The anatomical, functional and perceived location of the fovea in the human visual system

Markku Kilpeläinen[1,5,*], Nicole M. Putnam[2], Kavitha Ratnam[3] & Austin Roorda[4]

1) Department of Psychology and Logopedics, University of Helsinki, Helsinki, 00014, Finland

2) Arizona College of Optometry, Midwestern University, Glendale, AZ, 85308, USA

3) Facebook Reality Labs, Redmond, WA, 98052, USA

4) School of Optometry and Vision Science Graduate Group, University of California, Berkeley, CA, 94720, USA

5) Lead contact

*) Correspondence: markku.kilpelainen@helsinki.fi

Summary

Due to the dramatic difference in spatial resolution between the central fovea and the surrounding retinal regions, accurate fixation on important objects is critical for human visual behavior. It is known that the preferred retinal location for fixation (PRL) of healthy human observers does not exactly coincide with the retinal location with the highest cone density. It is not currently known, however, whether the PRL is consistent within an observer or subject to fluctuations and, moreover, whether observers' subjective fixation location coincides with the PRL. We studied whether the PRL changes between days. We used an adaptive optics scanning laser ophthalmoscope to project a Maltese cross fixation target on an observer's retina, and continuously imaged the exact retinal location of the target, while the observers fixated on it. We found that observers consistently use the same PRL across days, regardless of how much the PRL is displaced from the cone density peak location. We then showed observers small stimuli near the visual field location they were asked to fixate on, and the observers judged whether the stimuli appeared along their line of sight (i.e., in fixation) or not. Observers' precision in this task approached that of fixation itself. Observers based their judgement on both the external world coordinates and the retinal location of the stimuli. We conclude that, for a monocular fixation task, the PRL in a normally functioning visual system is fixed, and observers are relatively well aware of its location.

Introduction

The fovea of the human retina represents an evolutionary adaptation taken to an extreme. The spatial sampling resolution enabled by the dense packing, and narrowing of photoreceptors (and the displacement of post-receptoral neurons) within the human fovea is one of the best among all species [1] and, in fact, as good as allowed by the relatively average optics of the human eye [2]. Much like, for example, the built-for-speed anatomy of the cheetah, our retinal specialization comes at a cost. The omission of retinal vasculature from the fovea, for example, makes the neurons in that area very vulnerable to retinal disease [3]. Moreover, it is not feasible for foveal specialization to span a significantly larger area of the retina [4]. Thus, the photoreceptor density drops extremely steeply, when moving away from the center of the fovea. As a result, an oculomotor system capable of reliably and quickly placing images of objects of interest near the center of the fovea is necessary.

The adaptation has been put to good use, however, as humans heavily rely on the fovea in their normal visual behavior. Performance in many different visual tasks deteriorates dramatically when relying on retinal areas lying only a few degrees of visual angle outside the fovea [5–9].

Considering the importance as well as the high price of the fovea, both in terms of the anatomical tradeoffs and the need for a precise fixation system, it was quite surprising that Putnam et al. [10] first discovered that the preferred retinal locus for fixation (PRL) rarely coincides with the cone density peak, a finding that has been independently confirmed in more recent reports [11–13]. Similar, but much more pronounced, displacements of the PRL are caused by retinal diseases that lead to central vision loss. Patients must learn to fixate with another part of the retina, as the use of the central retina is precluded. A particularly interesting population in this respect are patients with an operable macular hole. Such patients often first learn to fixate relatively consistently with a retinal location just outside the hole. After surgery, the PRL moves back towards the center of the fovea [14,15]. A compelling medical condition is not necessary for the adoption of a substantially non-central PRL, however. Healthy observers with an artificial central scotoma can also learn to use a non-central PRL to carry out various visual tasks with surprisingly high performance [16–18].

Such findings, suggesting substantial plasticity of the PRL, lead one to think that perhaps the normal foveal PRL is also subject to some fluctuation, even if unaffected by real or artificial central scotomas. In this study, we tested this hypothesis by determining the PRL locations in healthy observers on 2-3 different days. Further, if the PRL is not an anatomically hard-wired retinal location, observers might be relatively poorly aware of its location. To test this hypothesis, we showed the same observers stimuli in various locations across their central retina, and asked the observers to judge whether the stimuli appeared in their line of sight (i.e., in fixation).

The present study shows that regardless of the amount of displacement of the PRL, it remains a fixed property of each observer's visual system, not subject to fluctuations. Further, observers are quite keenly aware of the location of their PRL, and use it to judge the precise location of visual stimuli.

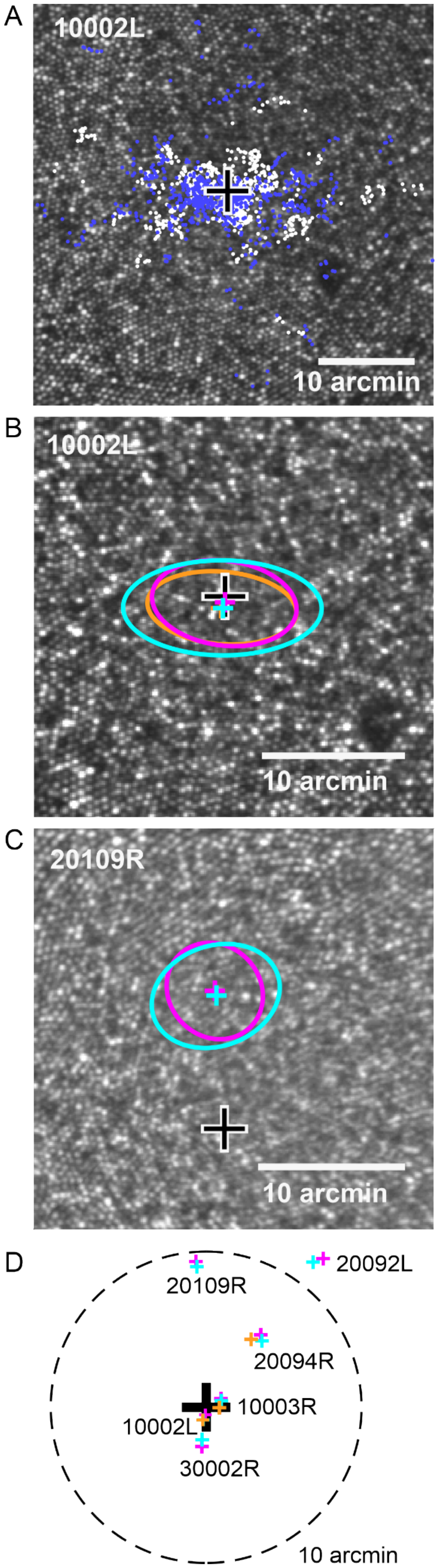
Results

The preferred retinal locus of fixation is stable across days

We imaged the retina and the retinal location of a Maltese cross target, while observers fixated on the target. Our fixation data consists of the retinal location of the target extracted from retinal imaging videos at 30 Hz. Figure 1A shows the retinal locations of the target extracted from two 30-second videos plotted on the cone image. One video is plotted with white markers, the other with blue markers.

The preferred retinal locus of fixation (PRL) is very stable across the days. Figures 1B,C show the mean and standard deviation of the 2-D Gaussian distributions fitted to the data of two observers. One can see that whether the PRL is near the cone density peak (the large black cross), like in observer 10002L, or further away, like in observer 20109R, there is very little difference in the mean locations from different days. Figure 1D shows the means from different days and different observers. The largest PRL difference across days for each observer ranged from 0.35 to 0.701 arcmin (mean 0.521, SD 0.162).

Although not one of the main interests of the study, we once again replicated the finding that the PRL is displaced from the cone density peak. Average distance of the PRL from the cone density peak is 5.08 arcmin (SD 4.39), very close to the 3.9 arcmin earlier observed by Li, Tiruveedhula and Roorda [11]. We also calculated the sampling frequency limits at the cone density peak and the PRL, assuming hexagonal packing [see 12]. The average sampling frequency limit was 72.4 cyc/deg (SD 2.19) for the cone density peak, and 69.8 cyc/deg (SD 2.57) for the PRL.



A
10002L
10 arcmin
B
10002L
10 arcmin
C
20109R
10 arcmin
D
20109R
20092L
20094R
10003R
10002L
30002R
10 arcmin

Figure 1. The preferred retinal locus of fixation is stable across days. A) Retinal locations of the target from two 30-second videos (white vs. blue markers) from the same day plotted on the observer's master cone image. The black and white cross indicates the cone density peak. B,C) Means (crosses) and SDs (ellipses) of the 2D Gaussians fitted to retinal target locations measured on different days (different colors) for two representative observers. The means (the colored crosses) represent the PRLs for different days. D) The PRLs relative to cone density peak for all observers. Some observers participated on two days (two crosses), some on three (three crosses). The dashed circle signifies a 10-arcmin distance from the cone density peak.

To test whether the small differences in PRL locations between days might yet be statistically significant, 2D Gaussian distributions were fitted to the data by means of maximum likelihood estimation (with Matlab fitgmdist function). More specifically, we compared a simpler model where one distribution was fitted to the combined data from both days and another model where the means were estimated separately for the two days (shared covariance). Akaike's information criterion (AIC) was used to compare the different models (see data analysis). The AICs produced by the single fit to the data were lower (better) for all observers, as the added parameters produced only small improvements in the fit. Average AIC for the single Gaussian model was 125542 (SD over observers 9071), and 125549 (SD 9071) for the two Gaussian model. The evidence ratio derived from the AIC analysis suggests that the single Gaussian model is on average 23 (SD 4.4) times more likely to be the better model than the model with different average locations for the two days. While not very strong evidence for either model, the analysis definitely does not support a view of different PRL locations for different days.

Observers have a strong sense of where they are looking

We presented small (2.3 arcmin) and short (1 frame) stimuli near the center of the raster, while observers maintained fixation near the center of the raster, and asked the observers to judge, whether the stimulus appeared along their line of sight (i.e., in fixation) or not. Observers were able to carry out the task quite reliably. Although stimuli were flashed only within a 22-arcmin wide area near the center of the stimulus raster, observers mainly gave the 'Yes' response only to stimuli landing on a very small region of the retina (green dots in Figure 2). Very few 'No' responses (red dots), in turn, were given to stimuli landing on the same region. The subjective fixation location for each observer was derived based on all the responses with the following logic. The probability of 'Yes' responses should decrease with distance from the subjective fixation location following a Gaussian distribution, the 'Maybe' -responses likewise, although probably with a larger SD, whereas the 'No' -responses should initially increase from the subjective fixation location and then decrease, as the stimulated locations become sparser. The subjective fixation location was then given by the common (x and y) center parameters of the three distributions. The SDs of the different distributions were allowed to vary independently. Figure 2 shows all the data from two representative observers, and the fits and the fitted functions. The ellipses correspond to 1 SD from the mean.

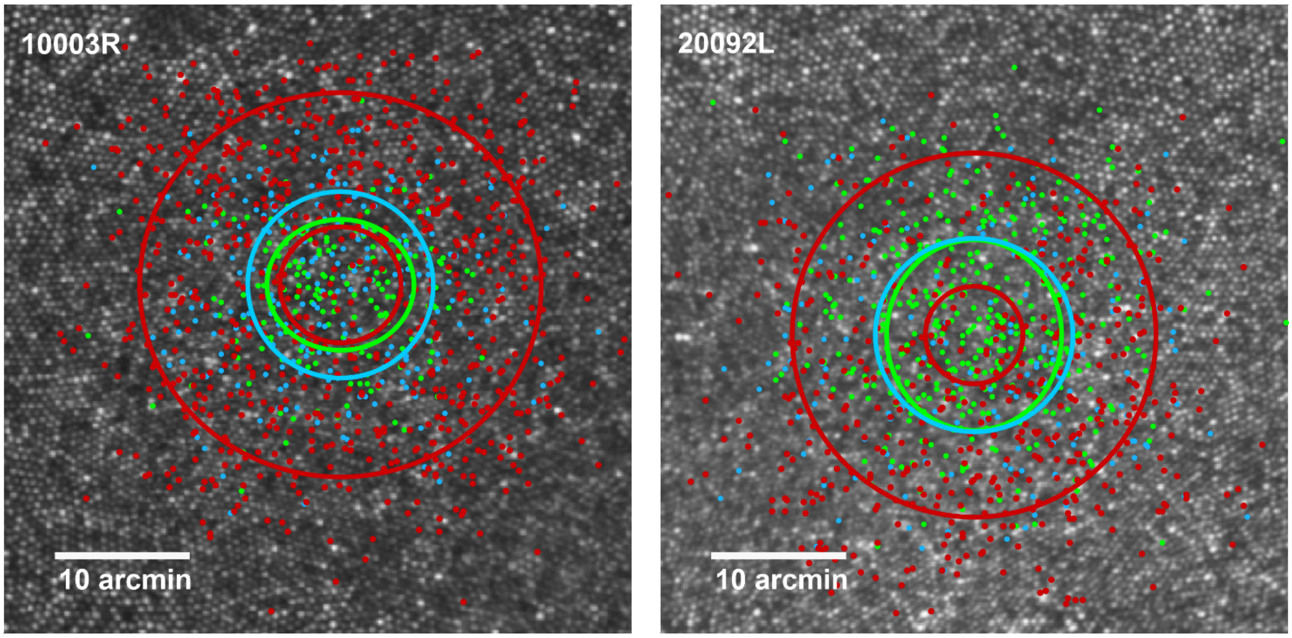


Figure 2. Observers have a strong sense of where they are looking. The psychophysics data of two representative observers (dots) and the commonly fitted functions (ellipses). Yes = green, maybe = blue, no = red.

Relationship between the cone density peak, the PRL and the subjective fixation location

Figure 3 shows the cone density peak, the PRL on different days, as well as the subjective fixation location. For most observers, the subjective fixation location differed somewhat from the PRL. However, the subjective fixation location was on average closer to the PRL (mean distance 3.04 arcmin, SD 1.90) than the cone density peak (mean distance 7.64 arcmin, SD 5.26). The difference was to the same direction in all observers, although not quite statistically significant ($t(4) = 1.64$, $p = 0.110$). Further, both were displaced from the cone density peak to a very similar direction. The correlation between the angles to which PRL and subjective fixation location are displaced from the cone density peak is quite high: $r(3) = 0.91$, $p = 0.032$.

The area where stimuli provoked a ‘Yes’ response in Experiment 2 appears to be somewhat larger than the area where observers held the stimuli during the fixation task in Experiment 1. The quadratic mean radius of the SD ellipses was on average 5.38 arcmin (SD 0.95) for the ‘Yes’ responses and 3.98 arcmin (SD 1.16) for the fixation data. The difference was to the same direction for all five observers and is statistically significant ($t(4) = 5.76$, $p = 0.005$).

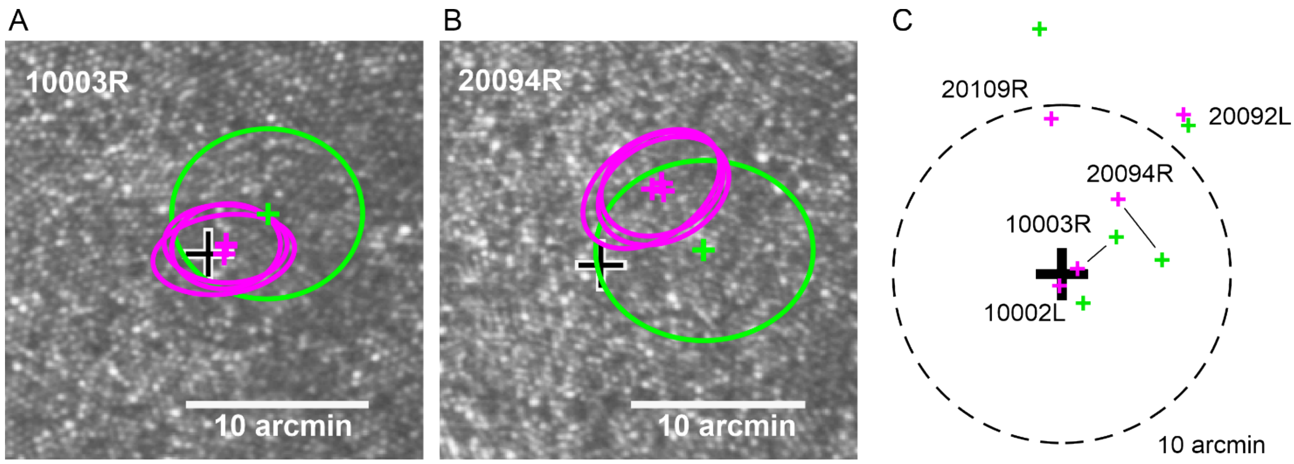


Figure 3. Relationship between the cone density peak, the PRL and the subjective fixation location. A,B) The cone density peak (Large black cross), the PRL (magenta crosses), and the subjective fixation location (green crosses) of two representative observers. Ellipses indicate SDs. C) All observers' average PRL and subjective fixation location relative to each observers' cone density peak. The subjective fixation location has been connected to the observers PRL location with a line in the two cases where it is not the closest one.

Observers rely on external world location and retinal location of stimulus in judging fixation location

When interpreting the results of the subjective fixation location experiment, it is important to understand that there is no precise retinal stimulus location, which we can a priori expect to provoke the "in line of sight" sensation. Nor is there a location where a 'Yes' response could be considered correct or incorrect. Rather, this explorative experiment sought to map which stimulus locations are more likely to provoke the "in light of sight" percept. That said, it is a reasonable hypothesis that stimuli landing very close to the PRL should provoke more 'Yes' responses. In addition, observers are likely to use the external reference frame in their judgments. For example, a stimulus might appear to the left of the raster center, and the observer's fixation might have drifted to that location, but the observer might still answer that it was not in the line of sight, if basing the response purely on external coordinates.

Since observers were instructed to keep fixation close to the center of the raster, and the locations of the stimuli were on average very close to the center of the raster, the distance of the stimulus from the raster center and from the PRL are unavoidably correlated ($r(5324) = 0.47$, $p<0.001$). Crucially, though, they also have a significant amount of independent variance due to fixational eye movements and the unpredictability of the stimulus position. Thus, we can distinguish the effects of the two factors on the the subjective fixation location. We constructed a generalized mixed linear model with the response as a dependent variable and stimulus distance from the raster center, stimulus distance from PRL and their interaction as predictors, and the observer as a random effect. Both the effects of the distance from raster center ($F(2,5318) = 62.46$, $p<0.001$) and the distance from PRL ($F(2,5318) = 34.58$, $p<0.001$) were statistically significant. The interaction effect was not significant ($F(2,5318 = 0.22$, $p = 0.803$). Figure 4A illustrates how the probability of the stimulus being perceived as appearing in fixation changes with increasing distance from raster center (black curve), with distance from the PRL (magenta curve), and with distance increasing simultaneously from PRL and raster center.

To illustrate how the responses are affected by both the raster position and the retinal location relative to the PRL, it is useful to compare the proportions of 'Yes' responses in situations where the stimuli are displaced equally from the raster center, but are at different distances from the PRL. In three of our observers, the PRL and the average retinal location of the raster center are sufficiently apart that one can compare responses in three conditions: stimuli presented near the raster center, stimuli displaced from the raster center such that they on average land on the PRL, and stimuli equally displaced from the raster center, but to the direction opposite from the PRL. The diameter of the analyzed region for each condition was equal to the distance between the raster center and the PRL, in retinal coordinates. Figure 4B shows the model predictions and the data for the three observers. In line with the predictions of the model, stimuli displaced towards the PRL lead to more 'Yes' responses than stimuli displaced to the opposite direction by the same amount. Although the purpose of this analysis was mainly to illustrate the effect of the distance from PRL, we point out that the difference is statistically significant for all three observers (10003R: $\chi^2(1, N = 106) = 7.57$, $p = 0.006$; 20094R: $\chi^2(1, N = 430) = 5.88$, $p = 0.015$; 20109R: $\chi^2(1, N = 330) = 17.25$, $p < 0.001$). Figure 4C shows data averaged over observers on a categorized (i.e., normalized) x-axis.

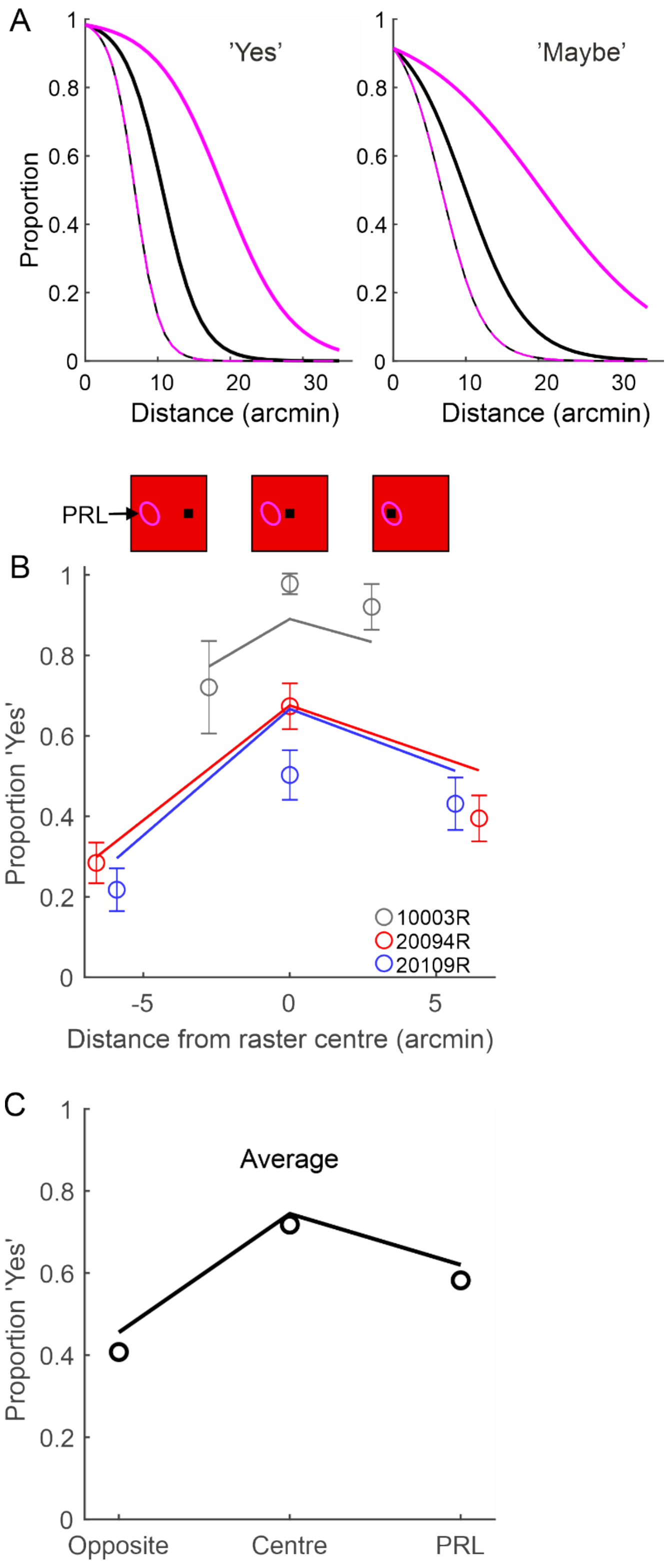


Figure 4. Observers rely on external world location and retinal location of stimulus in judging fixation location. A) Proportions of 'Yes' and 'Maybe' responses as a function of distance from the raster center (black line), from the PRL (magenta line), or both simultaneously (black-magenta) as predicted by the generalized linear model. B) Proportion of 'Yes' responses (± 95 % CI) for different stimulus locations for three observers. 'Yes' responses were most frequent for all observers when the stimuli were presented at raster center. 'Yes' responses decreased much less, however, if stimuli were displaced from the raster center towards the PRL (see illustrations above markers), than to the opposite side of raster center. C) Average over observers, where the distances between different locations have been normalized across observers.

Discussion

We studied the relationship between the preferred retinal locus of fixation (PRL), the subjective fixation location and the location of the cone density peak on the human fovea. The use of adaptive optics scanning laser ophthalmoscopy for stimulation and retinal imaging allowed us to determine very precisely the retinal location of every presented stimulus. We find that in human observers without retinal pathologies, the PRL is very stable across days. Further, observers are relatively keenly aware of their fixation direction, even when they are not fixating on a particular stimulus.

The PRL across days

Our data show that observers consistently use the same retinal location to fixate on a stimulus and that this does not in general coincide with the location of highest cone density. Although constant fixational eye motion moves the stimulus on the retina substantially, the average PRL is very much the same on different days. We did not find significant differences in PRL locations between days in any of our six observers. The largest observed difference between two days was 0.7 arcmin - roughly the diameter of two foveal cones. It is a very small difference, considering that a single microsaccade, for example, can move the stimulus across tens of foveal cones [19,20]. It is also important to note that in a case where the PRL would, in reality, be in the same exact point on two days, any measurement error can only displace the data further apart. No error, in contrast, can move them closer to each other, if they are in the same location to begin with. We conclude that we find no evidence of the PRL moving between days.

Since the PRL appears to be constant across days, it is very unlikely that the displacement of PRL from the cone density peak is a result of random fluctuations in the observer's fixation. Rather, each observer's PRL, no matter how much displaced from the cone density peak, is a fixed property of the observer's visual system. One might even suggest that, when observers intensely fixate on a target stimulus, they try to point a specific cone to its center. Why, then, should that cone not be in the retinal location with the highest cone density? One might suspect that the adaptive optics correction to the eye's natural aberrations might play a role. However, any lateral displacement of the image arising from unlikely prismatic effects of the adaptive optics system must be ruled out since the oculomotor system immediately compensates for these by refixating on the displaced image. Moreover, at this spatial scale, any meaningful improvement in higher order aberrations along the direction of the PRL compared to nearby locations (such as the cone density peak) is very unlikely. This is because the eye's isoplanatic patch (the area within which aberrations do not significantly change), spans one degree or more [21,22].

Besides the cone density peak, the anatomical center of the fovea has been defined based, for example, on the avascular zone and the foveal pit (void of post-receptoral neurons), but those do not coincide with the PRL any more than the cone density peak [13]. McGregor et al. [23] recently defined the foveal center based on the orderly arrangement of ganglion cell somas in macaque retina and found it to be displaced by roughly 8 arcmin from the cone density peak. They studied only one retina, however, and did not measure PRL. So, if there is a retinal determinant of the PRL, it remains elusive so far.

We suggest that the displacement is more likely to be a consequence of changes that take place during the development of the visual system. Both the maturation of the fovea [24,25] and the development of stereopsis [26,27] continue many (even more than ten) years into childhood. Since fixation is in everyday life a binocular process, it might not be possible to base the positions of the eyes solely on cone density. Instead, some small compromises are likely to be necessary along the way to enable well-functioning binocular vision. Considering such potentially conflicting developmental pressures, one might argue that our PRLs are quite well placed. The cone (or cones) that the visual system assigned the most attention to during development ended up being surprisingly close to the point of maximum cone density. The displaced PRLs that we observed are so close to the cone density peak that the sampling resolution is lower only to an inconsequential degree, as blurring due to the eye optics is the main limitation of spatial resolution in the central fovea [2].

The subjective locus of fixation

Our data indicate that observers are quite keenly aware whether an object appears in fixation. Although all stimuli were presented within a relatively confined area, both in retinal and external coordinates, observers would predominantly judge stimuli as being in fixation only if they appeared in a still much more restricted area near the raster center and near the PRL. Our data suggests that, at least in our task, observers emphasized the external world (raster) position of the stimulus somewhat more strongly than the retinal position. However, the retinal position clearly also had an effect. Our mixed model suggests that for stimuli presented in an identical raster location, observers would be quite confident of it being outside fixation (i.e., they would predominantly answer 'No'), if it landed approximately 0.35 degrees from the PRL. This is not in line with the findings of Wu and Cavanagh [28]. In their experiment, observers would most of the time feel, that they could "fixate" on a part of an afterimage, which actually lied 1.75 deg outside their fixation, and which they in fact could not move their fixation to (as the afterimage would also move the same amount). The very fact that observers in that study were asked to perform something that is strictly speaking impossible, makes a direct comparison of their results and ours difficult. Their study and the present one can be considered complementary as their data shows how crude the awareness of fixation location can be when there are no cues about retinal image slip. On the other hand, our study lacks that condition, but provides information of the roles of an external reference frame and retinal image in the (arguably more natural) condition where both information sources are present. One might argue that in our study the reference frame is exceptionally well constrained. However, Rattle [29] measured fixation accuracy in uniform fields up to 4 deg diameter and did not observe considerable reduction in fixation accuracy with increasing fixation target size.

Poletti, Rucci and Carrasco [30] recently showed that the classic perception boosting effect of a peripheral pre-cue also operates within the fovea. According to our results, however, even the 10-arcmin eccentricity of their experiments is sufficient that observers would rarely consider the stimuli as *in fixation*. Thus, it remains to be seen, whether there can be attentional effects among stimuli that are all perceived to be within the fixation locus.

The relationship between the cone density peak, the PRL and the subjective fixation location

The subjective fixation location is definitely not closer to the cone density peak than the PRL is, and both seem to be displaced to the same direction from the cone peak. This finding suggests that the displacement of the PRL from the cone density peak is not due to the oculomotor system's inability to correct a displacement that the visual system can detect.

In two of five observers, the PRL and the subjective fixation location are very near each other, but in the other three observers there is a clear difference in location. What might be the reason for this? We think that the most straightforward explanations is the following. During the subjective fixation location experiment, the observer is trying to fixate on the center of the raster, and perceives doing so successfully, but in reality the gaze direction is slightly biased. If the observer then uses the raster coordinates, along with the retinal stimulus location, to make a judgment of whether the stimulus appeared in fixation, this will cause a difference between the PRL and the subjective fixation location. Steinman [31] did observe some differences in fixation position with different-sized fixation targets, but not quite as large as those observed here. We do not have data to reveal whether that in fact is the cause of the location difference. Regardless of that, those three observers enabled a rather straightforward demonstration of how the judgement of the stimulus location relative to fixation is affected by retinal location of the stimulus (in addition to its raster location). The probability of the observers perceiving the stimulus as in fixation was higher, when the stimulus was displaced from the raster center towards the PRL, rather than the opposite direction (Figure 5). More generally, for all five observers in Experiment 2, our data suggests that while the external world reference frames have a strong effect on how the relationship between fixation and stimuli is perceived, the retinal location of the stimulus is also an important cue and would likely be dominant if the external reference frame cue were very weak (e.g., very large homogenous background). We point out, however, that both cues are usually present in normal visually guided behavior.

Our three-alternative-choice response format was designed to encourage observers to adopt a relatively strict criterion for the high confidence 'Yes' –response. An objective measure for the observer's criterion and sensitivity in detecting a stimulus in fixation cannot be derived, however, since there is no inherently wrong answer in this task. What we can say is that the SD for ‘Yes’ –responses is somewhat larger than the SD for fixation for all observers. That is not very surprising as such, as the oculomotor system tends to refoveate stimuli lying only a few arcmin from fixation, without the need for a conscious displacement percept [32,33]. Yet, rather than emphasizing the relatively modest difference, we would argue that the precision of observers’ subjective fixation location estimates comes surprisingly close to the precision of fixation itself, considering that the former, unlike the latter, is a skill that is hardly ever needed in everyday life.

Acknowledgements

The authors acknowledge technical support from Pavan Tiruveedhula. Related, but unpublished experiments on perceived location of the fovea were done in collaboration with Scott B. Stevenson and Girish Kumar. This work was supported by a grant from Oskar Öflunds foundation to MK, an NIH grant R01EY023591 to AR, NIH grants: P30EY002162, T32EY007043 to the UC Berkeley Vision Science Graduate Group and student support from the Minnie Flaura Turner Memorial Fund for Impaired Vision Research.

Author Contributions

MK, NP, KR and AR designed and conducted the experiments. MK analyzed and visualized the data and wrote the first draft of the manuscript. MK and AR wrote the final version of the manuscript, with input from NP and KR.

Declaration of Interests

AR has a patent (USPTO#7118216) assigned to the University of Houston and the University of Rochester which is currently licensed to Boston Micromachines Corp (Watertown, MA, USA). Both he and the company stand to gain financially from the publication of these results. AR is on the Board of Directors for C.Light Technologies, a company involved in retinal-image-based eye tracking. AR is a co-inventor on a patent (USPTO#10130253) assigned to UC Berkeley which is currently licensed to C.Light Technologies. Both he and the company stand to gain financially from the publication of these results.

STAR Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Markku Kilpeläinen (markku.kilpelainen@helsinki.fi).

Materials Availability

This study did not generate new unique reagents.

Data and Code Availability

The datasets generated during this study are available at Open Science Framework osf.io/2rd9z.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Observers

Six observers (3 female and 3 male, age 24-50 years) with normal visual acuity participated in Experiment 1, and five in Experiment 2. Observer 30002R was not available for Experiment 2. Observers 10002L, 10003R, 20094R and 30002R were authors of the paper, whereas observers 20092L and 20109R were naïve to the purposes of the experiment. All observers were relatively experienced as observers in retinal imaging experiments. Pupil dilation and paralysis of accommodation were achieved with one drop each of tropicamide (1%) and phenylephrine (2.5%), administered 15 minutes before the onset of imaging. Viewing was monocular in both experiments. The observers could freely choose the eye they used in viewing. The R or L in the end of the observer number indicates the eye (R = right, L = left) each observer used. The study adhered to the principles of the Declaration of Helsinki and was approved by the UC Berkeley Institutional Review Board. The observers signed written informed consent.

METHOD DETAILS

Retinal imaging and stimulation

The adaptive optics scanning laser ophthalmoscope (AOSLO) was used to image the retina and to project fixation targets and other visual stimuli to the retina. The principles of the AOSLO have been presented in detail elsewhere [34,35], but we describe the most relevant features of the current system here. A light beam from a supercontinuum laser (SuperK Extreme; NKT Photonics, Birkerød, Denmark) focused on the retina is transformed to a raster by a horizontal scanner (scan rate 16 kHz) and a vertical scanner (30 Hz). Light reflecting back from the eye is automatically descanned by the same two scanners, and then directed to a Shack-Hartmann wavefront sensor, which measures the aberrations of the observer's eye, as well as to a photomultiplier tube (Hamamatsu photonics, Hamamatsu, Japan), which records the intensity of light from each imaged pixel, enabling the reconstruction of a retinal image. In the current study, 680 nm light was used for all imaging and stimulation, and 940 nm light for wavefront sensing. The aberrations measured by the wavefront sensor are compensated for by a deformable mirror (7.2 mm diameter, 97 actuator membrane, ALPAO, Montbonnot-Saint-Martin, France).

Decrement stimuli (dark patterns on a red background) can be presented within the imaging raster by rapidly controlling the power of the scanning beam by means of an acousto-optic modulator (Brimrose Corp, Sparks, MD, USA). In the current study, all stimuli were (nominally) 100 % decrement patterns. Since such stimuli involve a structured omission of light within the raster, the stimulus shows in the video, and the retinal location of the stimulus can be extracted with absolute certainty, frame by frame, from the videos [36].

Procedure

Experiment 1 consisted of 30-second fixation runs. Each run started with a Maltese cross fixation target (diameter 5.5 arcmin) appearing in the center of the 0.93x0.93 degree raster. Once the observers felt they were fixating the center of the Maltese cross, the experiment operator started the fixation run. During the run, the Maltese cross shifted abruptly to a new, random location every 2-6 seconds, somewhere within a central 11 x 11 arcmin region of the raster. The observer's task was to carefully fixate on the center of the Maltese cross throughout the 30-second run, and to quickly shift fixation to the new location after the cross shifted. The aim of the motion was to more thoroughly engage the subject and to measure the PRL with a task more ecologically valid than continuously fixating on a completely stationary and predictable target – a task that is rarely, if ever, required in natural viewing. A video of the observer's retina (with the target location visible) was recorded throughout the fixation run. See supplemental video 1 for a short version of a retinal video of a fixation run. On each experimental day, the observers carried out eight fixation runs. Observers participated in 2-3 experimental days. The experimental days for each observer were on average 2.1 days apart (range 1-4, SD 1.05).

In Experiment 2, each trial proceeded as follows. The observer was instructed to fixate on the center of the raster (diameter 1.16 deg), according to their subjective estimate. The observer could then initiate a trial by pressing a key. After a random delay of 400-700 ms, a small black square (diameter 2.3 arcmin, distributed over 4 lines of the raster scan) was presented within a single frame. The location of the square varied pseudorandomly within a central, 22-arcmin wide, rectangular area. The stimulation (and imaging) sequence of each trial lasted for 1 second. After that, the observer's task was to judge whether the square had appeared along the observer's line of sight (i.e., in fixation), with a three-alternative response (Yes, Maybe, No). After giving the answer with a key stroke, the observer could initiate the next trial. There were 53 trials in one block. Altogether each observer completed between 1484-1749 trials over three experimental sessions.

QUANTIFICATION AND STATISTICAL ANALYSIS

The videos recorded from the experiments were processed offline according to the following process [see, 37]. Firstly, a composite reference frame was created by aligning and summing selected frames of each video such that the reference frame spanned all the imaged retinal locations of the video. Each frame of the video was then divided into 28 strips, each 9 pixels in height. These strips were registered with the composite reference, which yielded an eye motion trace with an 840 Hz sampling rate.

To generate a single image to which all data collected in this experiment could be referenced to, and also to determine the cone density across the fovea, a high-quality master cone image was created in the manner described above but using a 10-second video, where the observer was fixating a black square that was blinking in the center of the raster. The cone locations in the master cone image were manually determined across a 35-arcmin diameter area of the central fovea. To compute density across the mosaic, the cone locations were converted to a binary map with the same scale as in the cone counting image, where each cone location was assigned a single pixels with a value of 1. This binary image was then convolved with a circular window with a diameter of 8 arcmin. The output of the convolution generates a continuous density map across the image. The point of maximum cone density corresponds to the pixel location of the convolution maximum.

For Experiment 1, the retinal position of the Maltese cross in each video frame was determined by means of cross-correlation. We then used the above mentioned eye motion trace to determine the position of each video frame in the composite reference frame. Finally, to get the stimulus locations from different videos (including from different days) to the same coordinates, the composite reference frames of the different videos were co-registered with the master cone image and the resulting image transformation parameters (translation, scaling, rotation) applied to the stimulus locations (see Figure 5A). The black cross shows the cone density peak. For each observer and day, the four videos that produced the best co-registration results were included in the data.

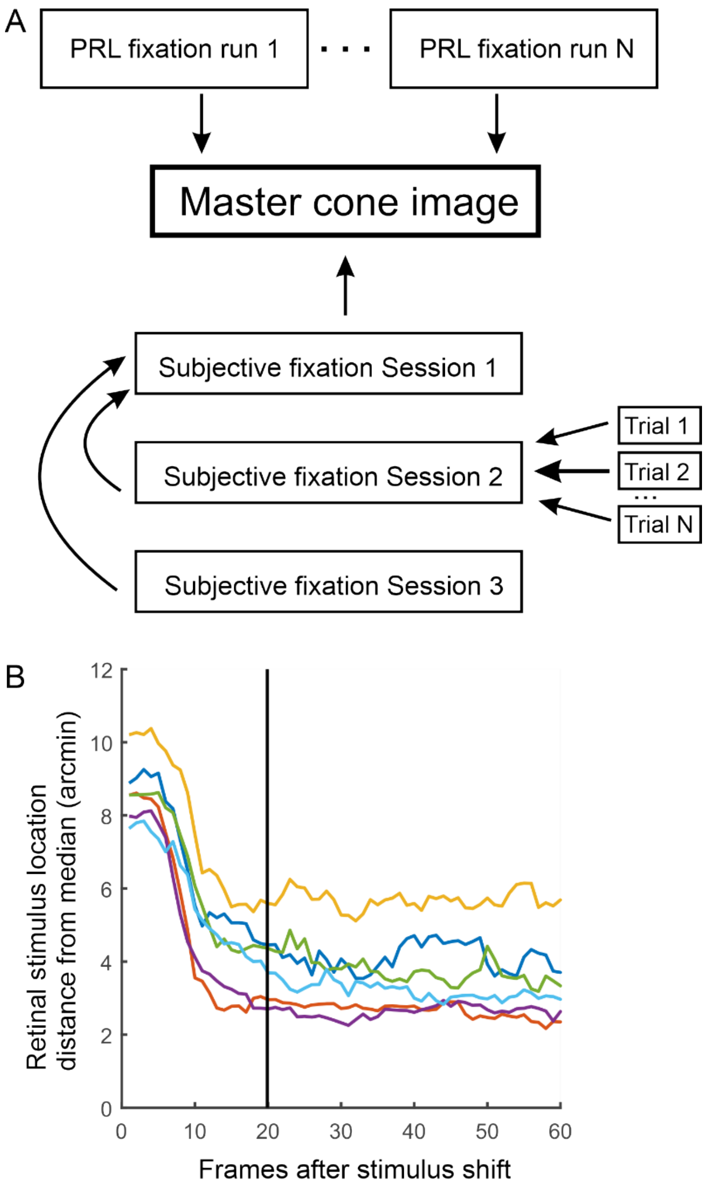


Figure 5. A) The procedure of bringing all data of the study to the same coordinates. Each box represents a reference frame image. The arrows point to the direction to which image the other was registered to. B) Average retinal stimulus location distance from median location as a function of time (in frames) from stimulus shift, separately for all observers (different line colors). Median was calculated over the 2-6 seconds where the stimulus stayed in one location. To exclude frames, where the observer's gaze had not yet moved to the new stimulus location, data from the first 20 frames (≈ 667 ms) after stimulus shifts was excluded from further analyses (black vertical line).

Since there is unavoidably a delay between the shift of the Maltese cross and the observer's saccade to the new location, some video frames after each stimulus shift needed to be excluded. We plotted the distance between the retinal location of the stimulus in each frame and the median retinal location as a function of time after stimulus shift (Figure 5B). One can observe that after 20 frames (≈ 667 ms), the saccade has been made and, as a result, including all data after that is likely to introduce very little noise. After removing the 20 first frames, and additional removal of frames due to blinks and other image quality degradations, the data included in the analyses had 2191 – 2992 samples (mean 2688, SD 258.5) per day per observer.

Differences in the PRLs between days were analyzed for each observer with Akaike's information criterion (AIC). The AICs were obtained by fitting 2D Gaussian distributions to the data with Matlab's fitgmdist function (Mathworks, Natick, MA, USA). In the simpler model (m1), fixation data from two days was fitted with shared parameters only. In the more complex model (m2), the mean (x,y) coordinates of the two distributions were allowed to differ. The evidence ratio is then calculated from the AICs for the more simple (m1) and the more complex model (m2) as P(m1 is best) / P(m2 is best), where P(m1 is best) = $\exp(-\Delta AIC/2) / (1 + \exp(-\Delta AIC/2))$ and P(m2 is best) = 1- P(m1 is best).

For Experiment 2, the extraction of retinal stimulus locations were done in the same ways as for Experiment 1. Bringing data to the coordinates of the master cone image (and thus the same coordinates as the data of Experiment 1) involved some additional steps (See Figure 5A). Firstly, each trial's reference frame was co-registered with the grand reference frame of the measurement session (produced from a 10-second video taken at the beginning of the session). Secondly, the grand reference images of sessions 2 and 3 were co-registered with the grand reference image from session 1, to bring all data of Experiment 2 to the same coordinates. Finally, the grand reference image from session 1 was registered with the master cone image. About 35 % of the trials of Experiment 2 could not be included in data, because either the cross-correlation found a wrong stimulus location or, predominantly, there was a poor alignment between the trial's reference frame and the grand reference frame of the measurement session. Due to the large number of trials, correcting these by hand was not feasible. However, these errors were expected and were mitigated by collecting a large number of trials.

A generalized mixed linear model was used to analyze the contributions of the raster location and the retinal location of the stimulus on the probability of different response alternatives. Since the response variable is an ordinal scale variable with three possible values, a multinomial logistic link function was used. The analysis was carried out with SPSS 25 software (IBM, Armonk, NY, USA). The repeated measures nature of the data was incorporated by adding observer as a random factor into the model.

Supplemental video 1. A shortened example video of the fixation task. The video shows how the fixation target location is rendered on the video of the observers retina. We advice the reader to pay attention to the dark spots on the retina to see how the retina follows the movements of the target.

References

1. Caves, E.M., Brandley, N.C., and Johnsen, S. (2018). Visual Acuity and the Evolution of Signals. Trends Ecol. Evol. *33*, 358–372.

2. Marcos, S., and Navarro, R. (1997). Determination of the foveal cone spacing by ocular speckle interferometry: Limiting factors and acuity predictions. JOSA A *14*, 731–740.

3. Provis, J.M., Penfold, P.L., Cornish, E.E., Sandercoe, T.M., and Madigan, M.C. (2005). Anatomy and development of the macula: specialisation and the vulnerability to macular degeneration. Clin. Exp. Optom. *88*, 269–281.

4. Provis, J.M., Dubis, A.M., Maddess, T., and Carroll, J. (2013). Adaptation of the central retina for high acuity vision: Cones, the fovea and the avascular zone. Prog. Retin. Eye Res. *35*, 63–81.

5. Mäkelä, P., Näsänen, R., Rovamo, J., and Melmoth, D. (2001). Identification of facial images in peripheral vision. Vision Res. *41*, 599–610.

6. Strasburger, H., Rentschler, I., and Harvey, L.O. (1994). Cortical magnification theory fails to predict visual recognition. Eur. J. Neurosci. *6*, 1583–1588.

7. Thibos, L.N. (1998). Acuity perimetry and the sampling theory of visual resolution. Optom. Vis. Sci. *75*, 399–406.

8. Rovamo, J., Virsu, V., Laurinen, P., and Hyvärinen, L. (1982). Resolution of gratings oriented along and across meridians in peripheral vision. Invest. Ophthalmol. Vis. Sci. *23*, 666–670.

9. Rossi, E.A., and Roorda, A. (2010). The relationship between visual resolution and cone spacing in the human fovea. Nat. Neurosci. *13*, 156–157.

10. Putnam, N.M., Hofer, H.J., Doble, N., Chen, L., Carroll, J., and Williams, D.R. (2005). The locus of fixation and the foveal cone mosaic. J. Vis. *5*, 3–3.

11. Li, K.Y., Tiruveedhula, P., and Roorda, A. (2010). Intersubject Variability of Foveal Cone Photoreceptor Density in Relation to Eye Length. Invest. Ophthalmol. Vis. Sci. *51*, 6858–6867.

12. Wang, Y., Bensaid, N., Tiruveedhula, P., Ma, J., Ravikumar, S., and Roorda, A. (2019). Human foveal cone photoreceptor topography and its dependence on eye length. eLife *8*, e47148.

13. Wilk, M.A., Dubis, A.M., Cooper, R.F., Summerfelt, P., Dubra, A., and Carroll, J. (2017). Assessing the spatial relationship between fixation and foveal specializations. Vision Res. *132*, 53–61.

14. Nakabayashi, M., Fujikado, T., Ohji, M., Saito, Y., and Tano, Y. (2000). FIXATION PATTERNS OF IDIOPATHIC MACULAR HOLES AFTER VITREOUS SURGERY. Retina *20*, 170–175.

15. Tarita-Nistor, L., González, E.G., Mandelcorn, M.S., Lillakas, L., and Steinbach, M.J. (2009). Fixation Stability, Fixation Location, and Visual Acuity after Successful Macular Hole Surgery. Invest. Ophthalmol. Vis. Sci. *50*, 84–89.

16. Kwon, M., Nandy, A.S., and Tjan, B.S. (2013). Rapid and Persistent Adaptability of Human Oculomotor Control in Response to Simulated Central Vision Loss. Curr. Biol. *23*, 1663–1669.

17. Varsori, M., Perez-Fornos, A., Safran, A.B., and Whatham, A.R. (2004). Development of a viewing strategy during adaptation to an artificial central scotoma. Vision Res. *44*, 2691–2705.

18. Walsh, D.V., and Liu, L. (2014). Adaptation to a simulated central scotoma during visual search training. Vision Res. *96*, 75–86.

19. Hauperich, A.-K., Young, L.K., and Smithson, H.E. (2020). What makes a microsaccade? A review of 70 years research prompts a new detection method. J. Eye Mov. Res. *12*. Available at: https://bop.unibe.ch/JEMR/article/view/JEMR.12.6.13 [Accessed July 21, 2020].

20. Nyström, M., Hansen, D.W., Andersson, R., and Hooge, I. (2016). Why have microsaccades become larger? Investigating eye deformations and detection algorithms. Vision Res. *118*, 17–24.

21. Bedggood, P., Daaboul, M., Ashman, R.A., Smith, G.G., and Metha, A. (2008). Characteristics of the human isoplanatic patch and implications for adaptive optics retinal imaging. J. Biomed. Opt. *13*, 024008.

22. Nowakowski, M., Sheehan, M., Neal, D., and Goncharov, A.V. (2012). Investigation of the isoplanatic patch and wavefront aberration along the pupillary axis compared to the line of sight in the eye. Biomed. Opt. Express *3*, 240–258.

23. McGregor, J.E., Yin, L., Yang, Q., Godat, T., Huynh, K.T., Zhang, J., Williams, D.R., and Merigan, W.H. (2018). Functional architecture of the foveola revealed in the living primate. PLOS ONE *13*, e0207102.

24. Hendrickson, A., Possin, D., Vajzovic, L., and Toth, C.A. (2012). Histologic Development of the Human Fovea From Midgestation to Maturity. Am. J. Ophthalmol. *154*, 767-778.e2.

25. Vajzovic, L., Hendrickson, A.E., O'Connell, R.V., Clark, L.A., Tran-Viet, D., Possin, D., Chiu, S.J., Farsiu, S., and Toth, C.A. (2012). Maturation of the Human Fovea: Correlation of Spectral-Domain Optical Coherence Tomography Findings With Histology. Am. J. Ophthalmol. *154*, 779-789.e2.

26. Giaschi, D., Narasimhan, S., Solski, A., Harrison, E., and Wilcox, L.M. (2013). On the typical development of stereopsis: Fine and coarse processing. Vision Res. *89*, 65–71.

27. Norcia, A.M., and Gerhard, H.E. (2015). Development of Three-Dimensional Perception in Human Infants. Annu. Rev. Vis. Sci. *1*, 569–594.

28. Wu, D.-A., and Cavanagh, P. (2016). Where are you looking? Pseudogaze in afterimages. J. Vis. *16*, 6–6.

29. Rattle, J.D. (1969). Effect of Target Size on Monocular Fixation. Opt. Acta Int. J. Opt. *16*, 183–190.

30. Poletti, M., Rucci, M., and Carrasco, M. (2017). Selective attention within the foveola. Nat. Neurosci. *20*, 1413–1417.

31. Steinman, R.M. (1965). Effect of Target Size, Luminance, and Color on Monocular Fixation*. JOSA *55*, 1158–1164.

32. Poletti, M., Listorti, C., and Rucci, M. (2013). Microscopic Eye Movements Compensate for Nonhomogeneous Vision within the Fovea. Curr. Biol. *23*, 1691–1695.

33. Ratnam, K. (2017). The effects of retinal image motion on the limits of spatial vision. Available at: https://escholarship.org/uc/item/80v0d4z5.

34. Mozaffari, S., Mozaffari, S., LaRocca, F., LaRocca, F., Jaedicke, V., Tiruveedhula, P., and Roorda, A. (2020). Wide-vergence, multi-spectral adaptive optics scanning laser ophthalmoscope with diffraction-limited illumination and collection. Biomed. Opt. Express *11*, 1617–1632.

35. Roorda, A., Romero-Borja, F., Iii, W.J.D., Queener, H., Hebert, T.J., and Campbell, M.C.W. (2002). Adaptive optics scanning laser ophthalmoscopy. Opt. Express *10*, 405–412.

36. Poonja, S., Patel, S., Henry, L., and Roorda, A. (2005). Dynamic Visual Stimulus Presentation in an Adaptive Optics Scanning Laser Ophthalmoscope. J. Refract. Surg. *21*, S575–S580.

37. Stevenson, S.B., Roorda, A., and Kumar, G. (2010). Eye tracking with the adaptive optics scanning laser ophthalmoscope. In Proceedings of the 2010 Symposium on Eye-Tracking Research & Applications ETRA '10. (Austin, Texas: Association for Computing Machinery), pp. 195–198. Available at: https://doi.org/10.1145/1743666.1743714 [Accessed July 9, 2020].