The effects of retinal image motion on the limits of spatial vision

by

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Abstract

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Vision is not a static process. Our perception of the world is not merely a sequence of fixed snapshots but rather involves a dynamic process in which the visual input is synthesized over time to provide a more detailed and informative signal than would otherwise be possible using a fixed array of sensors. This dynamic signal is largely a result of fixational eye motion, or the constant ocular jitter that creates an ever-changing signal in each photoreceptor cell. It is not known how the visual system potentially exploits such transient signals to serve our finest spatial acuity, and how the relationship between visual acuity and the photoreceptor sampling limit can be muddled because of this fact. We used an adaptive optics scanning laser ophthalmoscope to precisely control the spatiotemporal input on a cellular scale in human observers to assess how acuity differed as a function of retinal image motion. Additionally, we investigated the purpose of fixational eye motion, and in particular microsaccades, in relocating stimuli to a preferred region within the central foveal region. Combined, these results show the utility of fixational eye movements in high spatial vision.
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List of Abbreviations

ADRP  Autosomal-dominant retinitis pigmentosa
AOM  Acousto-optic modulator
AOSLO  Adaptive optics scanning laser ophthalmoscope
BCVA  Best-corrected visual acuity
CCD  Charge-coupled device
CD  Cone density
CHM  Choroideremia
CM  Curved mirror
CNTF  Ciliary neurotrophic factor
D  Diopters
dB  Decibels
DC  Dichroic mirror
DM  Deformable mirror
ETDRS  Early Treatment of Diabetic Retinopathy Study
FEM  Fixational eye movements
FM  Flat mirror
FOC  Fraction of cones
FS  Fast scanner
LGN  Lateral geniculate nucleus
MEMs  Microelectromechanical systems
MS  Microsaccade
NARP  Neurogenic weakness, ataxia, retinitis pigmentosa
Nc  Nyquist limit
OCT  Optical coherence tomography
OD  Right eye
OS  Left eye
PMT  Photomultiplier tube
PRL  Preferred retinal locus of fixation
RGC  Retinal ganglion cells
RP  Retinitis pigmentosa
SD  Standard deviation
SHWS  Shack-Hartmann wavefront sensor
SLO  Scanning laser ophthalmoscope
SS  Slow scanner
USH  Usher syndrome
VA  Visual acuity
<table>
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<td>Wavefront sensor</td>
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Chapter 1

Retinal imaging and stimulation with adaptive optics scanning laser ophthalmoscopy
Chapter 1
Retinal imaging and stimulation with adaptive optics scanning laser ophthalmoscopy

1.1 Introduction

1.1.1 Optical quality of the eye

For vision, the eye serves as the intermediary between the external world and our perceptual experience. Photons from physical objects are transmitted through the eye onto the retina; in spite of the perceived sharpness of our environment, this optical path is fraught with aberrations that reduce the fidelity of the resulting retinal image. As Helmholtz famously said, “Now, it is not too much to say that if an optician wanted to sell me an instrument which had all these defects, I should think myself quite justified in blaming his carelessness in the strongest terms, and giving him back his instrument” (translated from Helmholtz, 1962). Much work has been done to understand the optics of the eye, characterize their imperfections, and study their effects on visual perception (Gerald Westheimer, 2006).

When a photon of light arrives at the eye, it must pass through the cornea, lens, and vitreous before reaching the retina. Though imperfect, these transparent tissues form a focused image at the retinal plane in an emmetropic eye. A non-accommodating eye has a refractive power of about 60 diopters (D), which when focusing an image onto the retina, corresponds to an ocular focal length of approximately 22 mm (Larsen, 1971). Deviations from this axial length after eye growth result in hyperopia (undergrowth) or myopia (overgrowth), the latter of which is predicted to afflict 50% of the global population by 2050 (Holden et al., 2016).

Ocular imperfections are typically categorized into low-order and high-order aberrations (Figure 1.1). Low-order aberrations, defocus and astigmatism, are corrected with glasses or contact lenses. Defocus results from a discrepancy between the eye’s focusing power and axial length, while astigmatism is caused by radial asymmetries in the cornea and lens. While low-order aberrations typically account for most of the eye’s imperfections, high-order aberrations also contribute to noticeable retinal image blur (one such example being the asymmetrical light scatter observed when viewing a street lamp at night).

1.1.2 Adaptive optics for retinal imaging

The low- and high-order components of ocular imperfections are oftentimes quantified as Zernike polynomials, a set of orthogonal polynomials that constitute a wavefront function for the eye (Born & Wolf, 1989). The ability to deconstruct and quantify these aberrations was pertinent for the development of adaptive optics, a technique for wavefront
measurement and correction that has its origins in astronomy. When collecting images of
celestial objects using ground-based telescopes, image quality is degraded due to the
Earth’s atmospheric turbulence (the same reason that stars appear to “twinkle” in the night
sky). In order to compensate for atmospheric turbulence, or ocular imperfections in the
case of ophthalmic imaging, one needs to measure a wavefront passing through an optical
system and compensate for the distortions thereafter. An early method for measuring the
image quality of a telescope was developed by Johannes Franz Hartmann, in which multiple
holes were created in an opaque mask covering a telescope’s aperture. The relative
alignment of images seen through the holes were an indication of the image quality of the
telescope (Hartmann, 1900). Later, Roland Shack and Ben Platt replaced the holes with an
array of lenslets with equivalent focal lengths; the local shape of a wavefront could then be
determined by the position of each lenslet’s focal spot on a photon sensor (Platt & Shack,
2001). For a flat wavefront, resulting from undistorted light coming from optical infinity,
the lenslet spots would form a grid-like lattice at the sensor; any deviations from a flat
wavefront would result in deviations in this pattern, with larger aberrations resulting in
larger deviations. This device, termed the Shack-Hartmann wavefront sensor (SHWS),
would prove to be instrumental in the development of adaptive optics for ophthalmic
imaging. Shack-Hartmann wavefront sensing for the eye was first implemented at the
University of Heidelberg to make fast, objective measurements of ocular aberrations
(Liang, Grimm, Goelz, & Bille, 1994). Later, at the University of Rochester, SHWS was used
to characterize wavefront properties in the normal human eye (Liang & Williams, 1997;
Porter, Guirao, Cox, & Williams, 2001).
As previously mentioned, the low-order component of ocular aberrations can be corrected using prescription lenses. High-order aberrations, however, are more complex in shape and cannot easily be compensated for. A deformable mirror (DM), typically consisting of an array of smaller mirrors on individual actuators, can take the complex shape necessary for cancelling out errors in an incoming wavefront. Although DMs have been utilized for closed-loop control in astronomy since the 1970's (Hardy, Lefebvre, & Koliopoulos, 1977), it was not until two decades later that closed-loop AO systems were developed for the eye (Liang, Williams, & Miller, 1997).

Adaptive optics was initially integrated into flood-illuminated ophthalmoscopes, which provided the first high-quality in vivo images of the cone photoreceptor mosaic (Liang, Williams, & Miller, 1997). Correcting for aberrations over a larger, dilated pupil increased the numerical aperture and hence angular resolution of the system, enabling unprecedented visualization of the cone photoreceptor mosaic. Delivery of stimuli through this system resulted in improvements in contrast sensitivity and visual acuity, suggesting that the eye’s optical performance was improved with adaptive optics (Liang et al., 1997; Roorda & Williams, 1999, 2002; Yoon & Williams, 2002).
1.1.3 **Adaptive optics scanning laser ophthalmoscopy**

Although early adaptive optics ophthalmoscopes offered unprecedented *in vivo* images of the cone photoreceptor mosaic, these systems were limited in temporal resolution by the low capture rate of the charge-coupled devices (CCDs) available at the time. While early flood-illuminated AO systems had frame rates capping at 10 Hz, more recent systems have reached rates upwards of 100 Hz due to improvements in sensor semiconductor technology (Bedggood & Metha, 2012; Rha et al., 2006). Although flood-based AO systems have sufficient resolution for visualizing individual cones, the ability to track and stimulate specific retinal regions is impaired by the inability to measure and compensate for ongoing fixational eye movements. This limitation in the capabilities of flood-based system motivated the integration of AO into the scanning laser ophthalmoscope (SLO).

The SLO was invented in the 1980’s and is similar to a scanning laser microscope in that an image is collected pixel-by-pixel as the imaging beam moves across the tissue of interest. The primary difference between modalities is that the eye, rather than a fabricated lens, serves as the objective in an SLO (Webb, Hughes, & Delori, 1987). As a result, the presence of ocular aberrations set an upper limit to the image quality achievable by such a system.

To mitigate this problem, Roorda and colleagues developed the first adaptive optics scanning laser ophthalmoscope (AOSLO), improving lateral resolution from 5 to about 2.5 microns and axial resolution from 300 to better than 100 microns (Roorda et al., 2002). The enhanced axial resolution afforded by AOSLO enabled true optical sectioning of the retina’s vascular, photoreceptor and nerve fiber layers, improving *in vivo* characterization of retinal structure.

Subsequent generations of the AOSLO have upgraded deformable mirrors (Zhang, Poonja, & Roorda, 2006), multiple wavelengths for imaging and stimulation (Grieve, Tiruveedhula, Zhang, & Roorda, 2006), and the ability to visualize parafoveal rods and foveal cones (Dubra & Sulai, 2011; Merino, Duncan, Tiruveedhula, & Roorda, 2011). Most recently, implementation of non-confocal split-detection techniques have significantly improved visualization of retinal vasculature with the AOSLO (Sulai, Scoles, Harvey, & Dubra, 2014). Since its creation, the AOSLO has been used extensively to characterize retinal degenerations (Carroll, Kay, Scoles, Dubra, & Lombardo, 2013; Duncan et al., 2007; Roorda & Duncan, 2015; Roorda, Zhang, & Duncan, 2007; Talcott et al., 2011), correlate visual psychophysics with retinal structure (Harmening, Tuten, Roorda, & Sincich, 2014; Rossi & Roorda, 2010; Rossi, Weiser, Tarrant, & Roorda, 2007; Sabesan, Hofer, & Roorda, 2015; Tuten, Tiruveedhula, & Roorda, 2012), and to characterize chromatic aberrations across the visual field (Winter et al., 2016).
1.2 Methods

1.2.1 Retinal imaging with AOSLO

Figure 1.2 | Schematic of multi-wavelength AOSLO. Wavefront sensing is done with 940 nm light with 3 separate channels (543, 680, 840 nm) for imaging and stimulus delivery. CM, curved mirror; DC, dichroic mirror; DM, deformable mirror; FM, flat mirror; FS, fast scanner; PMT, photomultiplier tube; SS, slow scanner; WFS, wavefront sensor.

Figure 1.2 shows a schematic of a multi-wavelength AOSLO. The first experiment reported in this document (see chapter 2) was conducted with the AOSLOIII system located at the University of California, San Francisco. Subsequent experiments were respectively implemented with the AOSLOII (chapter 3) and AOSLOIV (chapter 4), located at the University of California, Berkeley.

The imaging source for the AOSLOII and AOSLOIII systems was 840 nm light from either a superluminescent diode (Superlum, Cork, Ireland) or broadband supercontinuum light source (SuperK Extreme, NKT Photonics A/S, Birkeød, Denmark); 680 nm light (source) was used for experiments conducted with AOSLOIV. Images were obtained by moving the imaging beam across the retina in raster pattern using resonant and galvanometric scanner. For the experiments described in this document, scanning amplitudes ranged from 0.9° (~260 µm) to 1.5° (~440 µm) of visual angle. Ocular monochromatic aberrations were dynamically measured with a custom-built Shack-Hartmann wavefront sensor and corrected with a deformable mirror (source) at a maximum rate of 24 Hz.
A photomultiplier tube (PMT; Hamamatsu, Japan) detected and recorded the imaging light intensity reflected from the eye; to block light scattered from other retinal layers from entering the PMT, a confocal pinhole was placed conjugate to the focus location of the ingoing beam. Each 512-by-512-pixel frame of an AOSLO video was created by assigning pixel intensities based on the position of the scanning mirrors, with a maximum frame rate of 30 Hz.

For the experiments reported in this document, stimuli for visual psychophysics were embedded directly into the raster by selectively turning off the laser (Poonja, Patel, Henry, & Roorda, 2005) at discrete points during the scan using an acousto-optic modulator (AOM; Brimrose Corp, Baltimore, MD, USA). Since the stimulus was generated pixel-by-pixel in the scanning raster, a given point in the retina was exposed to the stimulus for a short duration of time; subjects perceived the stimulus as appearing continuous.

1.2.2 Retinal eye tracking and stimulus delivery

The eye is constantly in motion, even during fixation on a static object. These fixational eye movements serve several purposes, including relocating objects of interest to the preferred retinal locus of fixation (PRL) and preventing neural adaptation and image fading to an unmoving object (for review see (Martinez-Conde, Macknik, & Hubel, 2004)). These eye movements, which can move an image across several to several dozen photoreceptor cells, impose difficulties when trying perform visual psychophysics at specific retinal locations.

Although the AOSLO video rate is 30 Hz, eye motion traces can be retrieved from AOSLO images at temporal frequencies exceeding 1000 Hz. Since AOSLO images are collected via raster scanning, eye movements occurring during the collection of a single frame induce compression, shearing, translation, or expansion in the final image. The positional differences between horizontal segments of an individual frame and reference image can then be used to quantify eye motion at much higher frequencies than the video rate (Stevenson & Roorda, 2005; Vogel, Arathorn, Roorda, & Parker, 2006). While this technique was initially performed exclusively during offline analyses, hardware and algorithmic improvements have made real-time eye motion tracking possible (Arathorn et al., 2007; Yang, Arathorn, Tiruveedhula, Vogel, & Roorda, 2010).

The accuracy of real-time AOSLO eye tracking depends on numerous factors, including image quality, field size, the amplitude of eye motion, small inaccuracies in predicting the eye's current location, and slight lags in preparing the AOM for stimulus delivery. In spite of these factors, AOSLO stimulus delivery has been shown to be precise to within 0.15 arcmin (Arathorn et al., 2007; Yang et al., 2010), less than half the diameter of a foveal cone. For stimuli directly encoded into the imaging scanning raster, there is an unambiguous record of stimulus retinal position during a given trial.
1.2.3 Eye motion analysis

AOSLO eye tracking depends on co-registration of individual frames to a standard reference retinal image. The reference image is assumed to be undistorted by eye movements; since individual frames contain distortions, the reference frame is an averaged composite of these frames, assuming net motion of the eye is zero over the course of the video (Stevenson & Roorda, 2005). Reference frames are generated iteratively by first creating an average image with good frames only. Additional frames without saccades are then added to improve the reference image; this final image is then used to register horizontal strips from each frame to the reference. If \( N \) horizontal strips are analyzed per image and the video frame rate is 30 Hz, the final eye tracking frequency is \( N \times 30 \) Hz. For the eye motion analyses cited in this document, each frame was divided into 28 strips, resulting in a temporal resolution of 840 Hz (Figure 1.3).

![Image-based eye motion trace.](image)

**Figure 1.3 | Image-based eye motion trace.** Example 840 Hz eye movement trace derived using an image-based cross correlation algorithm (Stevenson and Roorda, 2005 & 2010). The blue and orange traces indicate horizontal and vertical eye movements respectively. The high frequency spikes, most noticeable in the vertical trace, result from artifacts in the original reference image and tracking errors with the top strip of each frame.

Registration of each strip to the reference frame is performed by computing a two-dimensional cross correlation. To expedite this step, the search region within the reference frame is constrained based on motion traces from an earlier, coarser correlation step. Sub-pixel accuracy can be achieved by fitting a cubic spline function to the correlation peak in the finer resolution step.
If the reference frame has any distortions, such as those caused by torsion, the power spectrum of the eye movement trace will show artifacts at 30 Hz and at every subsequent harmonic. Additionally, large saccades may impair the cross-correlation analysis for two reasons: (1) the image shear within a strip resulting from a high-velocity movement and (2) minimal overlap between the retinal regions visible in the reference frame and the given image. In spite of these limitations, the eye tracking algorithm is typically accurate to < 1 arcmin and has been shown to outperform other conventional eye trackers (Stevenson & Roorda, 2010).

1.3 Summary

Monochromatic ocular aberrations have imposed limitations on the resolution achievable by conventional ophthalmoscopes. Within the past several decades, the development of adaptive optics has made it possible to quantify and compensate for the light scatter caused by these imperfections. The adaptive optics scanning laser ophthalmoscope offers unprecedented visualization of cells within the living human eye, and the integration of high-accuracy eye tracking and stimulus delivery make possible visual psychophysics at the scale of individual cells. Chapter 2 will describe how the AOSLO has been applied to correlate retinal structure with clinical measures of visual function. Chapters 3 and 4 will describe our efforts to better study the effects of fixational eye movements on high spatial resolution (Chapter 3), as well as the specific role of microsaccades during high-acuity tasks (Chapter 4).
Chapter 2

Relationship between foveal cone structure and clinical measures of visual function
Chapter 2

Relationship between cone structure and clinical measures of visual function

2.1 Abstract

The fovea is the retinal location with highest cone density and thus sharpest spatial resolution, making it a crucial region to study when monitoring the progression of retinal diseases. Due to difficulties in quantifying structural retinal changes, clinical measures of function, namely visual acuity (VA), are used to indirectly monitor disease progression. However, VA is known to be preserved until late stages of rod-cone degeneration due to factors such as intrasubject variability and the inherent subjectivity of such techniques. As such, efforts have been made to develop objective measures for quantifying foveal degeneration, but these attempts have been limited due to the low optical quality of conventional imaging systems. The integration of adaptive optics into the scanning laser ophthalmoscope has made it possible to directly visualize the in vivo photoreceptor mosaic and measure cone spacing and density in normal and diseased eyes. In the present cross-sectional study, we compare AOSLO cone structural measures with clinical measures of VA and foveal sensitivity in a cohort of patients with retinal degenerations. The results show that VA and sensitivity are less sensitive indicators of the integrity of the cone mosaic than direct, objective measures of cone structure. A recent longitudinal follow-up to the cross-sectional study, outlined in section 2.7, shows that structural cone loss precedes acuity changes over a period of 10 months to 5 years.

2.2 Introduction

The fovea, with its high cone density and sharp visual acuity, is the retinal location used for most everyday tasks, such as reading and driving. Because of its importance in fine visual resolution, foveal vision is commonly used to track the progression of retinal degenerations. In the case of rod-cone degenerations, in which cone loss works its way inward from the retinal periphery, the foveal function is preserved until late stages of disease, making it imperative to monitor vision loss before it encroaches on central vision. Since most imaging modalities have insufficient resolution to quantify structural changes in the cone photoreceptor mosaic, clinical measures of function, such as visual acuity and foveal sensitivity are used to monitor disease progression. However, patients with good Snellen VA (20/30 or better) have shown significant foveal cone abnormalities measured via contrast sensitivity (Akeo, Hilda, Saga, Inoue, & Oguchi, 2002; Lindberg, Fishman, Anderson, & Vasquez, 1981; Wolkstein, Atkin, & Bodis-Wollner, 1980) and foveal thresholds (Alexander, Hutman, & Fishman, 1986). The inherent subjectivity of psychophysical techniques lead to increased intrasubject variability in VA (Arditi & Cagenello, 1993; G A Fishman et al., 1994; Grover, Fishman, Gilbert, & Anderson, 1997;
Vanden Bosch & Wall, 1997) and sensitivity (Kim, McAnany, Alexander, & Fishman, 2007; Ross, Fishman, Gilbert, & Anderson, 1984; William Seiple, Clemens, Greenstein, Carr, & Holopigian, 2004) measures, making it difficult to objectively quantify the extent of foveal degeneration. Previous studies of inherited retinal degenerations have shown that conventional functional measures have anywhere from 5-year to 15-year half-life times of visual field loss (Berson, Sandberg, Rosner, Birch, & Hanson, 1985; Holopigian, Greenstein, Seiple, & Carr, 1996; Iannaccone et al., 2004; Massof, Dagnelie, Benzschawel, Palmer, & Finkelstein, 1990).

Due to the unreliability of clinical functional measures, objective measures of cone structure may serve as more robust and sensitive indicators of foveal degeneration. Previous studies have shown significant disparities between psychophysical and anatomical data. Cone photopigment optical density reductions have been observed in retinitis pigmentosa (RP) patients with normal acuity (Elsner, Burns, & Lobes, 1987; Kilbride, Fishman, Fishman, & Hutman, 1986; van Meel & van Norren, 1983), and Geller and Sieving reported that loss of 90% of foveal cones was necessary to significantly impair grating acuity (Geller & Sieving, 1993). Alexander and colleagues concluded that increased foveal cone spacing rather than reduced photopigment optical density was responsible for lowered grating, Vernier, and letter acuities (Alexander, Derlacki, Fishman, & Szlyk, 1992). A histologic study showed abnormal foveal cone spacing in an RP patient with normal acuity (Flannery, Farber, Bird, & Bok, 1989).

Due to monochromatic ocular aberrations, structural assessment of the living retina has been precluded by the low optical quality of conventional imaging systems. Within the past few decades, the development of non-invasive, high-resolution techniques such as optical coherence tomography and adaptive optics have revolutionized the field of clinical ophthalmology. AO-based studies of retinal degeneration have shown significant correlations between macular cone spacing and central visual function, but the unique anatomy (Ahnelt, 1998) and small diameter of foveal cones made it difficult to assess cone structure at the foveal center. Within the past few years, improvements in AOSLO optical design (Dubra & Sulai, 2011) have made it possible visualize the foveal cone mosaic in patients with retinal degeneration. Although there is a growing body of studies (Carroll et al., 2012; Merino et al., 2011; Yoon et al., 2009) on foveal cone anatomy, comparisons between foveal cone structure and clinical measures of function in a large cohort of patients with retinal degeneration have yet to be done.

In the present study, we quantified foveal cone spacing and density in 26 patients with retinal degeneration and compare these measures with best-corrected VA (BCVA) and foveal sensitivity. Cone density values below normal were compared to visual function to determine whether cone structure is a more sensitive indicator of disease severity.

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2.3 Methods

2.3.1 Study design

Research procedures followed the tenets of the Declaration of Helsinki, and informed consent was obtained from all subjects. The study protocol was approved by the institutional review board of the University of California, San Francisco; the University of California, Berkeley; and the Medical College of Wisconsin.

2.3.2 Subjects

Twenty-six patients (18 female and 8 male) with inherited retinal degenerations were characterized clinically (Table 2.1). Patients were excluded if they had other ocular or systemic conditions that could affect VA, including amblyopia, cataract, and foveal edema.

<table>
<thead>
<tr>
<th>Subj</th>
<th>Age/sex</th>
<th>Eye</th>
<th>Condition</th>
<th>Visual acuity</th>
<th>Humphrey 10-2 foveal sensitivity</th>
<th>PRL</th>
<th>Foveal cone spacing</th>
<th>% Cones below average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BC VA</td>
<td>ETDRS Logarithmic (dB); Linear (1/Lambert)</td>
<td>Location Fixational stability in arcmin (SDx SDy)</td>
<td>Z-score</td>
<td>Average eccentricity from PRL (arcmin [degrees])</td>
</tr>
<tr>
<td>1</td>
<td>17/M</td>
<td>OS</td>
<td>CHM</td>
<td>20/25</td>
<td>80 34*; 2511.89* 4.51, 3.86</td>
<td>Fixation target</td>
<td>2.07</td>
<td>1.62 [0.03]</td>
</tr>
<tr>
<td>2</td>
<td>38/F</td>
<td>OD</td>
<td>CHM carrier</td>
<td>20/16</td>
<td>88 36; 39810.7 3.32, 3.14</td>
<td>Fixation target</td>
<td>2.38</td>
<td>1.07 [0.02]</td>
</tr>
<tr>
<td>3</td>
<td>26/F</td>
<td>OS</td>
<td>CHM carrier</td>
<td>20/20</td>
<td>85 38; 6309.57 2.17, 5.52</td>
<td>Fixation target</td>
<td>2.97</td>
<td>1.8 [0.03]</td>
</tr>
<tr>
<td>4</td>
<td>27/F</td>
<td>OS</td>
<td>CHM carrier</td>
<td>20/20</td>
<td>85 36; 39810.7 3.56, 8.92</td>
<td>Fixation target</td>
<td>2.72</td>
<td>0.82 [0.01]</td>
</tr>
<tr>
<td>5</td>
<td>37/F</td>
<td>OD</td>
<td>ADRP</td>
<td>20/16</td>
<td>89 37; 50 11.87 1.95, 2.95</td>
<td>Fixation target</td>
<td>0.28</td>
<td>0.36 [0.01]</td>
</tr>
<tr>
<td>6</td>
<td>45/F</td>
<td>OD</td>
<td>ADRP</td>
<td>20/25</td>
<td>83 35; 31 62.28 Peak cone density N/A -0.97</td>
<td>0.00</td>
<td>-30.40</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>38/F</td>
<td>OD</td>
<td>Simplex RP</td>
<td>20/32</td>
<td>81 34*; 2511.89* 4.71, 2.54</td>
<td>Fixation target</td>
<td>5.88</td>
<td>8.08 [0.13]</td>
</tr>
<tr>
<td>8</td>
<td>48/F</td>
<td>OS</td>
<td>Simplex RP</td>
<td>20/25</td>
<td>80 34*; 2511.89* 1.09, 0.89</td>
<td>Fixation target</td>
<td>3.07</td>
<td>7.57 [0.13]</td>
</tr>
<tr>
<td>9</td>
<td>40/M</td>
<td>OD</td>
<td>Simplex RP</td>
<td>20/20</td>
<td>83 37; 50 11.87 4.20, 1.66</td>
<td>Fixation target</td>
<td>-0.20</td>
<td>0.40 [0.01]</td>
</tr>
<tr>
<td>10</td>
<td>28/F</td>
<td>OS</td>
<td>Simplex RP</td>
<td>20/25</td>
<td>82 39; 79 43.28</td>
<td>Fixation target</td>
<td>2.61</td>
<td>1.62 [0.03]</td>
</tr>
<tr>
<td>11</td>
<td>32/F</td>
<td>OD</td>
<td>Simplex RP</td>
<td>20/40</td>
<td>62* 12*; 15.85* 1.61, 2.37</td>
<td>Fixation target</td>
<td>5.44</td>
<td>0.90 [0.01]</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>12</td>
<td>40/F</td>
<td>OD</td>
<td>Simplex RP</td>
<td>20/16</td>
<td>89</td>
<td>37; 50 11.87</td>
<td>Fixation target</td>
<td>3.76, 0.55</td>
</tr>
<tr>
<td>13</td>
<td>30/M</td>
<td>OD</td>
<td>Multiplex RP</td>
<td>20/25</td>
<td>81</td>
<td>34*; 2511.89*</td>
<td>Fixation target</td>
<td>4.43, 2.67</td>
</tr>
<tr>
<td>14</td>
<td>30/M</td>
<td>OD</td>
<td>XLRP</td>
<td>20/50</td>
<td>63*</td>
<td>25*; 316.23*</td>
<td>Fixation target</td>
<td>1.65, 3.50</td>
</tr>
<tr>
<td>15</td>
<td>49/F</td>
<td>OS</td>
<td>XLRP carrier</td>
<td>20/12</td>
<td>93</td>
<td>35; 316.228</td>
<td>Fixation target</td>
<td>6.23, 3.30</td>
</tr>
<tr>
<td>16</td>
<td>20/F</td>
<td>OS</td>
<td>XLRP carrier</td>
<td>20/50</td>
<td>67*</td>
<td>32*; 1584.89*</td>
<td>Fixation target</td>
<td>5.49, 2.98</td>
</tr>
<tr>
<td>17</td>
<td>18/F</td>
<td>OD</td>
<td>NARP</td>
<td>20/50</td>
<td>65*</td>
<td>32*; 1584.89*</td>
<td>Fixation target</td>
<td>9.90, 4.08</td>
</tr>
<tr>
<td>18</td>
<td>22/F</td>
<td>OS</td>
<td>NARP</td>
<td>20/25</td>
<td>79*</td>
<td>35; 316.228</td>
<td>Fixation target</td>
<td>1.72, 2.65</td>
</tr>
<tr>
<td>19</td>
<td>50/F</td>
<td>OS</td>
<td>NARP</td>
<td>20/50</td>
<td>65*</td>
<td>27*; 501.19*</td>
<td>Fixation target</td>
<td>8.67, 3.07</td>
</tr>
<tr>
<td>20</td>
<td>27/F</td>
<td>OS</td>
<td>NARP</td>
<td>20/16</td>
<td>90</td>
<td>38; 63.0957</td>
<td>Fixation target</td>
<td>3.84, 3.63</td>
</tr>
<tr>
<td>21</td>
<td>26/M</td>
<td>OS</td>
<td>USH2</td>
<td>20/25</td>
<td>77*</td>
<td>33*; 1995.26*</td>
<td>Peak cone density</td>
<td>N/A</td>
</tr>
<tr>
<td>22</td>
<td>34/M</td>
<td>OD</td>
<td>USH2</td>
<td>20/20</td>
<td>85</td>
<td>36; 3981.07</td>
<td>Fixation target</td>
<td>1.53, 1.62</td>
</tr>
<tr>
<td>23</td>
<td>33/M</td>
<td>OD</td>
<td>USH2</td>
<td>20/20</td>
<td>85</td>
<td>39; 7943.28</td>
<td>Peak cone density</td>
<td>N/A</td>
</tr>
<tr>
<td>24</td>
<td>29/F</td>
<td>OS</td>
<td>USH2</td>
<td>20/30</td>
<td>80</td>
<td>32*; 1584.89*</td>
<td>Peak cone density</td>
<td>N/A</td>
</tr>
<tr>
<td>25</td>
<td>20/M</td>
<td>OS</td>
<td>USH3</td>
<td>20/16</td>
<td>90</td>
<td>36; 3981.07</td>
<td>Fixation target</td>
<td>3.60, 2.40</td>
</tr>
<tr>
<td>26</td>
<td>25/F</td>
<td>OD</td>
<td>USH3</td>
<td>20/20</td>
<td>85</td>
<td>35; 316.228*</td>
<td>Fixation target</td>
<td>2.77, 3.33</td>
</tr>
</tbody>
</table>

Table 2.1 | Summary of clinical and structural characteristics of patients studied. BCVA, best-corrected visual acuity; ETDRS, early treatment of diabetic retinopathy score, expressed as number of letters correctly identified; dB, decibels; PRL, preferred retinal locus for fixation; SD, standard deviation; SDx, horizontal standard deviation of fixation; SDy, vertical standard deviation of fixation; OD, right eye; OS, left eye; M, male; F, female; RP, retinitis pigmentosa; XL, X-linked; NARP, neurogenic weakness, ataxia, retinitis pigmentosa; USH, Usher syndrome. *Abnormal values for ETDRS score and foveal sensitivity. Adapted from Ratnam et al., 2013.

2.3.3 Clinical examination

BCVA was measured using a standard eye chart according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) protocol ("Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study report number 1," 1985). Foveal sensitivity thresholds were...
measured using a Goldmann III stimulus on a white background (10.03 cd/m²) and exposure duration of 200 ms (Humphrey Visual Field Analyzer HFA II 750-6116-12.6; Carl Zeiss Meditec, Inc., Dublin, CA). Foveal sensitivity was expressed in logarithmic decibel scale ($\text{dB} = 10 \times \log(1/\text{Lambert})$) and linearly ($1/\text{Lambert}$).

2.3.4 AOSLO image acquisition and cone structure analysis

Pupils were dilated with 1% tropicamide and 2.5% phenylephrine before AOSLO imaging. High-resolution AOSLO images of the macula were obtained for the 26 patients and 37 age-similar visually normal subjects. For patients measured at the University of California, Berkeley (n=22), the PRL was determined by recording 10-second videos during which patients looked at a fixation dot delivered via the AOSLO scanning raster. The mean and standard deviation (SD) locations of the PRL were analyzed to quantify fixational stability. For patients assessed at the Medical College of Wisconsin (n=4), PRL analysis was unavailable and hence it was assumed that patients fixated with the foveal location with maximum cone density. The PRL and location of maximum cone density are similar but have been shown to differ by 6-10 minutes of arc (arcmin) of visual angle (Li, Tiruveedhula, & Roorda, 2010; Putnam, Hofer, Chen, & Williams, 2005; Wilk et al., 2017). For each patient, the eye in which unambiguous cone mosaics could be visualized closest to the PRL was chosen for further analysis. Custom software was used to quantify cone spacing using previously described methods (Duncan et al., 2007), and cone spacing measurements for patients were compared with those of 37 visually normal subjects. For controls, the foveal center (eccentricity = 0°) was defined as the location of peak foveal cone density when known (n=11); for the remaining 26 normal subjects, the foveal center was identified as the PRL. Cone locations in control subjects were measured as eccentricity in degrees relative to PRL or location of peak cone density; cone spacing in patients was measured close to or at the PRL (mean [SD] eccentricity, 0.02 [0.03] degree; maximum eccentricity, 0.13 degree). Deviation from normal mean cone spacing was calculated as a Z-score, or the number of SDs from the mean. Z-scores between -2 and 2 were considered normal.

Cone spacing was converted to cone density using a previously published method (Duncan et al., 2007). Cone density was computed this way for two reasons. First, due to lower image quality at the fovea for reasons mentioned earlier, not all foveal cones are visible in the AOSLO image. As such, densities based on subjective identification of visible cones will likely be underestimated (see Figure 2.1). Second, fine spatial tasks are likely mediated by small patches of contiguous cones (Geller, Sieving, & Green, 1992), so our method of estimating cone density within small patches is adequate. Cone density (CD) was converted to fraction of cones (FOC) using the equation:
\[ FOC = \frac{CD_{\text{subject}}}{CD_{\text{normal, average}}} \]  

FOC was used to calculate the percentage of cones below average, or the difference in the patient’s cone density as a certain eccentricity compared to the average value from the normal controls. The relevant equation is:

\[ \% \text{ Cones Below Average} = 100(1 - FOC) \]

Negative percent values indicate cone density was greater than average. Cone spacing Z-scores within 2 SD at the foveal center correspond to cone densities up to 36.7% below or above the normal mean, which may be attributable to the high individual variability in human foveal cone density (Ahnelt, 1998; Chui, Song, & Burns, 2008; Chui, Song, & Burns, 2008; Curcio, Sloan, Kalina, & Hendrickson, 1990; Li et al., 2010; Song, Chui, Zhong, Elsner, & Burns, 2011). Therefore, percentage of cones below average does not necessarily indicate percentage of cone loss; Z-scores exceeding 2 however strongly suggest foveal cone loss has occurred.

### 2.3.5 Statistical analysis

Z-scores were compared to ETDRS scores and foveal sensitivity using Spearman rank correlation (\( \rho \)), which computes the correlation between the ranked order of variables and is unaffected by the nonlinearity of monotonic relationships between variables. \( P \) values were calculated using the Holm adjustment; \( P < 0.05 \) was considered statistically significant.

Percentage of cones below average was plotted against VA and foveal sensitivity. The cone percentage threshold after which the ETDRS score dropped below 85 letters (~20/20) and 80 letters (~20/25) (Ferris, Kassoff, Bresnick, & Bailey, 1982) was determined. Thresholds were similarly determined for foveal sensitivities below normal values (logarithmic scale: <35 dB; linear scale: <3162.28 1/Lambert). Data were fit to a locally weighted scatterplot smoothing curve with 95% confidence intervals (CI) generated using the cases bootstrap method (Davidson & Hinkley, 1997).

### 2.4 Results

Clinical characteristics of the patients are summarized in Table 2.2. Patients (18 female and 8 male) ranged in age from 17 to 50 years (mean [SD] age, 31.9 [9.6] years). Visually normal subjects (20 female and 17 male) were similar in age (age range, 14-58 years; mean [SD] age, 31.3 [12.2] years). Patients’ ETDRS acuity ranged from 93 to 62 letters (mean [SD] acuity, 80.5 [8.9] letters), and foveal sensitivities ranged from 39 to 12 dB (mean [SD] sensitivity, 33.8 [5.5] dB). Normal ETDRS acuity ranged from 93 to 80 letters, and normal
foveal sensitivity ranged from 39 to 35 dB. Patients’ mean (maximum) fixational SD was 3.84 (9.90) arcmin for the horizontal meridian and 3.63 (8.92) arcmin for the vertical meridian, which is similar to observations in normal subjects (Barlow, 1952; Ditchburn, 1973; Putnam et al., 2005; R. M. Steinman, Haddad, Skavenski, & Wyman, 1973). Cone selections were made on average within 1 SD of the PRL, with the exception of patients 7 and 8. Cone spacing Z-scores ranged from -0.97 (30.4% cones above the normal average) to 7.61 (74.6% cones below the normal average). Figure 2.1 shows examples of foveal cone mosaics with varying Z-scores.

<table>
<thead>
<tr>
<th></th>
<th>Spearman’s rank correlation</th>
<th>P-value (α = 5%)</th>
<th>%-Cones-Below-Average</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual acuity (ETDRS Score)</strong></td>
<td>-0.60</td>
<td>0.003</td>
<td>For &lt;85 letters (20/20 VA):</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.82%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI = 1.77 - 43.59%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>For &lt;80 letters (20/25 VA):</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI = 34.16 - 65.83%)</td>
</tr>
<tr>
<td><strong>Foveal Sensitivity, Logarithmic</strong></td>
<td>-0.47</td>
<td>0.017</td>
<td>51.66%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI = 17.90 - 67.27%)</td>
</tr>
<tr>
<td><strong>Foveal Sensitivity, Linear</strong></td>
<td>-0.47</td>
<td>0.017</td>
<td>61.85%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(95% CI = 46.58 - 69.90%)</td>
</tr>
</tbody>
</table>

**Table 2.2 | Summary of statistical analyses: correlation between cone spacing Z-scores and visual function.** Foveal sensitivities are in logarithmic (decibel) and linear (1/Lambert) scales. \( P < 0.05 \) is statistically significant; %-Cones-Below-Average, upper limits of cone density change before abnormal values were observed for ETDRS acuity (<85 letters and <80 letters) and foveal sensitivity (logarithmic, <35 decibels; linear, <3162.28 1/Lambert). Adapted from Ratnam et al., 2013.
Figure 2.1 | AOSLO images of foveal cone mosaics. 0.5° x 0.5° AOSLO images of foveal cone mosaics in six subjects’ eyes, centered around the preferred retinal locus of fixation (white dot). Patients arranged by increasing % cones below average from left to right and top to bottom. Red crosshairs indicate cone selections used to calculate cone spacing $z$-scores and percentage of cones below average, with blue diamonds indicating the average location of cone selections. Green and orange lines indicate 1 standard deviation of fixation from the average PRL location in the horizontal and vertical directions, respectively. White scale bar = 0.25°. Adapted from Ratnam et al., 2013.

Visual acuity (Figure 2.2) and foveal sensitivity (Figure 2.3) are plotted against $Z$-scores and percentage of cones below average; Table 2.2 summarizes the statistical analyses. Cone spacing $Z$-scores and ETDRS acuity were significantly correlated ($\rho = -0.60$, $P= 0.017$). Cone percentage reductions before abnormal acuity was observed were 24.82% (95% CI, 1.77-43.59%) for fewer than 85 letters and 51.75% (95% CI, 34.16-65.83%) for fewer than 80...
letters (Figure 2.2). Cone percentages below average for abnormal logarithmic and linear foveal sensitivities were 51.66% (95% CI, 17.90-67.27%) and 61.85% (95% CI, 46.58-69.90%), respectively (Figure 2.3).
For <85 letters (20/20 VA):

Threshold: 24.82%
95% CI: [1.77, 43.59]

For <80 letters (20/25 VA):

Threshold: 51.75%
95% CI: [34.16, 65.83]
**Figure 2.2 | Visual acuity as a function of cone spacing.** *(Top)* Visual acuity measured as ETDRS letter scores correlates with cone spacing Z-scores. Vertically-shaded grey region indicates range of normal Z-scores (within ±2); horizontally-shaded region indicates normal range of visual acuity (100-85 letters). *(Center)* Visual acuity plotted against percentage of cones below average. Vertically-shaded grey region indicates % cone values corresponding to the normal range of Z-scores; horizontally-shaded grey region indicates normal range of visual acuity. Red line indicates cone percentage after which ETDRS scores fall below 85 letters (20/20 acuity); red shaded region indicates 95% confidence intervals (95% CI). *(Bottom)* Percentage of cones below average with threshold value and 95% CI for EDTRS scores below 80 letters (20/25 acuity). Adapted from Ratnam et al., 2013.

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**Figure 2.3 | Foveal sensitivity as a function of cone spacing.** *(Top)* Foveal sensitivity in logarithmic (decibel; left column) and linear (1/Lambert; right column) scales do not correlate with cone spacing Z-scores; vertical grey regions indicate normal range of Z-scores (within ±2) and horizontal grey regions indicate normal range for sensitivity. *(Bottom)* Foveal sensitivity plotted against percentage of cones below average. Red vertical lines and shaded regions indicate cone percentage reductions that correspond to foveal sensitivity and 95% confidence intervals (95% CI) after which sensitivity in decibel (<35 dB) and 1/Lambert units (<3162.28 1/Lambert) become abnormal. Adapted from Ratnam et al., 2013.
2.5 Discussion

This study is the first cross-sectional assessment of *in vivo* foveal cone structure and clinical measures of visual function in patients with inherited retinal degenerations. Earlier AOSLO studies have compared cone structure metrics in normal and diseased eyes, yet none reported correlations between these measures and visual function (Chen et al., 2011; Choi et al., 2006; Duncan et al., 2007, 2012; Duncan, Ratnam, et al., 2011; Duncan, Talcott, et al., 2011; Li & Roorda, 2007; Merino et al., 2011; Ratnam, Västinsalo, Roorda, Sankila, & Duncan, 2013; Rha et al., 2010; Roorda et al., 2007; Talcott et al., 2011; Wolfing, Chung, Carroll, Roorda, & Williams, 2006; Yoon et al., 2009). Our study reports a significant correlation between increased AOSLO cone spacing Z-scores and decreased VA and foveal sensitivity at the fovea. Near-normal VA (>20/40) and normal foveal sensitivity were observed when cone density was up to 52-62% below the normal mean.

2.5.1 Normal variability of human foveal cone density

This study reports percentage of cones below normal, rather than cone loss, due to the high individual variability in foveal cone density, which precludes such a metric when comparing density across subjects (Ahnelt, 1998; Chui et al., 2008; Chui et al., 2008; Curcio et al., 1990; Li et al., 2010; Song et al., 2011). Cone spacing Z-scores within 2 SD were considered normal; when converted to density, this value corresponded to a cone percentage decrease of approximately 36.7% from the normal mean.

Histologic evidence suggests that in spite of this high intersubject variability in cone density, the total number of cones near the foveal center is relatively constant (Curcio et al., 1990). Age-dependent changes in foveal cone density have been previously reported (Panda-Jonas, Jonas, & Jakobczyk-Zmija, 1995; Song et al., 2011). Although our patients and normal controls were age-matched for the purpose of this study; comparison of patient and normative data by decade may have further reduced variability effects. The limited number of subjects in our normative dataset prevented more specific age-related comparisons. Despite these limitations, our calculated threshold for cone densities below which visual function became abnormal was lower than the lower bound of cone densities attributable to normal variability (~36.7% cones below average), with the exception of ETDRS acuity less than 85 letters (threshold of 24.8% cones below average; Table 2.1). Although these results do not provide exact measurements of cone loss, they suggest that VA and foveal sensitivity are preserved when cone density is significantly lower than normal.

2.5.2 Comparison of AOSLO normative cone measures with histologic data

AOSLO cone spacing measures at the foveal center were converted into density and compared with histologic data from seven subjects (mean [SD] histologic peak foveal cone
density, 199,200 [87,200] cones/mm², range, 98,200-324,100 cones/mm²) by Curcio et al. (Curcio et al., 1990). To convert values from angular cone density to retinal distances, the assumption of 289 µm/deg was used (Bennett, Rudnicka, & Edgar, 1994; Merino et al., 2011). Mean AOSLO foveal density was hence 127,774.27 cones/mm² (95% CI, 85,297.41-235,152.41 cones/mm²), which is within 1 SD of, yet reduced from, the data by Curcio et al. The source of this disparity may be the larger sample size of the AOSLO normative data set (n = 37), which may be less susceptible to variability of a smaller dataset. Additionally, the PRL was assumed to coincide with the anatomical foveal center for 26 of the 37 normal AOSLO eyes, so the mean density value was likely lower than if the peak cone location had been used, as was done for the histologic data. However, since cone spacing measurements for the present study were made at or near the PRL, it was appropriate that the normative database was similarly collected relative to the PRL.

2.5.3 Uncertainty of the relationship between PRL and the location of peak cone density

In four patients for whom the PRL was unknown, the location of peak cone density was used for analysis. Although the PRL is typically displaced from the location of peak cone density (Li et al., 2010; Putnam et al., 2005; Wilk et al., 2017), the eye’s optical blur reduces VA below the theoretical sampling limit of foveal cones (Marcos & Navarro, 1997), lessening the effect of absolute cone density on visual function. Weymouth and colleagues (Weymouth, Hines, Acres, Raaf, & Wheeler, 1928) mapped grating acuity in 11-arcmin intervals within the fovea and found that acuity was highest at the PRL, suggesting that the location of highest cone density does not necessarily indicate maximum function. Therefore, the substitution in the present study of peak cone density for comparison with visual function is appropriate, although it may underestimate the extent of cone density reductions occurring at the PRL. The four patients had peak cone densities of 40.83% below to 30.40% above the mean foveal density of visually normal subjects; because these were derived near the PRL rather than the location of peak cone density, these values likely reflect a lower bound of cone changes occurring at fixation.

2.5.4 AOSLO density measurements represent an upper bound of structural changes

In the present study, cone spacing was used to quantify foveal cone structure. Cone spacing represents a conservative measure of cone mosaic integrity (Duncan et al., 2007) since reliable spacing estimates can be made even if not all cones have been identified within the retinal region. Since the spacing to density conversion assumes a close-packed hexagonal mosaic, the cone density measures reported represent an upper limit of percentage of cone density differences from normal. Worded differently, actual cone densities are likely lower
than what is reported. Nevertheless, cone density thresholds observed in this study are in agreement with earlier studies in which significant cone loss was predicted to be necessary to cause measurable reductions in visual function. By analyzing the psychometric functions of patients with Stargardt disease, Geller and Sieving (Geller & Sieving, 1993) estimated that 90% of cones would be lost before significant functional changes occurred in these subjects. Additionally, Eagle and colleagues (Eagle, Lucier, Bernardino, & Yanoff, 1980) reported that a patient with juvenile macular degeneration maintained 20/30 acuity prior to his death, in spite of significant foveal deterioration. Seiple et al. (Seiple, Holopigian, Szlyk, & Greenstein, 1995) used pixel blanking in optotypes to simulate foveal cone dropout and determined that a loss of 80% of foveal cones was necessary to reduce acuity below 20/40. These results support our observations that VA is resilient to significant changes in foveal cone topography.

2.5.5 Longitudinal studies would facilitate accurate assessments of degeneration in individual subjects

The cross-sectional design of this study precluded tracking of longitudinal structural and functional changes in individual subjects. Because normal intersubject variability in foveal cone density prevents measurement of absolute photoreceptor loss, a longitudinal follow-up to the present study would facilitate accurate tracking of degenerative changes measured structurally and functionally. A longitudinal study of AOSLO cone measures published by Talcott et al. (Talcott et al., 2011) tracked three patients with inherited retinal degenerations treated with sustained-released ciliary neurotrophic factor (CNTF) over 30 to 35 months. Cone spacing increased by 2.9% and density decreased by 9.1% more per year in sham-treated versus CNTF-treated eyes, but VA and visual field sensitivity remained stable. These findings indicated preserved visual function despite significant cone loss in sham-treated eyes, and a longitudinal follow-up (summarized in section 2.7) to the present study showed similar findings.

2.5.6 Intrasubject variability of psychophysical measures

Due to the small size of the present study's dataset, Spearman rank correlation, which is more robust and insensitive to the effects of outliers than regression analysis, was used to evaluate correlations between cone spacing and visual function. The noise in the current dataset may be partially attributed to intrasubject variability in psychophysical examinations, which is worsened in patients with increased disease severity (Bittner, Ibrahim, Haythornthwaite, Diener-West, & Dagnelie, 2011; Grover, Fishman, Gilbert, et al., 1997; Kiser, Mladenovich, Esbraghi, Bourdeau, & Dagnelie, 2005). This amplified inconsistency with advanced stages of disease may be due to the irregular response of remaining foveal cones to light stimulation. Additionally, variations in test procedures (e.g.,
chart luminance, test distance, and examiner instructions) may also increase statistical error (Arditi & Cagenello, 1993). Although the ETDRS scoring protocol used in this study provides high test-retest ability (Arditi & Cagenello, 1993; Vanden Bosch & Wall, 1997), trained, visually normal subjects can still have inter-test variability of 3.5 to 5 letters (Arditi & Cagenello, 1993; Bailey & Lovie, 1976; Elliott & Sheridan, 1988). This variability reinforces the need for more objective measures such as cone structure for assessing retinal health.

### 2.5.7 Relationship between structural measures and VA

The present study found a significant relationship between foveal cone spacing and VA, which is consistent with previous structure-function correlations using optical coherence tomography (OCT). Previous reports have shown significant correlations between VA and foveal thickness (Ergun et al., 2005; Sandberg, Brockhurst, Gaudio, & Berson, 2005; Witkin et al., 2006), but they did not determine the extent of degeneration before abnormal values were observed psychophysically. Ergun and colleagues (Ergun et al., 2005) found significant linear relationships between VA and foveal thickness ($R^2 = 0.51$, $P = 1 \times 10^{-4}$). Sandberg et al. (Sandberg et al., 2005) compared ETDRS acuity and foveal thickness in RP patients using multiple models and found that second-order polynomial models provided the best fits, accounting for a decline in VA at smaller and larger retinal thicknesses because of cone loss and edematous thickening, respectively. They predicted a four-year time course before significant structural changes were observed with OCT, which is similar to the time needed to observe significant changes in ETDRS acuity ($0.9 \text{ letters/year} \times 4 \text{ years} = 3.6\text{-letter decrease over 4 years}$), which is within the threshold range for significant acuity change in normal subjects (3.5-5 letters; (Arditi & Cagenello, 1993; Bailey & Lovie, 1976; Elliott & Sheridan, 1988)). Talcott et al. (Talcott et al., 2011) reported significant reductions in cone density over 30 to 35 months in the absence of VA changes, suggesting that direct visualization of the cone mosaic may provide an earlier measure of structural changes.

### 2.5.8 Relationship between structural measures and foveal sensitivity

The present work found a significant correlation between cone spacing and foveal sensitivity. Cone density thresholds were 52-62% below normal before abnormal values were seen in sensitivity. This inconsistency is likely due to inability of perimetry stimuli to detect subtle changes in photoreceptor topography. The Goldmann III stimulus has a size of $0.12\text{ degree}^2\ (432\text{ arcmin}^2)$ on the retina from a distance of 0.33 meter ($m$) (Vislisel, Doyle, Johnson, & Wall, 2011). Since the diameter of a foveal cone is 0.5 arcmin (Putnam et al., 2005), approximately 2200 foveal cones would sample a Goldmann III stimulus. Since each foveal cone corresponds to a single receptive field (Dacey, 1993), functionally normal cones
may conceal dysfunctional regions. Smaller stimulus sizes such as the Goldmann I (0.0075 degree\(^2\) at 0.33 m) may increase sensitivity to subtle structural abnormalities, but these benefits may be compromised by the increased test-retest variability of smaller stimuli (Vislisel et al., 2011). Since AOSLO images showed 52-62% decrease in foveal cones before abnormal sensitivity was observed, these results suggest that structural measures may provide an earlier and more objective measure of degeneration.

### 2.5.9 AOSLO-based microperimetry for single-cell functional testing

Although AOSLO cone measures assess the integrity of the cone mosaic, they do not provide information on the health of individual cones. For AOSLO imaging to become a comprehensive and objective measure of disease progression, the structure-function relationship for individual foveal cones needs to be assessed. Makous and colleagues (Makous et al., 2006) used 0.75-arcmin AO-corrected stimuli to identify microscotomas and an estimated 30% cone loss in a deuteronopic patient with normal VA and visual field. This finding suggests that single-cone microperimetry may be necessary for evaluating subtle functional changes in the cone mosaic; to facilitate testing individual cone function, Tuten et al. (Tuten et al., 2012) have developed AOSLO-based microperimetry with real-time eye tracking. AOSLO microperimetry thus facilitates targeted, longitudinal functional testing of individual cones. Recent work using AOSLO-based microperimetry has shown visual sensitivity within retinal areas of ambiguous cone morphology, suggesting that single-cell functional testing is important for quantifying the health of cone photoreceptors within retinal lesions (Tu et al., 2017; Wang et al., 2015).

### 2.5.10 Structural measures may provide more reliable predictors of foveal degeneration than visual fields

For rod-cone degenerations in which cone loss begins in the periphery and encroaches inwards, natural history studies predict half-life times of Goldmann V-4e field loss ranging from 5 to 15 years (Holopigian et al., 1996; Iannaccone et al., 2004). Alexander and colleagues reported that VA loss in RP patients occurred following parafoveal photoreceptor degeneration, after which inner segment enlargement increased foveal cone spacing and decreased foveal sampling resolution (Alexander et al., 1992). Madreperla et al. showed that clinically significant VA loss (<20/40) in RP patients occurred after the visual field narrowed to a 15° radius, suggesting that visual field radius could be a useful marker for the onset of foveal dysfunction (Madreperla, Palmer, Massof, & Finkelstein, 1990). This prognosis however requires knowledge of the rate of visual field decay, which varies due to factors such as visual field loss pattern (Grover, Fishman, Anderson, Alexander, & Derlacki, 1997), critical age (Fishman, Bozbeyoglu, Massof, & Kimberling, 2007; Iannaccone et al., 2004), disease genotype (Sadeghi, Eriksson, Kimberling, Sjöström,
& Möller, 2006; Sandberg, Rosner, Weigel-DiFranco, Dryja, & Berson, 2007), and environmental and dietary factors (Hartong, Berson, & Dryja, 2006; Sunga & Sloan, 1967). Additionally, the rates of field loss can fluctuate over an individual’s lifetime (Sunga & Sloan, 1967). Because of these variations, the rate of visual field loss cannot be reliably predicted as a marker for VA decline. Instead, structural measures such as those provided by AOSLO may be used as an earlier indicator of parafoveal cone changes than visual function, enabling disease monitoring and treatment intervention before the fovea exhibits signs of degeneration.

2.5.11 Less commonly used clinical measures of function may be more sensitive to structural changes than VA or sensitivity

The purpose of the present study was to show that clinical measures of visual function, specifically VA and foveal sensitivity, are insensitive indicators of cone structural integrity. However, other psychophysical tests may provide improved sensitivity. Conventional tests such as ETDRS charts or Landolt rings use high-contrast figures to assess visual dysfunction, which may not be as sensitive to foveal degradation as contrast sensitivity tested at specific spatial frequencies. Akeo et al. (Akeo et al., 2002) reported systematic correlations between Landolt ring VA and contrast sensitivity at lower spatial frequencies (1.5, 3.0, and 6.0 cycles/degree) in RP patients with greater than 20/50 VA. At 18 cycles/degree however, a subset of these patients with 20/25 acuity had significantly reduced contrast sensitivity (<15 cycles/degree). Lindberg et al. (Lindberg et al., 1981) showed similar contrast sensitivity reductions at higher frequencies in patients with RP undetected by Snellen acuity, suggesting that abnormalities in patients with normal VA may be detectable with contrast gratings at high spatial frequencies. Despite these advantages, contrast sensitivity is difficult to implement routinely since test distance, lighting, and duration of grating presentation must be precisely controlled (Lindberg et al., 1981) and is more affected than conventional VA by ocular conditions such as cataract (Elliott, 1993).

In conclusion, direct, high-resolution images of cone structure such as those provided by AOSLO may provide more sensitive and reliable indicators of foveal degeneration than VA and foveal sensitivity. These findings support the use of AOSLO images as an outcome measure of disease progression and suggest that treatment intervention is best done before measurable functional loss occurs, at which point significant structural changes may already be present. Large cross-sectional and longitudinal assessments of patients are still necessary for better understanding the relationship between cone structure and standard measures of visual function.

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2.6 **Summary**

The fovea is the most crucial retinal location for vision due to its high spatial resolution and hence importance in everyday tasks. In rod-cone degenerations during which central vision is initially spared, visual acuity is preserved until late stages of disease in spite of observable cone abnormalities (Akeo et al., 2002; Alexander et al., 1986; Birch, Sandberg, & Berson, 1982; Lindberg et al., 1981; Wolkstein et al., 1980). Intrasubject variability in psychophysical techniques such as VA and sensitivity preclude accurate quantification of foveal degeneration and early therapeutic intervention. More objective measures of vision loss, such as the direct visualization of foveal cones, would provide a more reliable assessment of degeneration. In the present study, AOSLO-derived cone spacing and density measures were compared to best-corrected VA and foveal sensitivity in 26 patients with rod-cone degenerations. Cone density was reduced to up to 62% below normal before VA and sensitivity reached abnormal levels, suggesting that direct, objective measures of cone structure may be more sensitive indicators of disease severity.

2.7 **Longitudinal follow-up study and AOSLO-based acuity measures**

Since the preceding cross-sectional study was inadequate for measuring intrasubject changes in acuity with degeneration, a longitudinal follow-up study was necessary to quantify functional changes with respect to cone loss. Saud et al. (Saud et al., 2017) compared foveal cone spacing with BCVA in 15 eyes of 11 patients with inherited retinal degenerations and 10 eyes of 5 normal subjects monitored longitudinally over periods ranging from 10 months to 5 years. Similar to the current study, cone spacing measurements were made as close to the PRL as possible. There was no significant change in ETDRS acuity or foveal sensitivity across imaging sessions in normal subjects. In patients, however, significant increases in cone spacing (mean Z-score = +0.56) were observed while acuity (mean change, 0.13 letters; 95% CI, - 1.2 - 1.7) and foveal sensitivity (mean change, -0.6 dB; 95% CI, - 1.5 - 0.3) remained stable, suggesting that clinical measures of function inadequately reflect retinal structural changes.

Both the cross-sectional and longitudinal studies suggest that the relationship between foveal structure and psychophysical measures is not linear, which may be partially attributed to the redundancy or oversampling of foveal cones in relation to clinical measures of ‘normal’ function. Given reported foveal cone densities (Curcio, Sloan, Kalina, & Hendrickson, 1990), a loss of 40% of foveal cones is still adequate for obtaining 20/20 acuity levels (Geller et al., 1992). Additionally, the direct relationship between visual acuity and cone spacing is confounded by the presence of ocular aberrations, which blur the image on the retina and effectively make clinical acuity a blur-limited rather than sampling-limited task.
Since the AOSLO is a robust tool for compensating for ocular-blur and delivering near-diffraction limited stimuli to the retina, AOSLO-based measures of function would provide a more systematic way for measuring functional changes at the level of individual cone photoreceptor cells. We have recently implemented AOSLO-based acuity tests to better gauge how spatial resolution is affected by subtle changes in the cone topography of degenerative retinas (Loumou et al., 2016). AOSLO-based acuity measures thus allow a more precise measure of functional changes than clinical-based tests.

It is important to mention that visual acuity is not necessarily a static task. Fixational eye movements, or the normal jitter of the eye, perpetually moves an otherwise static retinal image across multiple photoreceptor cells, shifting spatial signals into the temporal domain. Recent work by Rucci et al. has shown that the dynamic signal conferred by eye movements can improve the discriminability of high spatial frequencies (Rucci, Iovin, Poletti, & Santini, 2007). In the next chapter, we investigate whether this benefit of fixational eye movements extend to spatial frequencies at the retinal sampling limit, which could help explain how acuity is preserved despite considerable cone loss.

2.8 Acknowledgements

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Chapter 3

Benefits of retinal image motion at the limits of spatial vision
Chapter 3

Benefits of retinal image motion at the limits of spatial vision

3.1 Abstract

Even during fixation, our eyes are constantly in motion, creating a dynamic signal in each photoreceptor cell. Neuronal processes can synthesize these temporal signals to serve spatial vision, but it is unknown how our finest visual acuity is maintained during this process. We used an adaptive optics scanning laser ophthalmoscope to control the spatiotemporal signal at the cone photoreceptor scale in human observers during a visual discrimination task under motion conditions in which normal, cancelled, or manipulated retinal image motion occurred. When stimuli moved, acuities were 25% better than in the case of stabilized presentation, regardless of whether the motion was self-induced, a playback of similar motion, or an external simulation of cone activation patterns. We argue that in our experimental condition, the visual system is able to synthesize a higher resolution percept from multiple views of a poorly resolved image; these results may extend the current understanding of how fixational eye motion affects high acuity vision.

3.2 Introduction

Even when fixating on a static object, our eyes are constantly moving. As a result, the human visual system has to incorporate methods that transform a dynamic retinal signal into a stable, acute percept. Classic theories of visual acuity postulated that fixational eye movements (FEM), small mostly involuntary movements of the eye, may enhance fine spatial detail through a dynamic sampling process (Ahissar & Arieli, 2012; Arend, 1973; Averill & Weymouth, 1925; Marshall & Talbot, 1942). Early experiments, limited by available technology, were unable to support these hypotheses (Kelly, 1979; Riggs, Ratliff, Cornsweet, & Cornsweet, 1953; Tulunay-Keesey, 1960, 1982; Tulunay-Keesey & Jones, 1976). Recent work by Rucci et al. (Rucci et al., 2007) showed FEM benefit discrimination of high spatial frequencies as high as 10 cycles/degree of visual angle. This benefit was attributed to the equalization or “whitening” of spatial energy across the temporal domain (Kuang, Poletti, Victor, & Rucci, 2012; Rucci et al., 2007), reducing the relative power of low spatial frequencies in natural scenes. While spectral equalization can account for improvements in perceptual contrast of retinal images resolvable by the cone mosaic (Rucci & Victor, 2015), this benefit cannot readily predict discrimination improvement of high-contrast images at the retinal sampling limit. Humans can resolve optotypes at the 20/10 acuity level and above (Rossi et al., 2010), suggesting that spatial resolution is not necessarily limited by the structural sampling limit of the retina (Curcio, Sloan, Kalina, & Hendrickson, 1990). The role of FEM in spatial resolution therefore remains unclear.
With recent improvements in eye tracking and stimulus delivery using adaptive optics scanning laser ophthalmoscopy (AOSLO), stimuli at the cellular scale can be delivered to the retina with an accuracy of 0.15 arcmin, smaller than the diameter of a foveal cone (Arathorn, Stevenson, Yang, Tiruveedhula, & Roorda, 2013; Harmening, Tuten, Roorda, & Sincich, 2014). By also correcting for ocular blur, the ability to deliver near diffraction-limited, retinally stabilized stimuli enables testing the effects of FEM on visual perception at the cone photoreceptor level. We find that FEM improve discrimination performance for poorly resolved images.

**3.2 Methods**

**3.2.1 Subjects**

In Experiments 1 and 2 (AOSLO-based), subjects were four adults (three males, one female; ages 30-38 years), who had no known visual issues and were naïve to the purpose of the study. Subject S4 was available for Experiment 1 only. A drop of 1% Tropicamide solution was instilled in the test eye 15 minutes prior to testing for pupil dilation and cycloplegia. For Experiment 3 (stimuli presented on LCD), eight additional subjects were recruited (six naïve, two of the authors; five female, three male; ages 25-37 years). Informed consent was obtained for each subject and experimental procedures adhered to the tenets of the Declaration of Helsinki.

**3.2.2 AOSLO imaging and stimulation**

Adaptive optics imaging and micro-stimulation were used to present retina contingent visual stimuli to targeted locations in cone-resolved retinas of human observers, a method described in detail elsewhere (Arathorn et al., 2007; Rossi & Roorda, 2010b). The light source was a supercontinuum laser (SuperK Extreme; NKT Photonics) with an infrared imaging wavelength of 842 ± 25 nm (luminance of ~ 4 cd/m^2^). Retinal images were derived by raster scanning a spot across the retina with horizontal and vertical scan rates of 16 kHz and 30 Hz respectively. High order aberrations were measured with a Shack-Hartmann wavefront sensor, and a 144-actuator, 5.5-micron stroke deformable MEMs mirror (Boston Micromachines Corp) corrected the computed wavefront error. Corrected light was captured with a photomultiplier tube, whose voltage output combined with positional signals from scanning mirrors created 512 x 512 pixel videos with a framerate of 30 Hz. A 1 second retinal video was recorded with every stimulus presentation trial (500 trials per condition per subject).

Stimuli were encoded into the scanning raster via 20 MHz acousto-optic modulation that switched off the laser beam at points in the raster corresponding to the stimulus location, producing stimulus decrements with high contrast (dark “E” on a red background).
Michelson contrast between full-on and full-off stimulation was 99.9%. Diffraction reduced the actual “E” contrast to between 60%-75%. Sampling resolutions for imaging and stimulus projection ranged from 0.12 to 0.16 arcmin per pixel, depending on the specific letter size chosen for each subject (see Results). The 30 Hz scanning rate has been shown to elicit neural signals in LGN parvocellular neurons (Sincich, Zhang, Tiruveedhula, Horton, & Roorda, 2009). Since AOSLO images provide unambiguous records of stimulus location during delivery, proper stimulus encoding and stabilization were verified during postprocessing. Retinal locations 0.8°-1.3° from the fovea along the horizontal meridian (nasally in S1 and S4; temporally in S2 and S3) were selected for testing (Figure 3.1A-C).

3.2.3 Eye movement analysis and trial rejection

Fixational eye movements were analyzed offline from recorded AOSLO videos with a sampling rate of 840 Hz (Stevenson, Roorda, & Kumar, 2010). Since microsaccades rarely occurred during the 750-ms stimulus presentation (Figure 3.2), and to avoid confounding effects due to microsaccadic suppression, trials in which microsaccades occurred were removed from further analysis. Eye movement characteristics described in this study are therefore due to drift and tremor only.

Proper stimulus delivery was verified by looking for distorted, improperly stabilized, or otherwise incorrectly delivered stimuli in the AOSLO videos. Trials with misdelivered stimuli were removed from further analysis.

3.2.4 Experiment 1: Natural versus manipulated retinal motion

In Experiment 1, we compared visual discrimination under two manipulated image motion conditions (stabilized and incongruent) with natural viewing. In natural viewing, retinal image motion was caused by ongoing FEM (Figure 3.3A, Figure 3.4A). In stabilized viewing, stimulus presentation was modulated to be locked onto a targeted set of cones (Figure 3.3B, Figure 3.4B). Incongruent motion was derived by (1) compensating for habitual FEM in real-time and (2) inducing net stimulus motion that followed a trajectory extracted from subjects’ earlier eye motion traces. Since subjects showed idiosyncratic eye movements, stimulus trajectories were selected randomly from a set of subjects’ earlier eye motion traces. The resulting net movement of the stimulus relative to the retina was a path similar to typical eye motion but incongruent to eye motion occurring at the time of presentation (Figure 3.4C).

In a four-alternative-forced-choice task, subjects reported the orientation of an “E” optotype with maximum negative contrast. The height and width of the letter were five times the line thickness. Subjects gazed at a fixation laser target while attending to the peripheral stimulus. The parafoveal location was chosen for two reasons: (1) to prevent the
subjects from trying to follow a stabilized stimulus, which would appear to move relative to the scanning raster, and (2) because image stabilization is better in locations just off the fovea where cone photoreceptors are easier to resolve. The exact retinal location of stimulus delivery was determined from AOSLO videos for each subject (Figure 3.1C). Stimulus size was selected to be undersampled by the underlying cone mosaic, with an average number of cones sampling the image at any given time being ~21, compared to 23 for a stimulus at the cone sampling limit. Illustrations and an equation demonstrating this relationship are shown in Figure 3.1D-E. Subjects correctly discriminated about 40%-60% of letters presented at this stimulus size in an earlier acuity experiment; this performance range was selected to ensure subjects performed above the 25% guessing rate but below the performance plateau. The stimulus gap size used was about 0.6 arcmin on average, corresponding to a Snellen optotype of 20/12, which, for an “E”, has a dominant spatial frequency of 50 cycles/degree. The stimulus was presented for 500 trials in each viewing condition, pseudorandomly interleaved and divided into ten experimental blocks. Comparison of stabilized and incongruent motion with natural viewing was done over two successive experimental sessions.

3.2.5 Experiment 2: Contrast matching and discrimination performance

Experiment 2 consisted of a contrast matching task and discrimination task. For the contrast matching task, subjects matched perceptual contrast for stimuli presented under stabilized and incongruent viewing conditions; the purpose of this task was to quantify the perceptual fading of the stabilized stimulus compared to the moving stimulus. Stimulus duration, size, and retinal location were identical to those from Experiment 1 except that square stimuli were used instead of optotypes (Figure 3.5A). The use of a square allowed subjects to focus on stimulus contrast, rather than orientation, for the matching task. Two vertically offset squares were simultaneously presented, one stabilized and the other incongruently moving. Simultaneous presentation ensured that stimulus motion relative to the AOSLO scanning raster were similar, so the stimuli would not be easily differentiated other than by perceptual contrast. For each trial, subjects indicated which stimulus appeared darker. The contrast of the stabilized square was fixed while the incongruently moving square’s contrast was adjusted over repeated staircases to converge onto the value for which it appeared similar to the stabilized square.

Seven one-down-one-up staircases were used for the contrast matching task. Staircases terminated after seven reversals, and the threshold was calculated as the mean value from the last four reversals. For the initial three staircases, stimulus contrast started at the maximum physical contrast. The mean threshold value from these staircases was doubled and then used as the initial value for the next three staircases. For the final staircase, the starting value was the mean of the previous six staircase thresholds; contrast step intervals
were made smaller as to provide finer resolution when determining the final contrast value.

This reduced contrast value was used for the second part of Experiment 2. The protocol for Experiment 1 was repeated except that discrimination of naturally moving, maximum contrast and naturally moving, reduced contrast “E” optotypes were compared. Maximum and reduced contrast conditions were pseudorandomly interleaved for 250 trials each.

### 3.2.6 Experiment 3: External computer-based simulation

To better understand the amount of visual information needed to benefit from image motion, simulations of cone activation patterns were constructed and presented to a separate subject cohort in a monitor-based discrimination experiment. Stimuli were computed with custom written Matlab scripts (Figure 3.6A-B). Spatial representations of cone apertures were constructed using randomly jittered hexagonal arrays with center-to-center distances equaling those from cone outer segment distances (Curcio et al., 1990). Cone apertures were represented by a two-dimensional Gaussian whose full-width at half-maximum was 48% of the inner segment diameter for the mean eccentricity from the AOSLO experiments (MacLeod, Williams, & Makous, 1992). A binary image of an at-threshold “E” stimulus was spatially convolved with a two-dimensional Gaussian to represent residual blur due to diffraction. The stimulus was then filtered by the simulated cone array and summed across each cone aperture to generate activation values ranging from 0 to 1 for each cone. The model cone array was then replaced by a Voronoi diagram representing cone locations. Each Voronoi cell had a gray value representing the cone activation value. The size of the simulated activation patterns was magnified on the computer screen such that visual acuity did not limit performance on the discrimination task. Eight subjects (six naïve, two of the authors) discriminated stimulus orientation via a liquid-crystal display at 2 m viewing distance. Subject positioning was stabilized using a head and chin rest. 150 trials of each static and dynamic viewing condition were presented pseudorandomly interleaved. In static viewing, cone and stimulus locations were held fixed. In dynamic viewing, the position of each cone relative to the stimulus was updated at 30 Hz based on motion paths drawn from the AOSLO experiments. All subjects were presented with the same motion paths but in random sequences. Inter-trial progression was self-paced and stimulus presentation time was 750 ms.
3.3 Results

3.3.1 Nature of FEM during the acuity task and quality of stabilization

AOSLO imaging and micro-stimulation enabled us to study the exact nature of FEM during a given task (Figure 3.3A), as well as provide unambiguous records of tracking performance (Figure 3.3B). FEM behavior shown here represent FEM that occur within 1-second epochs of stimulus presentation and that occur when the eye is fixating on a target while attending to a peripheral task. Additionally, analyzed trials do not include those containing microsaccades or poorly tracked trials, which comprised between 10% and 20% of the trials.
Figure 3.2 | Distribution of microsaccades over experimental trials. Normalized distribution of microsaccades (MS) for all trials in the natural condition containing MS, which constituted 10-20% of total trials. Gray region indicates stimulus presentation time; dashed line indicates distribution if MS occurred uniformly across trial. Subjects S1-S3 show propensities for suppressing MS until latter stages of each experimental trial.

In Experiment 1, of the total 929 trials analyzed with a naturally moving stimulus, FEM showed idiosyncratic differences across subjects. Some subjects exhibited relatively random FEM directions between each trial (Figure 3.3C, subjects S1 and S2). FEM trajectories from S3 and S4 showed directionality of motion during the task (Figure 3.3C, S3 and S4). Absolute trajectory length across subjects was similar. Relative to retinal cone mosaic, the stimulus traversed a retinal distance equaling about 10.5 unique cones during each 750-ms presentation during natural viewing. In 600 analyzed trials from the stabilized condition, residual stimulus motion from tracking and stabilization techniques was small. On average the stimulus traversed 0.4 cones across all subjects. This analysis confirms that the exact same set of cones was stimulated during the stabilized as opposed to natural condition.

Given the nature of the discrimination task, we wondered if the eye can adjust FEM relative to orientation of the letter optotype to maximize temporal information content, and whether specific motion traces improve performance on the discrimination task compared to others. The same motion paths as in Figure 3.3C are plotted in Figure 3.3D, but rotated relative to optotype orientation during presentation and with indication of correct and incorrect psychophysical responses. No clear trends were observed in this analysis; the eye
does not seem to adjust FEM behavior according to letter orientation under a short period of time, and specific motion directions do not appear to confer clear benefits.

**Figure 3.3 | Retinal image motion due to FEM and motion manipulation.** (A) Projected stimuli are directly encoded into the AOSLO video, allowing for an unambiguous record of the relative locations of the retina and the stimulus over the course of each trial. Here, the path of the stimulus over the course of one trial (duration: 750 msec, colored dots denote stimulus location in each of 23 video frames) of a naturally moving eye is shown. Due to fixational eye motion, the ‘E’ moves over many photoreceptors. (B) When stimuli were presented stabilized, residual stimulus movement was smaller than the diameter of single cones. (C) Retinotopic stimulus trajectories for natural (blue) and stabilized (orange) conditions are shown across subjects S1-S4; subjects exhibit idiosyncratic differences in FEM, sometimes with micro-nystagmus type orientation preferences (e.g., S3). Concentric circles represent 5, 10, and 15 arcmin radii of visual angle around the retinal location of stimulus starting location (compare Fig. 3.1C). (D) Trajectories from the natural condition corresponding with correct (blue) and incorrect (red) psychophysical responses are replotted relative to stimulus orientation. There is no clear relation between how the stimulus is sampled and discrimination performance. The size of the letter for each subject is superimposed for reference. Adapted from Ratnam et al., 2017.

3.3.2 Experiment 1: Discrimination benefits from FEM at the resolution limit

Discrimination performance dropped by an average of 23% with retinal image stabilization (Figure 3.4D; p < 0.05, two-tailed binomial z test). Fine spatial resolution was impaired in the absence of retinal image motion due to FEM. Visual resolution achieved was higher than predicted by spatial sampling models of the cone mosaic. For each subject, the distance between adjacent bars of the “E” was compared to the Nyquist limit ($N_c$) of the tested retinal location (Figure 3.1E). The stimulus gap constitutes the primary image detail subjects use to discriminate orientation (Rossi & Roorda, 2010b). The gap size was smaller than $N_c$ for each subject (gap size/$N_c$ = 0.61/0.90, 0.74/0.85, 0.63/0.80, 0.57/0.94 arcmin for S1 through S4, respectively).
Subjects performed similarly or better under the incongruent than natural condition (Figure 3.4E; S1, $p < 0.01$; S2 and S3, $p > 0.05$; two-tailed binomial $z$ test, $n = \sim 450$). These findings demonstrate that the visual system can benefit from retinal image motion even when activity is mismatched with FEM at the time of stimulus presentation.

![Figure 3.4 | Stimulus motion improves acuity at the resolution limit.](image)

**Figure 3.4 | Stimulus motion improves acuity at the resolution limit.** (A) In natural viewing, the stimulus ('E') is fixed in space and the retinal cone mosaic (circles) moves due to fixational eye motion (FEM, light blue arrow). (B) In stabilized viewing, the stimulus moves with the retina (orange arrow), such that it stays locked on the same cones during presentation. (C) In the incongruent motion condition, the stimulus moves - while the eye performs its habitual FEM - in a path according to a previously recorded FEM trace. (D) Stimulus stabilization reduced discrimination performance in all subjects by an average of 23%. (E) Relative to the natural viewing condition, subjects performed equally well or better when incongruent motion was employed. Asterisk (*) denotes $p$-value $< 0.05$. Adapted from Ratnam et al., 2017.

### 3.3.3 Experiment 2: Contrast reduction during stabilization is not critical

To quantify whether contrast was reduced by stabilized stimulus delivery and how performance may have been affected, we devised a pair of experiments. The perceived contrast of stabilized versus moving stimulus was reduced by about 20%, but performance was similar ($p > 0.05$, two-tailed binomial $z$ test, $n = \sim 250$) for discrimination of naturally moving stimuli presented at full and reduced (80%) contrast (Figure 3.5). These results suggest reduced contrast was not responsible for decreased performance under stabilized conditions.
Figure 3.5 | Contrast matching and discrimination at reduced contrast. (A) Two squares with identical dimensions to the ‘E’ stimuli in experiment 1 were simultaneously presented retinally stabilized and in an incongruent motion similar to subjects’ own eye movements. Over multiple staircases, the contrast of the moving square was updated until both squares appeared perceptually similar to the subject. These reduced contrast values (percentages indicated in (B)) were used in the second part of the experiment. (B) Discrimination performance for naturally moving, maximum contrast and naturally moving, reduced contrast ‘E’s were compared. Reduced contrast values, indicated as a percentage of maximum contrast, are shown for each subject. Subjects performed similarly for both conditions. Adapted from Ratnam et al., 2017.

3.3.4 Experiment 3: Dynamic cone activation patterns suffice for discrimination benefit

We tested whether dynamic information presented at the photoreceptor level, effectively a series of poorly sampled “snapshots”, is sufficient for improving discrimination under the natural motion condition. Using an external monitor, subjects viewed simulations of cone activation patterns for moving and stabilized stimuli (Figure 3.6A-B).

Subjects performed on average 27% worse in the static stimulus condition (Figure 3.6C; S1 and S3, \( p < 0.01 \); remaining subjects, \( p < 0.001 \); two-tailed binomial z test, \( n = 150 \)). This degradation is similar to the performance reduction (23%) in Experiment 1; performance ratios of the natural versus stabilized AOSLO experiment and dynamic versus static simulation experiment were not significantly different (mean ratio: 1.30 and 1.38, respectively; \( p = 0.57 \); Wilcoxon rank sum test). These results reinforce the fact that the retinal output via dynamic cone excitation patterns, regardless of ongoing FEM, contains sufficient information to improve discrimination of images at the sampling resolution limit.
Figure 3.6 | Modelled dynamic cone activation produces a similar benefit to feature discrimination as actual retinal motion. (A) A model of cone activation was derived by convolution of size-matched stimuli with a Voronoi patch of cone photoreceptor positions (see Methods for details). (B) Presented on a standard computer display, stimuli were either computed on a non-moving model mosaic (Static), or on one that moved based on fixational eye movements from the AOSLO experiments (Dynamic). (C) Similar as in natural vs. stabilized viewing, discrimination performance of all subjects dropped when stimuli were presented statically. Asterisk (*) denotes P-value < 0.05. Adapted from Ratnam et al., 2017.

3.4 Discussion

Our results demonstrate that discrimination of high contrast optotypes at the retina’s resolution limit benefit from image motion similar to or caused by motion due to FEM. The benefits of eye motion observed in this study are restricted to those caused by ocular drift.
and tremor only. Subjects rarely exhibited microsaccades during the stimulus presentation interval and trials in which microsaccades occurred were removed to eliminate the effects of microsaccadic suppression on stimulus visibility. Given the current understanding of the functional consequences of FEM on vision, our findings offer cause to extend such theories.

Theories of spatial whitening postulate that temporal modulations induced by FEM serve to spectrally equalize the power of natural images across spatial frequencies by decorrelating low spatial frequencies and enhancing high spatial frequencies. Temporal modulations induced by typical FEM amplitudes can improve contrast thresholds for stimuli up to 10 cycles/degree in the presence of lower frequency noise or natural image statistics (Kuang et al., 2012; Rucci et al., 2007). Additionally, since stabilized stimuli fade due to neural adaptation (Ditchburn & Ginsborg, 1952; Riggs & Ratliff, 1952; Riggs et al., 1953), we first needed to explore the extent to which degraded performance under stabilized image presentation could have been due to reduction in perceptual contrast of the stimulus. An important aspect of AOSLO stimulus delivery is that stimuli delivered via the AOSLO scanning raster are continuously modulated at 30 Hz, corresponding to the system's frame rate (see Methods). It is known that such temporal modulation is preserved in visual signals up to postretinal stages, as those 30 Hz signals in neural activity, including those measured under stabilized stimulus conditions, have been observed in LGN parvocellular neurons (Sincich et al., 2009). While the raster refresh rate may have minimized fading, perceptual fading of relatively stable but flickering stimuli is still known to occur (Schieting & Spillmann, 1987). The minor amount of fading for the stabilized condition could not explain the performance reduction observed in Experiment 1, and we generally observed that contrast did not limit discrimination performance (Experiment 2). Additionally, our visual stimuli were undersampled by the photoreceptor array, a situation that is not explicitly considered in previous studies.

It is unclear that whitening theories can readily explain the results of our study, and alternative explanations may be necessary for how FEM enhance acuity. One potential mechanism can be found in multiframe superresolution algorithms in the field of computer vision, in which a high-resolution image is reconstructed from a series of lower resolution frames, enabling the synthesis of images surpassing the spatial resolution of the original camera (Ben-Ezra, Zomet, & Nayar, 2005; Farsiu, Robinson, Elad, & Milanfar, 2004). Superresolution techniques include multi-exposure noise reduction and subpixel image location, in which the centroid of light distribution, blurred due to undersampling, can be computed with subpixel accuracy. Both mechanisms are feasible within the visual system. Shifter circuits (Anderson and Van Essen, 1987), interpolation circuits (Barlow, 1979; Crick, Marr, & Poggio, 1981), neural networks (Pitkow, Sompolinsky, & Meister, 2007), and neuronal phase locked loops (Ahissar & Arieli, 2012) have all been proposed as mechanisms by which the signals from a moving retinal image can be correctly integrated.
The visual system is capable of a form of subpixel resolution in a phenomenon known as hyperacuity (Westheimer, 1987), in which relative stimulus positioning can be judged at a resolution three to five times higher than the cone sampling limit (Klein & Levi, 1985) and is robust against retinal image motion (Westheimer & McKee, 1977).

Although FEM are large enough in amplitude to be perceptually visible, our world appears stable (Murakami, 2003). Conversely, if stimuli are presented with similar amplitudes but incongruent to actual FEM, they are perceived as moving (Arathorn et al., 2013). In order to correct for ocular jitter and provide a stable percept of the external world, it has been suggested that the visual system decodes retinal signals relative to FEM (Burak, Rokni, Meister, & Sompolinsky, 2010; Coakley, 1983; Eizenman, Hallett, & Frecker, 1985). If cone signals are integrated relative to ongoing FEM, then the benefits of FEM may be limited to image motion caused by natural eye movements. In Experiment 1, we created an incongruent viewing condition in which the stimulus moved in trajectories derived from subjects’ earlier eye movements. Discrimination performance was similar under natural and incongruent motion conditions, so efference-based processes are unlikely to contribute to the integration of dynamic cone signals from the incongruent condition. Efference copies, which are generated at central motor stations, are not expected to have sufficient resolution for resolving spatial ambiguities of details (<1 arcmin) presented in the current study (Havermann, Cherici, Rucci, & Lappe, 2014). Even if their resolution was sufficient, their involvement would not explain why similar performance was achieved in the natural and incongruent conditions. Afferent based mechanisms for encoding retinal image motion, such as proposed models of elongated arrays of retinal ganglion cells (Ahissar, Ozana, & Arieli, 2015) would better explain performance in the incongruent viewing condition.

Our results, demonstrating benefits from eye motion at the visual acuity limits, may have practical implications. It may explain how patients with retinal degenerative diseases maintain excellent visual acuity despite massive reductions in foveal cone density (Chapter 2) or how the increased FEM in patients with central vision loss could be a mechanism to optimize the benefits of image motion for larger receptive fields outside the fovea (Hennig & Worgotter, 2004). Cone activations during FEM may also serve as a biomimetic principle for the refinement of image processing algorithms in computer vision and the design of retinal prosthetics (Dagnelie, 2012). It can also help explain why performance improves with duration on a visual acuity task (Baron & Westheimer, 1973).

3.6 Follow-up neural model of high-acuity vision in the presence of fixational eye movements

In an attempt to better understand the neural circuitry that may give rise to the present study’s psychophysical results, Alexander et al. proposed a computational model based on a Bayesian ideal observer that approximates the retinal image given simulated retinal
ganglion cell (RGC) spikes (Anderson, Ratnam, Roorda, & Olshausen, 2016). Eye motion traces, cone spacing measurements, and stimulus size from the AOSLO experiments were integrated into the model, in which RGC spikes were simulated as if a diffraction-limited ‘E’ were presented to the retina. RGC spike patterns were used to perform a maximum likelihood estimate of eye motion and the inferred retinal image, the latter of which had a stronger signal to noise ratio with the presence of retinal image motion. These simulations corroborate our psychophysical findings to suggest neural circuitry improve acuity in the presence of retinal image motion.

3.7 Acknowledgements

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Chapter 4

The stability of fixation during high-spatial vision
Chapter 4

The stability of fixation during high-spatial vision

4.1 Abstract

Since our eyes are never at rest, the act of “fixation,” or gazing at a specific object, does not utilize a fixed location in the fovea but actually employs a small foveolar subregion whose relative location and expanse is variable across individuals. Since cone density and hence the maximum sampling limit of the photoreceptor mosaic drops rapidly even within the fovea, it may seem sub-optimal that fixation is not necessarily restricted to the foveal region with the theoretical maximum sampling resolution. However, due to the imperfections of the typical eye, natural acuity is limited by the eye’s optics rather than sampling density of the foveal cone mosaic, thus effectively homogenizing acuity within the fovea and making optimal fixational behaviors unnecessary. It is hence unknown how fixational patterns may differ when acuity is limited by the cone photoreceptor sampling limit. We used an adaptive optics scanning laser ophthalmoscope to compensate for ocular blur and deliver near-diffraction limited stimuli to the retina, enabling us to quantify the foveal region used for fixation, or the preferred retinal locus (PRL), measure acuity within foveal subregions, and determine the role of fixational eye movements in relocating stimuli presented within discrete foveal locations to a preferred fixational locus.

4.2 Introduction

Even when fixating on a static object, our eyes are constantly moving. As a result, our preferred retinal locus of fixation (PRL) spans a region rather than fixed point within the fovea (Steinman, 1965), whose center does not necessarily correspond to the location of maximum cone density (Li et al., 2010; Putnam et al., 2005; Wilk et al., 2017). Although it may seem suboptimal for the visual system to utilize a fixation locus deviant from the location of peak cone density, blurring due to the eye’s imperfect optics reduces visual acuity below the cone Nyquist limit (Marcos & Navarro, 1997), minimizing the need to use this theoretically optimal location for everyday vision. Early work reported that the location of maximum acuity was not displaced from fixation (Weymouth et al., 1928), but these results reflect acuity blurred by the eye’s optics and not that limited by the true cone sampling limit.

With the advent of adaptive optics techniques for retinal imaging, it has become possible to compensate for ocular blur, obtain high-resolution images of foveal cones, and deliver near-diffraction-limited stimuli to the retina, allowing a more direct comparison between fixation behavior and the location of peak cone density. Putnam et al. used an adaptive optics (AO) ophthalmoscope to present subjects with a 1° maltese cross and quantified the
spread of the PRL in relation to the location of maximum cone density (Putnam et al., 2005). They found the mean standard deviation of fixation to be 3.4 arcminutes of visual angle, consistent with previous reports (Barlow, 1952; Ditchburn, 1973; Steinman et al., 1973). The center of fixation was found to be approximately 8-11 arcmin from the location of peak cone density. Li et al., using an AO scanning laser ophthalmoscope, found the PRL to be displaced from peak cone density by approximately 5 arcmin (Li et al., 2010). Since cone density, and presumably acuity, drops off rapidly from the peak location (Curcio et al., 1990), subjects’ fixational behaviors did not appear to utilize the optimal location for maximum acuity, even when the confounding effects of ocular blur were minimized.

A potential conjecture for this finding is that traditional fixation stimuli, such as a maltese cross or solid square, lack high-frequency features that necessitate use of the region of maximum acuity. Additionally, subjects participated in a passive fixation task rather than actively discriminating a feature of the fixation target, a subtle difference in instructions that could potentially change fixational behavior. Most critically, visual acuity within subregions of the foveal center (~0.5 degrees in diameter) has not been systematically measured before while compensating for ocular blur. It is therefore unknown whether or how quickly acuity drops off within this region and whether current fixational patterns are sub-optimal, especially when viewing a high-frequency stimulus.

By using adaptive optics to compensate for ocular blur and deliver near-diffraction-limited retinal stimuli, the purpose of the current study was to systematically map acuity within the central 0.5° of the fovea, enabling a more direct comparison between fixational spread, the location of peak cone density, and sub-foveal differences in acuity. An additional objective of this study was to assess the potential role of eye movements in optimizing fixational behavior when discriminating stimuli at the limits of spatial vision. The role of FEM, and in particular microsaccades, in high-spatial tasks has long been debated, with multiple studies (including Chapter 3 of this document; see Figure 3.2) showing that microsaccades are suppressed during high-acuity tasks (Bridgeman & Palca, 1980; Winterson & Collewijn, 1976). Conflicting work, however, has shown that microsaccades tend to be more frequent and smaller during strict rather than relaxed fixation (Cherici, Kuang, Poletti, & Rucci, 2012) and less frequent when stimuli are presented stabilized rather than unstabilized at the PRL (Poletti & Rucci, 2010), all suggesting that microsaccades serve to relocate stimuli within the fovea. In particular, microsaccades have been shown to precisely relocate gaze within the foveola during high-acuity tasks such as the threading of a needle (Ko, Poletti, & Rucci, 2010) and discrimination of high frequency gratings shown at specific intervals within the central fovea (Poletti, Listorti, & Rucci, 2013). Although these studies showed the utility of microsaccades in relocating stimuli within the fovea, they were unable to determine how this function potentially reflected differing visual function at discrete locations within the central fovea.
In the current study, we presented high-frequency stimuli at discrete intervals within the fovea to determine whether microsaccades relocate stimuli to a specific retinal location, and determined how the specificity of relocation related to visual acuity at specific regions within the fovea. Since subjects were instructed to gaze directly at the stimulus during this relocation task, we were also able to quantify the fixational “dead zone”, or the distance from the fixational center at which eye motion statistics changed to relocate stimuli closer to the fixational center. The results of this study support the theory that microsaccades serve to precisely relocate stimuli within the central fovea.

4.2 Methods

4.2.1 Subjects

Subjects were six adults (three males, three females; ages 25-29 years), who had no known visual issues and were naïve to the purpose of the study. A drop of 1% Tropicamide solution was instilled in the test eye, chosen as the eye with less refractive error, 15 minutes prior to testing for pupil dilation and cycloplegia. Informed consent was obtained for each subject and experimental procedures adhered to the tenets of the Declaration of Helsinki.

4.2.2 AOSLO imaging and stimulation

Adaptive optics imaging and micro-stimulation were used to present retina-contingent visual stimuli to targeted locations in cone-resolved retinas of human observers, a method described in detail elsewhere (Arathorn et al., 2007; Rossi & Roorda, 2010b). The light source was a supercontinuum laser (SuperK Extreme; NKT Photonics) with an infrared imaging wavelength of 842 ± 25 nm (luminance of ~ 4 cd/m²). Retinal images were derived by recording the light scattered from a focused spot, raster-scanning across the retina with horizontal and vertical scan rates of 16 kHz and 30 Hz respectively. High order aberrations were measured with a Shack-Hartmann wavefront sensor, and a 97-actuator, 25-micron stroke deformable mirror (ALPAO) corrected the computed wavefront error. Corrected light was captured with a photomultiplier tube, whose voltage output combined with positional signals from scanning mirrors created 512 x 512 pixel videos with a framerate of 30 Hz. For Session 1, a 1-second retinal video was recorded with every stimulus presentation trial; a 2-second video was recorded per trial during Session 2.

Stimuli were encoded into the scanning raster via 20 MHz acousto-optic modulation that switched off the laser beam at points in the raster corresponding to the stimulus location, producing stimulus decrements with high contrast (dark ‘E’ on a red background). Michelson contrast between full-on and full-off stimulation was 99.9%. Diffraction reduced the actual “E” contrast to between 60%-75%. 1.3° and 1.4° raster fields were used for
Sessions 1 and 2 respectively, resulting in sampling resolutions for imaging and stimulus projection of ~0.15-0.16 arcmin per pixel.

During both experimental sessions, subjects’ heads were immobilized using customized bite bars. At the beginning of every experimental session, defocus induced by the deformable mirror was optimized for foveal imaging and stimulus delivery. First, an estimate of ideal defocus was derived from wavefront sensor measurements. Videos at 0.025 D defocus intervals away from the estimated value were acquired and analyzed. The defocus value with the sharpest images of foveal cones was then used as the baseline level for the remainder of the session.

4.2.3 Session 1: Mapping acuity within the central fovea

During Session 1, we mapped visual acuity at 5-arcmin intervals from the center of fixation. First, the spread of the PRL was quantified by asking subjects to fixate on a flashing dot (diameter = 5 arcmin) for 10 seconds. The retinal location of the fixation dot in each video frame was extracted using custom MATLAB scripts, which was then used to quantify the mean location (hereby referred to as “fixation center”) and spread of fixation (standard deviation, SD) for that session. The fixation center (PRL$_1$) served as the central coordinates (0') from which acuity measurements were taken. In addition to at the fixation center, acuity was measured 5 and 10 arcmin in either direction along the horizontal and vertical meridians (Figure 4.1) for a total of 9 test locations. Acuity thresholds were measured using the QUEST protocol, an adaptive psychometric procedure in which the stimulus size for each trial is the current most probable Bayesian estimate of the threshold (Watson & Pelli, 1983). QUEST is generally a more efficient threshold-determining procedure than method of constant stimuli or other staircase techniques, minimizing the number of trials necessary to estimate threshold. During each trial, the ‘E’ was presented retinally stabilized in one of four directions for 750 msec, after which subjects indicated the perceived orientation. Trials were self-initiated by the subject. For each tested location, 40 trials were used to determine threshold, set to be the stimulus size at which subjects correctly discriminated the ‘E’ 72% of the time. Trials for all locations were pseudo-randomly interleaved, split into 80-trial blocks and repeated to get two threshold measurements per location, for a total of 720 trials.
4.2.4 Session 2: Stimulus refoveation within the central fovea

During Session 2, we repeated the protocol to determine the mean location and spread of fixation (PRL2), which was then used as the central coordinates from which stimuli were presented for the remainder of the session. In this session we were interested in potential differences in fixation characteristics depending on where stimuli were initially presented relative to the fixation center. During each trial, the ‘E’ optotype was presented for 1 second at locations 0, 5, 10, or 15 arcmin away from the fixation center along the horizontal meridian (Figure 4.1). Stimulus size was fixed to be the smallest ‘E’ threshold from Session 1, or in other words the stimulus threshold from the location with potentially maximum acuity. Subjects were instructed to indicate the orientation of the ‘E’ for a given trial, and more crucially, told that they were free to gaze directly at the stimulus during this session. This clarification was important since in the first session, during which stimuli were stabilized to specific locations eccentric from the PRL, subjects were told to avoid trying to directly gaze at the stimulus if it was presented eccentrically. In the current session, the ‘E’ was initially presented to a tracked retinal location but then untracked so the stimulus could move on the retina according to subjects’ eye movements. This condition, with initial retinal tracking followed by unstabilized stimulus presentation, is referred to as ‘gain clamping’. A total of 500 trials were collected, each condition pseudorandomly interleaved.

The stimulus was presented for 1 second, rather than 750 msec, during Session 2 to account for potential delays in the onset of eye movement changes in response to stimulus presentation. Increasing stimulus duration allowed sufficient eye movement data to be
collected for analysis. Since longer stimulus exposure duration can affect acuity thresholds due to an increase light summation or signal integration, this difference in duration across Sessions 1 and 2 could have introduced inconsistencies in visual performance. Earlier work however suggests that acuity performance plateaus at 400-500 msec stimulus durations (Baron & Westheimer, 1973), suggesting that acuity was already maximized with the 750-msec presentation and should have been consistent across sessions.

4.2.5 **Measurement of location of peak cone density**

Cones were manually selected within the central 1.3 degrees of the AOSLO foveal images for each subject. Cone coordinates were then fed into a custom MATLAB script in which a moving sampling window was convolved with the cone locations to determine density across the foveal region. The peak cone density location was then determined and compared to the relative location of the PRL from both experiment sessions.

4.2.6 **Eye movement analysis and trial rejection**

Fixational eye movements that occurred during stimulus delivery in Session 2 were analyzed offline from recorded AOSLO videos with a sampling rate of 840 Hz (Stevenson, Roorda, & Kumar, 2010). Epochs of microsaccades and drift were segregated and analyzed separately.

Proper stimulus delivery was verified by looking for distorted or otherwise incorrectly delivered stimuli in the AOSLO videos. Trials with misdelivered stimuli were removed from further analysis.

4.3 **Results**

4.3.1 **Stability of PRL across sessions and distance from location of peak cone density**

Subjects in the current study, in which the two sessions were separated by an average of 7 days, showed very stable fixation behaviors, with displacement across sessions ranging from 0.2 to 4.1 arcmin (mean, 1.2 arcmin; Table 4.1). The distance of the fixation center to location of peak cone density ranged from 1.7 to 7.2 arcmin (mean, 3.8 arcmin). Figure 4.2 shows contour maps depicting the PRL spread for both sessions overlayed on AOSLO foveal images, with the location of peak cone density marked in red.
Table 4.1 | PRL characteristics across sessions. Fixation patterns of subjects across experiment sessions (Session 1, PRL₁; Session 2, PRL₂). Values are in arcminutes.

<table>
<thead>
<tr>
<th>Subject</th>
<th>SDₓ</th>
<th>SDᵧ</th>
<th>Distance to peak cone density</th>
<th>SDₓ</th>
<th>SDᵧ</th>
<th>Distance to peak cone density</th>
<th>Mean distance PRL₁ to PRL₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>1.5</td>
<td>1.8</td>
<td>6.1</td>
<td>2.1</td>
<td>1.7</td>
<td>6.0</td>
<td>0.2</td>
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<tr>
<td>S2</td>
<td>1.8</td>
<td>1.6</td>
<td>1.9</td>
<td>1.9</td>
<td>2.1</td>
<td>1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>S3</td>
<td>0.9</td>
<td>2.5</td>
<td>4.5</td>
<td>2.4</td>
<td>2.4</td>
<td>4.7</td>
<td>1.8</td>
</tr>
<tr>
<td>S4</td>
<td>6.3</td>
<td>2.4</td>
<td>7.2</td>
<td>3.3</td>
<td>2.1</td>
<td>3.3</td>
<td>4.1</td>
</tr>
<tr>
<td>S5</td>
<td>4.1</td>
<td>2.4</td>
<td>2.6</td>
<td>4.0</td>
<td>2.9</td>
<td>2.6</td>
<td>0.2</td>
</tr>
<tr>
<td>S6</td>
<td>5.4</td>
<td>1.8</td>
<td>1.7</td>
<td>3.1</td>
<td>1.4</td>
<td>2.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean</td>
<td>3.3</td>
<td>2.1</td>
<td>4.0</td>
<td>2.8</td>
<td>2.1</td>
<td>3.5</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Figure 4.2 | PRL topography and location relative to peak cone density. Fixation patterns of subjects across experiment sessions (Session 1, PRL₁ (solid contours); Session 2, PRL₂ (dashed contours)) overlayed on 0.5° x 0.5° AOSLO images. Dark blue, light blue, green, and yellow contours encapsulate regions containing 25, 50, 75, and 100% of the retinal regions used for fixation,
respectively. Session 2 images contain a subtle gray outline of the outermost contour from PRL as a reference. Red diamond indicates location of peak cone density. Scale bar = 5 arcmin.

4.3.2 Session 1 results: Acuity within the central fovea

Two acuity thresholds were measured at each of 9 retinal locations using the QUEST protocol. The mean values of these thresholds are shown in Figure 4.3. The standard deviation (SD) of each threshold distribution was ±0.18 arcmin. Threshold values across all stimulus locations were within 1 SD of each other, suggesting that acuity as measured in the current study was relatively homogeneous within a 10-arcmin radius from the center of fixation, as measured during Session 1.

![Figure 4.3](image)

**Figure 4.3 | Acuity measurements within central fovea.** The mean of two QUEST-acuity thresholds measured at retinal locations 0, 5, and 10 arcmin away from the fixation center in Session 1. Values are in units of arcmin. Central square corresponds to foveal center with adjacent squares being 5 arcmin intervals away (see Figure 4.1 for reference diagram). SD of measurements are ±0.18 arcmin.

4.3.3 Session 2 results: Fixational eye movements by initial stimulus location

Fixational eye movement data was binned by initial stimulus location and analyzed for epochs of microsaccades and drift. For trials in which microsaccades occurred, the orientation (θ) and amplitude (ρ) of the initial microsaccade following stimulus presentation were analyzed and plotted in Figure 4.4. It is visually apparent that subjects S1, S2, and S6 had more trials with microsaccades than S3, S4, and S5, noticeable by the
density of data points in the polar plots. It is also visually apparent that the orientation and amplitude of the initial microsaccade shifted depending on initial stimulus location, seen as a rightward shift of the scatterplot distribution for a given subject as stimulus location goes from -15 to 15 arcmin (Figure 4.4). Table 4.2 quantifies and summarizes the data shown in Figure 4.4.
Figure 4.4 | First microsaccade orientation and amplitude relative to stimulus start position. Polar scatter plots depicting orientation and amplitude of the first microsaccade per trial for given initial stimulus positions (-15 to 15 arcmin along horizontal meridian). Subjects S1, S2, and S6 appear to have a bias in microsaccade characteristics given the stimulus start point, visualized as a rightward shift of the scatterplot distribution as the stimulus location goes from -15 to 15 arcmin. Colored points are individual data points; black points are mean values. Rho values for polar plots are in arcmin; angle values are in degrees.
In addition to looking at spatial characteristics of initial microsaccades per trial, we plotted microsaccade frequencies as a function of stimulus start location in Figure 4.5. Subjects S1, S2, S5, and S6 exhibited fewer microsaccades per trial when the initial stimulus location was closer to the fixation center. This trend is most visible in S1 and S6. Subjects S3 and S4 rarely made microsaccades, irrespective of stimulus start point, with microsaccades occurring in ~20% of their trials.

Since subjects S3 and S4 mainly made drift eye movements, we analyzed the orientation of drift by stimulus start point to see whether the drift trajectory was biased to move fixation in the direction of the stimulus. Drift trajectories generally exhibit the properties of a self-avoiding random walk, or a stochastic process that consists of random steps that avoid reverting to the location of the previous step (Engbert, Mergenthaler, Sinn, & Pikovsky, 2011). Each step of a random walk for a single drift epoch would therefore have approximately equal probability of moving across all orientations, which would manifest in a histogram with equal distribution of steps across all orientations. We plotted the orientations of all drift steps, binned by stimulus start position, in Figure 4.6, for subjects S3 and S4. Although drift showed slight orientation bias in both subjects, this bias was relatively consistent across and independent of stimulus start position, suggesting that subjects S3 and S4 did not utilize drift to reorient eccentric stimuli closer to the fixation center.

Next we looked at drift characteristics across all subjects to see whether the orientation of drift, including during inter-saccadic epochs, would shift depending on the relative location of the stimulus at that time. We analyzed all drift segments from each subject and binned each step orientation depending on whether the stimulus location was currently to the left or right of fixation. This allowed us to assess whether drift served to compensate for saccadic under- or overshoots relative to stimulus position, as well as to observe whether drift could generally be modulated to relocate gaze. Figure 4.7 shows drift characteristics for a given subject were remarkably consistent regardless of stimulus direction and that all subjects exhibited idiosyncrasies in drift orientation. Our finding is consistent with those of Poletti et al., who similarly showed that drifts did not serve to reorient gaze towards stimuli (Poletti et al., 2013). Additionally, idiosyncratic differences in fixational eye movements such as those exhibited by our subjects are widely cited in the literature, although these traits have not been systematically characterized.
Figure 4.5 | Microsaccade frequency by initial stimulus location. Stacked histograms showing microsaccade frequencies per trial as a function of initial stimulus position. Subjects S1, S2, S5, and S6 appeared to make fewer microsaccades when the initial stimulus location was closer to the fixation center (0), with S1 and S6 most strongly showing this trend. Subjects S3 and S4 rarely made microsaccades during all trials, regardless of stimulus start position. Microsaccade frequencies, shown in figure legends, are in Hz.
Figure 4.6 | Polar histograms of drift step orientation for Subjects S3 and S4. Normalized polar histograms showing directionality of each drift step binned by stimulus location for subjects S3 and S4. A random walk would exhibit approximately uniform probability of directionality across all orientations. Subject S3 exhibited a subtle bias in drift directionality towards the lower left quadrant while S4 showed a tendency to drift towards the upper right. These biases were relatively consistent across stimulus start positions however and would not have reoriented fixation towards the stimulus, suggesting that drift was not utilized to systematically reorient gaze in these subjects.
Figure 4.7 Drift step orientation relative to location of stimulus. Normalized polar histograms showing directionality of each drift step binned by relative location of stimulus (to the left or right of fixation). Subjects showed consistent idiosyncratic drift orientation patterns independent of stimulus direction, suggesting that drift, even during inter-saccadic epochs, was not utilized to reorient gaze towards the stimulus.

Since the initial stimulus locations in Session 2 were displaced along the horizontal meridian, we performed a more thorough analysis of microsaccade amplitude along the horizontal axis only, done by decomposing the amplitude vector into its horizontal and vertical projections. Figure 4.8 shows boxplots depicting the distribution of the horizontal amplitude data. An analysis of variance (ANOVA) and Tukey-Kramer test were performed on the horizontal amplitude data to look for differences in mean microsaccade amplitudes based on initial stimulus location. Coupled with ANOVA, the Tukey-Kramer test is a multiple-comparisons test used to compare all possible pairs of means for significant differences (Tukey, 1949). The Tukey-Kramer method is conservative when there are unequal sample sizes per group, a necessity with the current dataset in which microsaccade occurrences were variable across subjects. Table 4.4 summarizes the results from the Tukey-Kramer analyses and a visualization of the significance of difference-of-means is shown in Figure 4.9.

The probability distribution over the course of stimulus presentation for initial microsaccades is shown in Supplementary Figure S4.1. The mean onset time for all subjects was 400 msec and 315 msec if S3 and S4 are excluded.
Figure 4.8 | Boxplots of horizontal microsaccade amplitude data. Box plots showing distribution of horizontal microsaccade amplitude data by stimulus start location. Red line indicates median value; top and bottom line of box indicate 75th and 25th-percentile values respectively. Height of box indicates interquartile range. Horizontal markers connected to box by dashed lines indicate highest and lowest data values within 1.5*25th or 75th-percentile value respectively. Outliers are indicated as red crosses. Microsaccade amplitudes are in units of arcmin.
Figure 4.9 | Visualization of results from Tukey-Kramer analysis. The Tukey-Kramer method, a post-hoc analysis for doing multiple comparisons of difference of means, was used to compare mean horizontal amplitudes of microsaccades between stimulus start points. The red regions in each matrix shows group pairs in which the means were significantly different (P < 0.05). Quantitative values are available in Table 4.4.
<table>
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<tr>
<th>Stim. Loc.</th>
<th>Mean θ; ρ</th>
<th>Mean X; Mean Y (± SEM)</th>
<th>Mean θ; ρ</th>
<th>Mean X; Mean Y (± SEM)</th>
<th>Mean θ; ρ</th>
<th>Mean X; Mean Y (± SEM)</th>
<th>Mean θ; ρ</th>
<th>Mean X; Mean Y (± SEM)</th>
<th>Mean θ; ρ</th>
<th>Mean X; Mean Y (± SEM)</th>
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</thead>
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<td>-9.1 (± 0.7)</td>
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<td>-13.8 (± 6.5)</td>
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<td>-7.7 (± 4.3)</td>
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<td>-9.0 (± 2.5)</td>
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<tr>
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<td>-3.2 (± 0.9)</td>
<td>-1.9 (± 3.3)</td>
<td>-13.8 (± 11.2)</td>
<td>349.8; 14.0</td>
<td>-2.5 (± 4.3)</td>
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</tr>
<tr>
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<td>-1.7 (± 0.9)</td>
<td>3.0 (± 3.0)</td>
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<td>3.4 (± 0.8)</td>
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<td>205.3; 17.2</td>
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<td>14.1 (± 2.2)</td>
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Table 4.2 | First microsaccade orientation and amplitude relative to stimulus start position. Summary of microsaccade characteristics depicted in Figure 4.4. Subjects S1, S2, and S6 in general had more trials with microsaccades per stimulus location than S3, S4, and S5. The mean microsaccade orientation (θ) and amplitude (ρ) for S1, S2, and S6 systematically shifted with stimulus location. A statistical analysis of this data is presented in Table 4.4. Units of orientation (θ) and amplitude (ρ) are in degrees and arcmin, respectively.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
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<td>-2.0; -0.7</td>
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<tr>
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<td>2.1; 8.9 [9.2]</td>
<td>-7.9; -9.2 [12.1]</td>
<td>6.0; -2.4 [6.5]</td>
<td>4.0; -2.4 [4.6]</td>
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</table>

Table 4.3 | Stimulus location relative to fixation center and location of peak cone density after initial microsaccade, averaged over all locations. Mean horizontal and vertical displacements of stimulus relative to fixation center and location of peak cone density following initial microsaccade. Values are averaged over all trials and stimulus locations. Positive values indicate stimulus is to the right (X) or above (Y) fixation center or peak cone density location; negative values indicate locations to the left (X) or below (Y). Units are in arcminutes.

Table 4.4 | Tukey-Kramer analysis of microsaccade amplitudes (horizontal only). The Tukey-Kramer method is a post-hoc analysis for determining the significance of differences between multiple means. Each row corresponds to a comparison of means from two groups, differentiated by the stimulus start location. The difference of means and confidence intervals (CI) have units of arcmin. Comparisons with a significant difference in means (P-value < 0.05) are shaded in red. A visual depiction of these regions of significance are shown in Figure 4.9.
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Table 4.4 (see previous page for caption)
4.4 Discussion

4.4.1 Fixation displacement across sessions and relationship to location of peak cone density

Previous work looking at the stability of the fixation locus shows relative stability of the fixation center, with a shift of ~2-4 arcmin across days (Putnam, 2012). The standard deviation of fixation has been reported to be anywhere between 1-5 arcmin (Barlow, 1952; Ditchburn, 1973; Putnam et al., 2005; Steinman, 1965). In the current study, fixation shifted by an average of 1.2 arcmin across days; the fixation spread had a mean standard deviation of approximately 2.6 arcmin (Table 4.1). The mean displacement of fixation from location of peak cone density has been previously shown to be between 5 and 9.8 arcmin (Li et al., 2010; Putnam et al., 2005) whereas in the current study mean displacement was 3.8 arcmin (Table 4.1; Figure 4.2). A potential reason for the variability in findings across studies could be the characteristics of the stimulus used, which has been shown to affect fixation behavior (Steinman, 1965). The stimulus employed in Putnam et al.’s 2005 study was a 1 degree Maltese cross (Putnam et al., 2005), a 6 arcmin square in Li et al. (Li et al., 2010), and a 5 arcmin spot in the current study. The larger size of the Maltese cross coupled with a larger spectral bandwidth may have necessitated a less specific fixation location across days than the significantly smaller target used in our study. Additionally, different adaptive optics systems were used in the 2005, 2010, and current studies, so differences in stimulus resolution, luminance, and delivery wavelength can also explain discrepancies with the present work.

Cone density measurements in the 2005, 2010, and current studies were derived by manually identifying cones within an approximately 1-2° region in the central retina, the coordinates of which were then scanned into a moving sampling window in which the number of cones was measured and converted into cone density (Curcio et al., 1990). Inconsistencies in sampling window size across studies or errors in manual cone selection can affect estimates of peak cone density location. The potential error in determining peak cone density can therefore result in dissimilar results across reports.

4.4.2 Mapping acuity within the central fovea

In the current study we were interested in measuring acuity in discrete locations within the central 0.5° of the fovea by stabilizing stimuli in regions at 5-arcmin intervals. Weymouth, et al. mapped grating acuity at 11-arcmin intervals across the central fovea and found that acuity was highest at and declined away from the PRL when ocular blur is uncompensated (Weymouth et al., 1928). Since ocular blur precludes the cone sampling limit from being the limiting factor for acuity, we were interested in mapping acuity within a central small foveal region (~20-arcmin in diameter) to assess whether acuity declined at smaller intervals when ocular blur was compensated for and performance was truly sampling-
limited. Histology shows that cone density declines as close as 10-arcmin from the location of peak cone density, although the steepness of the decline varies greatly across subjects (Curcio et al., 1990). Since acuity is closely matched to the sampling limit within the central 0.5° of the fovea (Rossi & Roorda, 2010a), we would expect acuity to reflect the drop-off in cone density within this region. Our findings, however, did not indicate any significant differences in acuity across the central 20-arcminute region (Figure 4.3). This observation may be due to multiple factors, including intersubject variability in cone density distribution within the fovea which may affect where cone density and acuity begin to noticeably decline. Another factor could be the nature of the protocol and stimulus used in the current study to measure acuity thresholds. For consistency with our previous study (Chapter 3) and due to difficulties in presenting and analyzing stimuli with varying contrast using the AOSLO, we decided to use a maximum-contrast tumbling ‘E’ for the current work. Thresholds measured with a tumbling ‘E’ are based on the width of each ‘leg’, presumably the main feature used to discriminate orientation. However, the tumbling ‘E’ also has lower frequency cues that can be used for orientation discrimination (Bondarko & Danilova, 1997), thus muddling the relationship between stimulus size and spatial frequency threshold. It is unlikely our subjects relied solely on lower frequency cues during the acuity task since their thresholds more closely matched the spatial frequency corresponding to the legs of the ‘E’. However, it should be emphasized that the broader spectrum of our stimuli makes the relationship between cone sampling limit and stimulus threshold more complex.

The QUEST paradigm is an adaptive psychometric procedure in which the stimulus size chosen for each trial is based on the most probable Bayesian estimate of threshold (Watson & Pelli, 1983). This allows one to quickly and efficiently determine threshold within several dozen rather than several hundred trials, an important distinction when measuring thresholds across several locations and when the number of trials required becomes prohibitively large. The downside of using a Bayesian procedure is that the shape of the psychometric function must be initially assumed; since the true shape is never measured, any discrepancies between the assumed and actual shape can lead to errors in threshold determination. Classical methods such as method of constant stimuli have fewer assumptions but are much more time consuming, which would have been prohibitive in the current study in which acuity was measured twice over 9 locations in a single session. Therefore QUEST was the best option for threshold determination in spite of its limitations.

4.4.3 Fixational eye movements by initial stimulus location
During Session 2 we presented stimuli at the lowest threshold derived from Session 1 at 5-arcmin intervals from the fixation center along the horizontal meridian. The purpose of this experiment was to observe the characteristics of fixational eye movements depending on stimulus start point and whether microsaccades played a role in refoveating stimuli to an
ideal location. Whereas subjects S3 and S4 made microsaccades in less than ~20% of all trials, S1, S2, S5, and S6 showed a bias toward exhibiting more microsaccades the further away the stimulus start point was from the fixation center (Figure 4.5). When the orientations and amplitudes of the initial microsaccades per trial were analyzed, these four subjects showed a systematic trend in microsaccade orientation and amplitude depending on stimulus start point (Figure 4.4; Tables 4.2 & 4.4), with an overall tendency for the initial microsaccade to bring the stimulus closer to the fixation center (Table 4.3). Since subjects S3 and S4 rarely made microsaccades, we analyzed their drift characteristics (Figure 4.6) to determine whether drift had a tendency to relocate peripheral targets closer to the fixation center. We did not find this to be the case with these two subjects and in fact drift orientation appeared to be mostly random with a slight orientation bias that was consistent across stimulus locations. A larger analysis of drift across all subjects also showed idiosyncracies in drift orientation that were independent of relative stimulus orientation (Figure 4.7). Thus, it appears that microsaccades alone served to relocate stimuli closer to the fixation center, even if the targets were displaced from fixation by several arcminutes only, results that are consistent with earlier findings (Ko et al., 2010; Poletti et al., 2013). An analysis of microsaccade onset relative to stimulus presentation showed the mean onset time for subjects S1, S2, S5, and S6 to be 315 msec (Supplementary Figure S4.1); in comparison, saccadic latencies in response to peripheral stimuli are generally 200-250 msec (Cohen & Ross, 1978; Saslow, 1967). The latencies observed in this study are similar but slightly longer than those observed for larger saccades, but this variation can be caused by numerous factors, including stimulus saliency and observer training. Overall, our results support the theory that microsaccades serve a similar, but more precise, purpose to larger saccades in relocating gaze.

We also analyzed whether there was tendency for the initial microsaccade to relocate the stimulus closer to the location of peak cone density than to the original fixation center (Table 4.1 & 4.3). We found this to be the case with S1 only. Since acuity was relatively homogeneous within the central 20 arcmin of fixation, it is unsurprising that subjects did not necessarily shift their fixation behavior to use the retinal location with highest cone density for this task.

A key point in Session 2’s protocol was that subjects were allowed to gaze directly at the stimulus, a contrast from Session 1 in which stimuli were stabilized at retinal locations peripheral to the PRL and subjects had a strong sense that they weren’t gazing directly at the stimulus. Assuming in Session 2 that subjects opted to gaze directly at the stimulus and that the initial microsaccade served to refoveate peripheral stimuli, the analysis from Table 4.4 (visualized in Figure 4.9) can provide insight into the expanse of the fixational ‘dead zone,’ or the region surrounding the fixation center in which a stimulus appears to be within the subjective location of gaze. Table 4.4 and Figure 4.9 provide statistical analyses
in which microsaccade characteristics were compared across different stimulus start points. Comparing microsaccade amplitudes at the fixation center to those at more peripheral locations can serve as a determinant for the smallest eccentricity at which fixation behavior, i.e. microsaccade amplitude, significantly changed. Of the four subjects that showed shifting microsaccade behavior with stimulus location, S1 and S2 exhibited significant changes in microsaccade amplitude beginning at a 5-arcmin eccentricity from fixation. In other words, their fixational 'dead zone' was restricted to a 5-arcmin radius from the fixation center (Figure 4.9). Subject S5 did not exhibit a boundary to their dead zone within the tested 15-arcmin-radius region, and S6's dead zone had a 10-arcmin radius. The extent of the fixational dead zone, or subjective line-of-sight, appears to be correlated with the spread of the PRL measured during Session 2, as S1 and S2 exhibited the smallest fixational SD, followed by S6 and lastly S5. The smaller the SD, the narrower the dead zone appeared to be. S3 and S4, the two subjects who made minimal microsaccades, had smaller fixational spread than S5, so it is unclear why they failed to employ microsaccades to relocate gaze. An interesting observation is that subjects S1 and S2, who exhibited the smallest PRL and strongest inclination for using microsaccades to refoveate eccentric stimuli, were the only two individuals with prior experience as AOSLO subjects, especially with performing high-acuity discrimination tasks. The remaining subjects had no prior experience participating in AOSLO experiments. It could be then that with additional experience, these subjects would eventually optimize their fixational behavior and employ microsaccades more systematically for relocating gaze, which would be in line with earlier observations showing that microsaccade characteristics vary with observer training on a fixation task (Cherici et al., 2012).

Our results show that while the preferred retinal locus is relatively stable across days, the spread of fixation is variable across subjects. Mapping acuity at 5-arcmin intervals across the central fovea did not show a noticeable decline in performance, although these tests were restricted to a 10-arcmin radius from fixation. Future work should repeat sub-foveal acuity measurements with a more suitable stimulus than the tumbling 'E' to better restrict the spatial frequencies available for orientation discrimination. The majority of our subjects employed microsaccades to relocate peripherally-presented stimulus closer to the fixation center, even when stimuli were restricted to a 15-arcmin radius from the fixation locus. This finding supports the theory that microsaccades serve a similar function to larger saccades, even for stimuli presented within the central 0.5° of the fovea. Our subjects displayed fixational 'dead zones' as small as 10-arcmin in diameter, and more experienced subjects appeared to have smaller subjective regions of gaze. It is possible then that with continued experience, subjects may develop fixational behaviors that employ microsaccades for optimizing visual performance.
Overall, our study is consistent with earlier reports showing that the visual system has a preferential location for fixation, demonstrated by the consistency in PRL location across days (Putnam, 2012; Putnam, Hammer, Zhang, Merino, & Roorda, 2010) and the utility of microsaccades in relocating peripheral stimuli as close as 5 arcminutes from the fixation center (Ko et al., 2010; Poletti et al., 2013). Although this location does not appear to match the location of maximum sampling and is impervious to the relative homogeneity of acuity within the fovea, there are other factors unmeasurable with the current experimental setup that may influence the foveal location used for fixation. Photopigment optical density, a function of the pigment’s chromatic spectrum, concentration, and path length, is variable within the foveola and affects a cone’s capacity for absorbing light. Receptive field pooling, or the number of photoreceptors that provide input to higher retinal neurons, dictates the effective retinal sampling limit at the level of ganglion cells. Additionally, cortical magnification, or the area in the visual cortex that processes stimuli within a specific region of the visual field, can affect visual attention and saliency in a manner that optimizes a specific foveal location for fixation. The interaction of these factors in determining a preferred retinal locus is still unknown, although it is clear that eye movements, and in particular microsaccades, serve a crucial role in maintaining a specific retinal location for fixation.

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**Supplementary Figure**

Figure S4.1 | Initial microsaccade probability relative to stimulus onset. Normalized probability of the initial microsaccade per trial relative to stimulus onset. Shaded regions indicate standard error of mean. Written values indicate mean (± SEM) onset time in milliseconds of first microsaccade.


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