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Assessing the spatial relationship between fixation and foveal specializations

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ABSTRACT

Increased cone photoreceptor density, an avascular zone (FAZ), and the displacement of inner retinal neurons to form a pit are distinct features of the human fovea. As the fovea provides the majority of our vision, appreciating how these anatomical specializations are related is important for understanding foveal development, normal visual function, and retinal disease. Here we evaluated the relationship between these specializations and their location relative to the preferred retinal locus of fixation (PRL). We measured foveal pit volume, FAZ area, peak cone density, and location of the PRL in 22 subjects with normal vision using optical coherence tomography and adaptive optics scanning light ophthalmoscopy. Foveal pit volume was positively correlated with FAZ area; however, peak cone density was not correlated with pit volume. In addition, there was no systematic offset of the location of any of these specializations relative to PRL, and there was no correlation between the magnitude of the offset from PRL and the corresponding foveal specialization measurements (pit volume, FAZ area, peak cone density). The standard deviation of our PRL measurements was consistent with previous measurements of fixational stability. These data provide insight into the sequence of events during foveal development and may have implications for visual function and retinal disease.

1. Introduction

The normal fovea is a highly specialized region of the human retina, characterized by the foveal avascular zone (FAZ), complete displacement of inner retinal neurons (creating the characteristic foveal "pit"), increased cone packing, and an absence of rod photoreceptors (Hendrickson, 2005; Provis, Dubis, Maddess, & Carroll, 2013). While the fovea itself represents a relatively small area of the retina, it drives the majority of our visual function. Developmental disruption of the fovea in conditions such as albinism, aniridia, isolated foveal hypoplasia, and premature birth are linked with a decrease in visual function throughout life (Nelson, Spaeth, Nowinski, Margo, & Jackson, 1984; Quinn & Dobson, 1996; Summers, 1996). Likewise, alterations to the foveal region in adulthood by conditions such as diabetic retinopathy and age-related macular degeneration result in a similar reduction in vision (Cunha-Vaz, Ribeiro, & Lobo, 2014; Zarbin, Casaroli-Marano, & Rosenfeld, 2014). Examination of the different aspects of foveal specializations and how they are related to one another will aid in the understanding of the anatomical basis of visual dysfunction in such conditions, as well as clarify models of normal foveal development.

In addition, while many tests of visual function are intended to test central vision (i.e., at the fovea), the preferred retinal locus of fixation (PRL) is actually the target of many of these tests. However, it is not known how the PRL relates to the different foveal specializations. Putnam and colleagues (Putnam et al., 2005) have shown that the PRL is offset from the location of peak cone density by about 50 μm, with no consistency in the direction of offset across the five subjects tested. However, this study only assessed fixation relative to the cone mosaic, not the FAZ or pit. It remains to be seen how the PRL is associated with other features of the fovea.

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Due to the heterogeneity in methods of defining and assessing foveal morphology, it is also important to understand the relationships between each of the existing foveal measurements. Here we suggest metrics for objective quantification of foveal pit size, avascular area, and photoreceptor mosaic specialization, and examine the relationships between them. In addition, we evaluated the location of each specialization (center of FAZ, bottom of pit, and location of peak cone density) relative to the PRL. The quantification of these metrics and relationships will allow better comparison of foveal morphology across studies and could provide an improved understanding of visual function and retinal development and disease.

2. Methods

2.1. Subjects

This study followed the tenets of the Declaration of Helsinki and was approved by the Medical College of Wisconsin Institutional Review Board. Written informed consent was obtained from all subjects (or an adult guardian for minors) after explanation of the nature and possible consequences of the study. Twenty-two subjects (6 female, 16 male) aged 13–67 years (average ± standard deviation = 31 ± 16 years) were recruited to participate in this study (Table 1). Subjects with refractive error of 10 diopters or more, or with other vision-limiting pathology were excluded from the study. Subjects' self-reported ethnicities were Asian (n = 2), African American (n = 2), or Caucasian (n = 18). For the imaging experiments, each subject had one eye dilated and accommodation suspended using one drop each of Phenylephrine Hydrochloride (2.5%) and Tropicamide (1%). Axial length, used for calibrating the lateral scale of all retinal images, was measured using an IOL Master (Carl Zeiss Meditec, Dublin, CA).

2.2. Quantifying foveal pit metrics

Volumetric images of the macula were acquired using the Cirrus High Definition (HD)-OCT (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA). Volume scans were nominally 6 × 6 mm (assuming a 24.46 mm axial length) and consisted of 128 B-scans (512 A-scans/B-scan). Foveal pit volume was calculated from topographical maps of retinal thickness as previously described (Wilk et al., 2014). The bottom of the pit was located using the automatic "Fovea Finder" of the Cirrus software.

2.3. Assessing the foveal avascular ZONE (FAZ)

Subjects' FAZs were imaged using OCT Angiography (RTVue XR 100 Avanti, Optovue, Inc., Fremont, CA; nine subjects), adaptive optics scanning light ophthalmoscopy (AOSLO; 11 subjects), or the Retinal Function Imager (RFI, Optical Imaging Ltd., Rehovat, Israel; two subjects). When possible, multiple OCT Angiography images were acquired, aligned, and averaged in ImageJ to achieve better signal-to-noise ratio (Schneider, Rasband, & Eliceiri, 2012; Thévenaz, Ruttimann, & Unser, 1998) (Fig. 1A). AOSLO images were registered and averaged as previously described (Cooper et al., 2011; Dubra & Harvey, 2010) prior to manual montaging in Photoshop (Adobe Systems, San Jose, CA). For all imaging modalities, the boundaries of the FAZ were manually identified by a single observer (MAW) using ImageJ (Schneider et al., 2012) (Fig. 1A). A mask was then constructed from the identified boundary points to create a closed contour defining the FAZ (Fig. 1B) using MATLAB software (Mathworks, Natick, MA). The area of the FAZ was calculated by multiplying the area (mm^2) of one pixel, adjusting for ocular magnification, by the number of pixels encompassed by the mask. Similarly, the perimeter of the FAZ in millimeters was also computed from the mask. Acircularity was defined as the ratio of FAZ perimeter to the circumference of a circle with an area equal to that of the FAZ, as previously described (Tam et al., 2011). In this approach, an acircularity of 1 corresponds to a perfect circle, and values greater than 1 indicate an increasingly oblong or irregular shape.

2.4. Measuring peak cone density

Confocal reflectance AOSLO images of the foveal cone mosaic were registered and montaged as described in Section 2.3. Peak cone density was estimated as previously described (Wilk et al.,

Table 1
Subject demographics and foveal metrics.

Subject	Age	Sex	Ethnicity	Eye	Axial length (mm)	Method for FAZ	FAZ area (mm^2)	FAZ perimeter (mm)	Pit volume (mm^3)	Peak cone density ($cones/mm^2$)	Horizontal SD of PRL (μm)	Vertical SD of PRL (μm)	Number of frames for PRL*
JC_0002	28	M	Caucasian	OD	24.72	RFI	0.1558	1.635	0.0641	147,550	15.7	18.4	90
JC_0007	37	M	Caucasian	OD	27.45	OCTA	0.0788	1.375	0.0375	106,650	19.2	17.1	103
JC_0138	25	F	Asian	OD	22.75	RFI	0.4110	2.613	0.1374	195,030	12.2	14.7	105
JC_0200	26	M	Caucasian	OD	24.72	OCTA	0.2242	1.902	0.0826	128,560	19.7	11.5	144
JC_0571	25	M	Caucasian	OD	24.08	AO	0.1428	1.570	0.0706	137,330	15.8	9.2	95
JC_0616	23	M	Caucasian	OD	24.35	OCTA	0.2394	1.997	0.1046	167,280	15.0	11.1	118
JC_0628	67	F	Caucasian	OD	22.92	AO	0.0634	1.243	0.0207	165,080	15.7	13.6	98
JC_0629	63	M	Caucasian	OD	23.29	AO	0.2068	1.976	0.0563	160,700	9.6	14.7	73
JC_0645	20	M	Caucasian	OD	23.76	AO	0.2480	2.525	0.1163	177,500	9.9	10.0	128
JC_0654	25	F	Caucasian	OD	23.57	AO	0.1405	2.049	0.0561	214,020	12.9	7.9	131
JC_0661	23	M	African American	OD	25.52	AO	0.2521	2.307	0.0549	132,210	16.4	13.2	76
JC_0677	24	F	Caucasian	OD	24.03	OCTA	0.4902	2.675	0.1060	165,080	9.4	10.7	131
JC_0692	40	M	Caucasian	OD	24.54	AO	0.2582	2.308	0.0400	142,440	19.7	11.6	87
JC_0769	21	F	Caucasian	OD	24.29	OCTA	0.3111	2.204	0.1068	127,830	15.9	10.8	118
JC_0905	21	M	Caucasian	OD	22.46	OCTA	0.2764	2.107	0.1231	125,640	15.8	14.3	87
JC_10119	22	M	Asian	OD	25.90	OCTA	0.1902	1.825	0.0657	108,110	19.7	14.0	120
JC_10121	23	M	African American	OS	23.93	AO	0.3692	2.389	0.1773	144,630	14.5	9.3	134
JC_10145	49	F	Caucasian	OD	24.66	OCTA	0.2784	2.104	0.0709	120,530	20.8	13.7	133
JC_10147	13	M	Caucasian	OS	24.66	AO	0.2105	1.771	0.0699	134,400	18.6	17.5	111
JC_10311	62	M	Caucasian	OD	22.86	OCTA	0.1698	2.282	0.0348	153,400	12.9	8.6	100
JC_10312	15	M	Caucasian	OS	26.88	AO	0.2284	2.172	0.1055	128,560	20.2	14.2	85
JC_10329	22	M	Caucasian	OS	24.46	AO	0.2548	2.504	0.0887	127,830	11.4	10.5	141

RFI = Retinal Function Imager; OCTA = OCT angiography; AO = adaptive optics scanning light ophthalmoscopy; OD = right eye; OS = left eye.

* Number of frames is per video location. Total number of fixation points is 4 times the number listed for each subject. The total number of frames recorded for each location, and thus the maximum possible fixation points per location, was 150 for each subject.

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