duncan JL, Advances in imaging of Stargardt disease, *Adv Exp Med Biol*, 2010;664:333–40.

43. Chen Y, Ratnam K, Sundquist SM, et al., Cone photoreceptor abnormalities correlate with vision loss in patients with Stargardt disease, *Invest Ophthalmol Vis Sci*, 2011;52:3281–92.

44. Yoon MK, Roorda A, Zhang Y, et al., Adaptive optics scanning laser ophthalmoscopy images in a family with the mitochondrial DNA T8993C mutation, *Invest Ophthalmol Vis Sci*, 2009;50:1838–47.

45. Gelfand JM, Duncan JL, Racine CA, et al., Heterogeneous patterns of tissue injury in NARP syndrome, *J Neurol*, 2011;258:440–8.

46. Carroll J, Neitz M, Hofer H, et al., Functional photoreceptor loss revealed with adaptive optics: an alternate cause for color blindness, *Proc Natl Acad Sci U S A*, 2004;101:8461–6.

47. Baraas RC, Carroll J, Gunther KL, et al., Adaptive optics retinal imaging reveals S-cone dystrophy in tritan color-vision deficiency, *J Opt Soc Am A*, 2007;24:1438–46.

48. Carroll J, Baraas RC, Wagner-Schuman M, et al., Cone photoreceptor mosaic disruption associated with Cys203Arg mutation in the M-cone opsin, *Proc Natl Acad Sci U S A*, 2009;106:20948–53.

49. Torti C, Povazay B, Hofer B, et al., Adaptive optics optical coherence tomography at 120,000 depth scans/s for non-invasive cellular phenotyping of the living human retina, *Opt Express*, 2009;17:19382–400.

50. Wagner-Schuman M, Neitz J, Rha J, et al., Color-deficient cone mosaics associated with Xq28 opsin mutations: a stop codon versus gene deletions, *Vision Res*, 2010;50:2396–402.

51. Makous W, Carroll J, Wolfing JI, et al., Retinal microscotomas revealed with adaptive-optics microflashes, *Invest Ophthalmol Vis Sci*, 2006;47:4160–7.

52. Rha J, Dubis AM, Wagner-Schuman M, et al., Spectral domain optical coherence tomography and adaptive optics: imaging photoreceptor layer morphology to interpret preclinical phenotypes, *Adv Exp Med Biol*, 2010;664:309–16.

53. Alexander JJ, Umino Y, Everhart D, et al., Restoration of cone vision in a mouse model of achromatopsia, *Nat Med*, 2007;13:685–7.

54. Carvalho LS, Xu J, Pearson R, et al., Long-term and age-dependent restoration of visual function in a mouse model of CNGB3-associated achromatopsia following gene therapy, *Hum Mol Genet*, 2011;20:3161–75.

55. Komáromy A, Alexander JJ, Rowlan JS, et al., Gene therapy rescues cone function in congenital achromatopsia, *Hum Mol Genet*, 2010;19:2581–93.

56. Carroll J, Choi SS, Williams DR, *In vivo* imaging of the photoreceptor mosaic of a rod monochromat, *Vision Res*, 2008;48:2564–8.

57. Genead MA, Fishman GA, Rha J, et al., Photoreceptor structure and function in patients with congenital achromatopsia, *Invest Ophthalmol Vis Sci*, 2011; [Epub ahead of print]

58. Carroll J, Rossi EA, Porter J, et al., Deletion of the X-linked opsin gene array locus control region (LCR) results in disruption of the cone mosaic, *Vision Res*, 2010;50:1989–99.

59. Fulton AB, Albert DM, Craft JL, Human albinism. Light and electron microscopy study, *Arch Ophthalmol*, 1978;96:305–10.

60. Guillery RW, Hickey TL, Kaas JH, et al., Abnormal central visual pathways in the brain of an albino green monkey (Cercopithecus aethiops), *J Comp Neurol*, 1984;226:165–83.

61. Wilson HR, Mets MB, Nagy SE, et al., Albino spatial vision as an instance of arrested visual development, *Vision Res*, 1988;28:979–90.

62. Marmor MF, Choi SS, Zawadzki RJ, et al., Visual insignificance of the foveal pit: reassessment of foveal hypoplasia as fovea plana, *Arch Ophthalmol*, 2008;126:907–13.

63. McAllister JT, Dubis AM, Tait DM, et al., Arrested development: high-resolution imaging of foveal morphology in albinism, *Vision Res*, 2010;50:810–7.

64. Mohammad S, Gottlob I, Kumar A, et al., The functional significance of foveal abnormalities in albinism measured using spectral-domain optical coherence tomography, *Ophthalmology*, 2011;118:1645–52.

65. Vilupuru AS, Rangaswamy NV, Frishman LJ, et al., Adaptive optics scanning laser ophthalmoscopy for *in vivo* imaging of lamina cribrosa, *J Opt Soc Am A*, 2007;24:1417–25.

66. Ivers KM, Li C, Patel N, et al., Reproducibility of measuring lamina cribrosa pore geometry in human and nonhuman primates with *in vivo* adaptive optics imaging, *Invest Ophthalmol Vis Sci*, 2011;52:5473–80.

67. Lee EJ, Kim T-W, Weinreb RN, et al., Visualization of the lamina cribrosa using enhanced depth imaging spectral-domain optical coherence tomography, *Am J Ophthalmol*, 2011;152:87–95.

68. Strouthidis NG, Fortune B, Yang H, et al., Longitudinal change detected by spectral domain optical coherence tomography in the optic nerve head and peripapillary retina in experimental glaucoma, *Invest Ophthalmol Vis Sci*, 2011;52:1206–19.

69. Yang H, Williams G, Downs JC, et al., Posterior (outward) migration of the lamina cribrosa and early cupping in monkey experimental glaucoma, *Invest Ophthalmol Vis Sci*, 2011;Epub ahead of print.

70. Nork TM, Ver Hoeve JN, Poulsen GL, et al., Swelling and loss of photoreceptors in chronic human and experimental glaucomas, *Arch Ophthalmol*, 2000;118:235–45.

71. Werner JS, Keltner JL, Zawadzki RJ, et al., Outer retinal abnormalities associated with inner retinal pathology in nonglaucomatous and glaucomatous optic neuropathies, *Eye*, 2011;25:279–89.

72. Choi SS, Zawadzki RJ, Lim MC, et al., Evidence of outer retinal changes in glaucoma patients as revealed by ultrahigh-resolution *in vivo* retinal imaging, *Br J Ophthalmol*, 2011;95:131–41.

73. Zawadzki RJ, Cense B, Zhang Y, et al., Ultrahigh-resolution optical coherence tomography with monochromatic and chromatic aberration correction, *Opt Express*, 2008;16:8126–43.

74. Kocaoglu OP, Cense B, Jonnal RS, et al., Imaging retinal nerve fiber bundles using optical coherence tomography with adaptive optics, *Vision Res*, 2011;51:1835–44.

75. Romero-Borja F, Venkateswaran K, Roorda A, et al., Optical slicing of human retinal tissue *in vivo* with the adaptive optics scanning laser ophthalmoscope, *Appl Opt*, 2005;44:4032–40.

76. Joeres S, Jones SM, Chen DC, et al., Retinal imaging with adaptive optics scanning laser ophthalmoscopy in unexplained central ring scotoma, *Arch Ophthalmol*, 2008;126:543–7.

77. Godara P, Siebe C, Rha J, et al., Assessing the photoreceptor mosaic over drusen using adaptive optics and SD-OCT, *Ophthalmic Surg Lasers Imaging*, 2010;41:S104–S8.

78. Godara P, Rha J, Tait DM, et al., Unusual adaptive optics findings in a patient with bilateral maculopathy, *Arch Ophthalmol*, 2010;128:253–4.

79. Bessho K, Fujikado T, Mihashi T, et al., Photoreceptor images of normal eyes and of eyes with macular dystrophy obtained *in vivo* with an adaptive optics fundus camera, *Jpn J Ophthalmol*, 2008;52:380–5.

80. Kitaguchi Y, Kusaka S, Yamaguchi T et al., Detection of photoreceptor disruption by adaptive optics fundus imaging and Fourier-domain optical coherence tomography in eyes with occult macular dystrophy, *Clin Ophthalmol*, 2011;5:345–51.

81. Massamba N, Querques G, Lamory B, et al., *In vivo* evaluation of photoreceptor mosaic in type 2 idiopathic macular telangiectasia using adaptive optics, *Acta Ophthalmologica*, 2010; [Epub ahead of print].

82. Ooto S, Hangai M, Takayama K, et al., High-resolution photoreceptor imaging in idiopathic macular telangiectasia type 2 using adaptive optics scanning laser ophthalmoscopy, *Invest Ophthalmol Vis Sci*, 2011;52:5541–50.

83. Sallo FB, Leung I, Chung M, et al., Retinal crystals in type 2 idiopathic macular telangiectasia, *Ophthalmology*, 2011; [Epub ahead of print].

84. Ooto S, Hangai M, Sakamoto A, et al., High-resolution imaging of resolved central serous chorioretinopathy using adaptive optics scanning laser ophthalmoscopy, *Ophthalmology*, 2010;117:1800–9.

85. Kitaguchi Y, Fujikado T, Bessho K, et al., Adaptive optics fundus camera to examine localized changes in the photoreceptor layer of the fovea, *Ophthalmology*, 2008;115:1771–7.

86. Ooto S, Hangai M, Takayama K, et al., High-resolution imaging of the photoreceptor layer in epiretinal membrane using adaptive optics scanning laser ophthalmoscopy, *Ophthalmology*, 2011;118:873–81.

87. Chui TY, Thibos LN, Bradley A, et al., The mechanisms of vision loss associated with a cotton wool spot, *Vision Res*, 2009;49:2826–34.

88. Kitaguchi Y, Fujikado T, Kusaka S, et al., Imaging of titanium:sapphire laser retinal injury by adaptive optics fundus imaging and Fourier-domain optical coherence tomography, *Am J Ophthalmol*, 2009;148:97–104 e2.

89. Venkateswaran K, Romero-Borja F, Roorda A, Design of an adaptive optics scanning laser ophthalmoscope. In: Porter J, Queener H, Lin JH, et al. (eds), *Adaptive Optics for Vision Science*, Hoboken: Wiley-Interscience, 2006; 417–46.

90. Choi SS, Zawadzki RJ, Keltner JL, et al., Changes in cellular structures revealed by ultra-high resolution retinal imaging in optic neuropathies, *Invest Ophthalmol Vis Sci*, 2008;49:2103–19.

91. Choi SS, Zawadzki RJ, Greiner MA, et al., Fourier-domain optical coherence tomography and adaptive optics reveal nerve fiber layer loss and photoreceptor changes in a patient with optic nerve drusen, *J Neuroophthalmol*, 2008;28:120–5.

92. Telander DG, Choi SS, Zawadzki RJ, et al., Microstructural abnormalities revealed by high resolution imaging systems in central macular arteriovenous malformation, *Ophthalmic Surg Lasers Imaging*, 2010;9:1–4.

93. Sarda V, Nakashima K, Wolff B, et al., Topography of patchy retinal whitening during acute perfused retinal vein occlusion by optical coherence tomography and adaptive optics fundus imaging, *Eur J Ophthalmol*, 2011;21:653–6.

94. Audo I, El Sanharawi M, Vignal-Clermont C, et al., Foveal damage in habitual poppers users, *Arch Ophthalmol*, 2011;129:703–8.

95. Stepien KE, Martinez WM, Dubis AM, et al., Subclinical photoreceptor disruption in response to severe head trauma, *Arch Ophthalmol*, 2011;in press.

96. Stepien KE, Han DP, Schell J, et al., Spectral-domain optical coherence tomography and adaptive optics may detect hydroxychloroquine retinal toxicity before symptomatic vision loss, *Trans Am Ophthalmol Soc*, 2009;107:28–34.

97. Michaelides M, Hunt DM, Moore AT, The cone dysfunction syndromes, *Br J Ophthalmol*, 2004;88:291–7.

98. Michaelides M, Rha J, Dees E, et al., Integrity of the cone photoreceptor mosaic in oligocone trichromacy, *Invest Ophthalmol Vis Sci*, 2011;52:4757–64.

99. Talcott KE, Ratnam K, Sundquist S, et al., Longitudinal study of cone photoreceptors during retinal degeneration and in response to ciliary neurotrophic factor treatment, *Invest Ophthalmol Vis Sci*, 2011;52:2219–26.

100. Jiang M, Zhang X, Puliafito CA, et al., Adaptive optics photoacoustic microscopy, *Opt Express*, 2010;18:21770–6.

101. Hunter JJ, Masella B, Dubra A, et al., Images of photoreceptors in living primate eyes using adaptive optics two-photon ophthalmoscopy, *Biomed Opt Express*, 2010;2:139–48.

102. Zawadzki RJ, Jones SM, Pilli S, et al., Integrated adaptive optics optical coherence tomography and adaptive optics scanning laser ophthalmoscope system for simultaneous cellular resolution *in vivo* retinal imaging, *Biomed Opt Express*, 2011;2:1674–86.

103. Kocaoglu OP, Lee S, Jonnal RS, et al., Imaging cone photoreceptors in three dimensions and in time using ultrahigh resolution optical coherence tomography with adaptive optics, *Biomed Opt Express*, 2011;2:748–63.

104. Yang Q, Arathorn DW, Tiruveedhula P, et al., Design of an integrated hardware interface for AOSLO image capture and cone-targeted stimulus delivery, *Opt Express*, 2010;18:17841–58.

105. Ferguson RD, Zhong Z, Hammer DX, et al., Adaptive optics scanning laser ophthalmoscope with integrated wide-field retinal imaging and tracking, *J Opt Soc Am A*, 2010;27:A365–A277.

106. Yoon GY, Williams DR, Visual performance after correcting the monochromatic and chromatic aberrations of the eye, *J Opt Soc Am A*, 2002;19:266–75.

107. Artal P, Manzanera, Piers P, et al., Visual effect of the combined correction of spherical and longitudinal chromatic aberrations, *Opt Express*, 2010;18:1637–48.

108. Arathorn DW, Yang Q, Vogel CR, et al., Retinally stabilized cone-targeted stimulus delivery, *Opt Express*, 2007;15:13731–44.

109. Sincich LC, Zhang Y, Tiruveedhula P, et al., Resolving single cone inputs to visual receptive fields, *Nat Neurosci*, 2009;12:967–9.

110. Hofer H, Singer B, Williams DR, Different sensations from cones with the same photopigment, *J Vis*, 2005;5:444–54.

111. Rossi EA, Roorda A, The relationship between visual resolution and cone spacing in the human fovea, *Nat Neurosci*, 2010;13:156–7.

112. Rosolen SG, Lamory B, Harms F, et al., Cellular-resolution *in vivo* imaging of the feline retina using adaptive optics: preliminary results, *Vet Ophthalmol*, 2010;13:369–76.

113. Biss DP, Sumorok D, Burns SA, et al., *In vivo* fluorescent imaging of the mouse retina using adaptive optics, *Opt Lett*, 2007;32:659–61.

114. Geng Y, Greenberg KP, Wolfe R, et al., *In vivo* imaging of microscopic structures in the rat retina, *Invest Ophthalmol Vis Sci*, 2009;50:5872–9.

115. Gray DC, Merigan W, Wolfing JI, et al., *In vivo* fluorescence imaging of primate retinal ganglion cells and retinal pigment epithelial cells, *Opt Express*, 2006;14:7144–58.

116. Gray DC, Wolfe R, Gee BP, et al., *In vivo* imaging of the fine structure of rhodamine-labeled macaque retinal ganglion cells, *Invest Ophthalmol Vis Sci*, 2008;49:467–73.

117. Carroll J, Gray DC, Roorda A, et al., Recent advances in retinal imaging with adaptive optics, *Opt Photonics News*, 2005;16:36–42.