PERGAMON

Vision Research 38 (1998) 2685–2696

Vision Research

Entoptic image quality of the retinal vasculature

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Received 27 November 1996; received in revised form 27 August 1997

Abstract

Spatial details of entoptically visible retinal vessels were investigated using transcleral and Maxwellian-view stimulators. Nine normal subjects provided detailed drawings of the entoptic images which were digitized and superimposed onto digitized fundus photographs and fluorescein angiograms from the same eyes. Subjects also used a tracing method to locate visible entoptic features. The trans-scleral method provided images similar in detail to standard fundus photography (lacking capillary detail, but capturing larger arteries, veins, arterioles and venules) in the macula and around the disk. The Maxwellian-view method illuminated the fovea (7.7 degree field) and provided foveola capillary detail (capillaries traversing the foveola, the capillary arcade forming the FAZ) as well as the larger foveal vessels supplying the foveola, and often contained more foveal detail that available with fluorescein angiography.

Keywords: Entoptic; Vascular; *Maxwellian-view*

1. Introduction

Accurate imaging of the retinal vasculature is an essential component in the successful diagnosis and treatment of numerous retinal diseases (e.g. diabetic retinopathy), and accordingly considerable technology has been developed to enhance the clinician's view of retinal vessels. A typical retinal exam employs ophthalmoscopy which provides a resolution at the retina of approximately 20 microns or worse [1]. Unfortunately, since retinal capillary diameters are generally smaller than 10 microns [2], even good quality color fundus photography fails to capture any of the capillary details. Although some of the better fluorescein angiograms (FAs) clearly show capillary details around the foveal avascular zone [3–11], others fail to capture any capillary detail [12]. Capillary details should be most easily resolved around the foveal avascular zone (FAZ), where capillary density is lower [2,13,14]. A comparative histological and angiographic study of monkey retina has recently shown that, outside of the fovea, high quality FAs fail to image the majority of the capillaries, particularly in the deeper retinal layers, and even miss some of the capillaries forming the FAZ [11].

In addition to the objective photographic techniques for imaging retinal vessels, self-examination of the retinal vasculature is possible using entoptic techniques [15]. Three entoptic methods for viewing intraocular structures were described by Helmholtz [16]: (i) moving a bright point source (such as a candle) around near to the eye in an otherwise dark room; (ii) imaging a moving small point of light onto the sclera temporal to the temporal limbus; and (iii) imaging a moving small point source in the pupil plane. Most twentieth century studies of entoptic visualization of the retinal vasculature have adopted variants of either the trans-scleral (TS) illumination method [17–19] or through-the-pupil (TP) illumination [20–24]. In addition, a fourth method, which requires subjects to view a bright blue uniform field ('blue-field entoptoscope') has been used to examine macular vessels by following the path of 'flying corpuscles' [25,26].

All entoptic methods have similar requirements for success. In order for intra-ocular structures to be

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0042-6989/98/$19.00
PII: S0042-6989(97)00345-3

viewed entoptically, they must cast moving and visible shadows at the plane of the photoreceptor entrance apertures. The blue field entoptoscope is unique in that the motion of the shadow is achieved by the motion of the retinal blood cells and not, as in the other methods, the light source. A moving light source has the advantage that entoptic images of stationary opacities, such as blood vessels and pathological opacities can be seen [18,27–29].

Although no formal comparison between the different entoptic methods has been published, Helmholtz [16] comments on the superior quality of the entoptic image of the foveal vessels obtained with the through-the-pupil (TP) method. Applegate et al. [22] argue that the spatial detail within the shadow pattern cast by the retinal vessels will be optimized by using a small light source imaged in the pupil plane. They suggest that internal scattering within the sclera will expand any light imaged onto the sclera and hence the TS method may not be able to create a shadow pattern with as much spatial detail. Detailed observations of the capillary arcade surrounding the foveal avascular zone obtained with the TP technique support these ideas [23,24]. However, Bird and Weale [17], using a TS method, were also able to see capillaries traversing the center of the foveola.

In addition to questions about the relative accuracy and sensitivity of the TS and TP entoptic methods, it can be argued that the entoptic image may be superior to images created with objective photographic methods because the entoptic shadow does not have to be imaged by the imperfect optics of the eye in order to be seen. Of course, the accuracy of the entoptic methods will be limited by the spatial resolution of the retina in addition to the optical quality of the shadow pattern. Therefore, we expect entoptic methods to provide the most detailed image at the fovea and lower quality images with increasing eccentricity. To ascertain whether or not these expectations are correct, we have conducted a comparative study to assess the quality of two entoptic methods (trans-scleral and through-the-pupil) for viewing retinal vasculature in comparison to standard clinical objective images of retinal vasculature (color fundus photography and fluorescein angiography).

2. Methods

We have compared two entoptic methods (trans-scleral illumination (TS) and a through-the-pupil Maxwellian-view method that images a small point source in the pupil plane (TP)) for viewing the retinal vasculature in the living human eye with two photographic methods (color fundus photography (FP) and Fluorescein Angiography (FA)).

2.1. Subjects

Nine adult subjects with normal visual function and no sign of retinal pathology served as subjects. None had any formal training in drawing, and all subjects were unaware of their objective fundus images while drawing their entoptic images. Six of the subjects were familiar with the general pattern of retinal vessels found in human eyes. The study was conducted in accordance with the tenets of Declaration of Helsinki after IRB approval, and informed consent was obtained from each subject.

2.2. Entoptic stimulators

2.2.1. Trans-scleral (TS) method

Using a modified slit lamp in which the fixed mirror was replaced by a rotating mirror [19], a small 2–3-mm point of white light was imaged onto the sclera approximately 5 mm temporal to the temporal limbus. The bright point of light was translated in a circular path at about three times per second. Subjects sat in a dark room. Light source intensity and size were adjusted to achieve the subjectively 'best' entoptic image.

2.2.2. Trans-pupil (TP) method

Using a Maxwellian-view optics vascular entoptoscope [22], subjects viewed a central 7.7 degree field formed by a short wavelength ($\lambda = 447$ nm) point source (1 mm diameter) imaged in the plane of the pupil and rotating through a circular path of 4 mm at 4 Hz.

2.3. Color photographic and angiographic methods

Standard color slide images of field 2 (centered approximately on the macula), were taken using a Cannon 60UV fundus camera (45 by 60°) or with a Zeiss FF-3 30 degree fundus camera. Each slide was digitized using a Nikon LS 3500 scanner with 256 gray levels. Fluorescein angiograms (FAs) were obtained from the eyes of three of the authors (AB, RA and LT) and two other subjects undergoing fluorescein angiography for a different study using a scanning laser ophthalmoscope and recorded on Super VHS. Later, the best image of the foveal area capillary phase captured during the FA was digitized and stored on disk.

2.4. Data collection and analysis

2.4.1. Drawing

With both entoptic illumination methods (TS and TP), subjects observed and then drew their retinal vascular patterns. Subjects were allowed as much time, repeated entoptic views and drawing modifications as necessary to obtain a drawing that they felt accurately

represented their entoptic image. One subject (L.N. Thibos) used direct tracing when drawing the entoptic image seen with the TS method. He viewed a dimly illuminated sheet of white paper oriented orthogonal to his visual axis to trace the entoptic image. The tracing method is quite difficult because inadvertent eye movements continually change the relative location of the entoptic image and the drawing making registration difficult. The advantage of this method, however, is that it produces a drawing of known angular scale. As shown by Sharpe [21], initially clear entoptic images will begin to fade if viewed for a long period of time. This perceptual fading was avoided by intermittent breaks.

2.4.2. Feature location

Specific vascular details (e.g. branchings or obvious bends in a specific vessel) were chosen that were clearly visible in both the entoptic and the photographic images. Using the TS illumination technique subjects viewed a sheet of white paper in a dark room. Subjects fixated a dim red fixation point located at the center of a sheet of drawing paper which was oriented orthogonal to the subjects' visual axis. Subjects holding a pencil with a LED at its tip positioned the pencil tip to coincide with the entoptic image of a series of pre-chosen vascular details while maintaining fixation on the central fixation point. Once the pencil light was aligned with one of the pre-chosen vascular features, the position was marked on the paper. The locations of these marks were then compared to the locations of the pre-chosen vascular features in the photographic images as described below.

3. Image manipulation

3.1. Super-position of entoptic and photographic images

The entoptic drawings were digitized using a scanner (Apple) and retraced to produce a high contrast version of the entoptic drawing. High contrast versions of the retinal vasculature were obtained from the digitized color fundus photographs of the macular area and capillary phase of the FA by tracing each under high magnification using an object-based graphics program (McDraw, Claris). Both high contrast tracings could then be super-imposed to examine differences and similarities between the entoptic and photographic images. In order to prevent bias, tracings of the photographic images were performed independently from the entoptic images.

Before superimposing the entoptic and photographic images, the entoptic drawings, in which superior represents the inferior retina, had to be flipped vertically. This process converts the entoptic image to the same coordinates observed by the clinician and shown routinely in the photographic images (right in the image is the subject's left retina while the top of the image is superior on the retina). Since drawings could be mislocated, of different sizes and potentially rotated with respect to the subject's perceived entoptic pattern, the two appropriately oriented tracings (entoptic and photographic) were then superimposed and manipulated using strictly isotropic affine transformations (shift, magnify and rotate) to try and obtain the best subjective match. Such image transformations will not correct for any non-isotropic drawing errors such as individual errors in feature location and/or local magnification differences within the drawing or photograph.

3.2. Quantification of feature location and vessel diameter

Using the data obtained from the drawing phase of this study, we ascertained a series of vascular features that were visible in both the entoptic and photographic images. These features were used to assess: (i) the width of visible vessels; and (ii) the accuracy with which visible features can be localized. Using high magnification digital imaging, vessel widths and feature locations were determined from fundus photographs and FAs using a locatable cursor under visual control. The cursor was centered on the feature when measuring the location, and positioned on opposite edges of the vessels when measuring the vessel width. Vessel widths and distances from the foveola center were converted from computer screen coordinates to microns in retinal space using a 4.822-mm estimate for the foveola center to disk center separation. The angular eccentricities of the selected vessels features were calculated using a 3.43° of visual angle per retinal mm conversion assuming the distance from the secondary nodal point to retina is 16.67 mm.

Using the same isotropic scaling used to match drawings to the photographic images, we adjusted the digitized LED wand tracings of the selected features to obtain the best match with the photographic locations of the same features. Location in retinal Cartesian and angular units of each feature in the resulting entoptic image were then calculated using the same location and scaling procedures described for the photographic images.

4. Results

4.1. Entoptic drawings

4.1.1. Trans-scleral method

Right and left eye scans of drawings made by four subjects using the TS entoptic method are shown in

Fig. 1. Although each drawing is unique, there are obvious similarities and each drawing has many characteristics common to the familiar vascular patterns seen in fundus photographs. All subjects tested with the TS method (ten eyes from five subjects) were able to see and draw the large vessels emerging from the optic disk. In addition, most subjects (e.g. the right eyes of subjects AB, HZ and PM) observed and drew much smaller vessels emerging from the disk and running temporally, directly towards the fovea. Every subject drew large superior and inferior temporal vessels traversing above and below the macula. Some subjects were able to identify a pair of these vessels (Fig. 1C and D) indicating that both arteries and veins are visible.

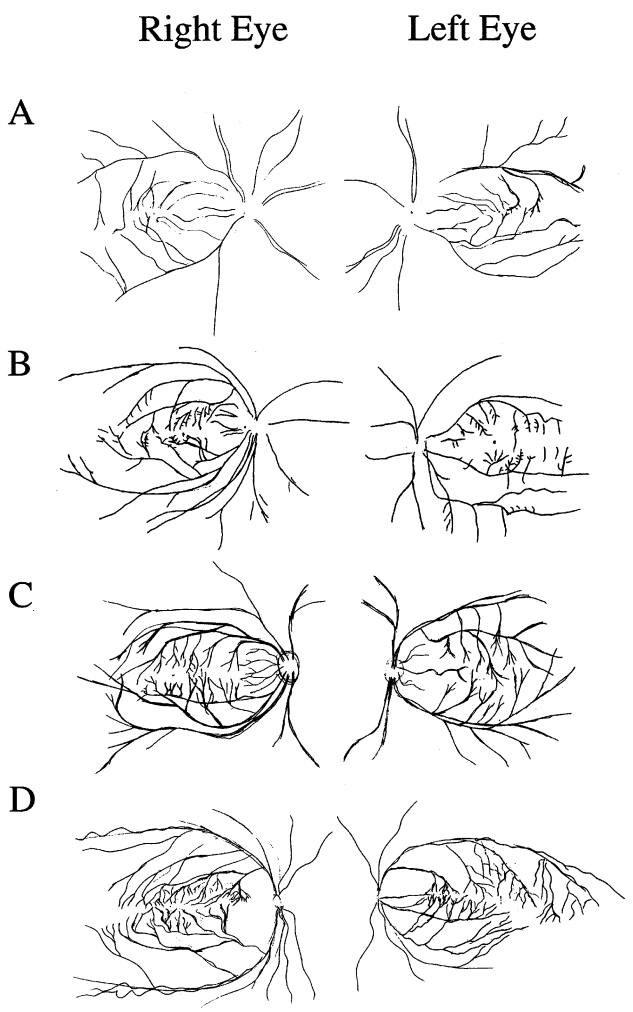

Fig. 1. Four pairs (RE and LE) of digitized entoptic drawings made with the Trans-scleral (TS) technique from subjects AB (A), HZ (B), PM (C) and SM (D). The images are shown as seen and drawn by the subjects, that is, the left side of the retina projects to the right side of the field and hence the right side of the drawing. The top of each drawing represents the inferior retina. The right and left eye drawings are arranged to conform with the clinician's view of the eyes. Subject PM (panel C) has drawn circles representing the edge of the optic nerve head reflecting his prior knowledge of ocular anatomy since there is no circular opacity at this location.

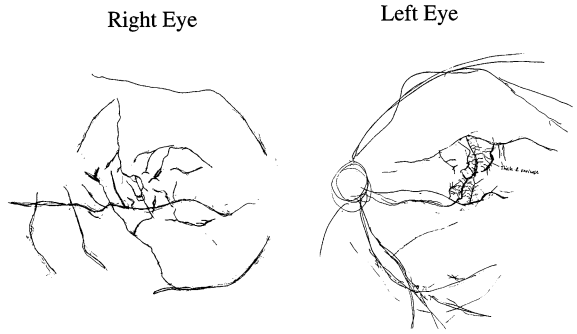

Fig. 2. Digitized right and left eye entoptic drawings made by subjects LT with the TS entoptic method. Tracing instead of free-hand drawing was used by this subject (see Methods for details).

The drawings of every tested eye showed a decrease in the number and branching of vessels with increasing distance from the central fovea, and few if any vessels were visible beyond about 20–25° from the fovea.

The drawings of subject LT (Fig. 2) who used the tracing method show a similar vascular pattern to those seen in Fig. 1. In addition to the large superior and inferior temporal vessels, there are large horizontal vessels drawn slightly above (left eye) and below (both eyes) the fovea. This subject was able to see an FAZ in the left eye, and this can be seen in as a small diamond shape in the left eye drawing temporal to the disk.

4.1.2. Trans-pupil method

Within the 7.7 degree central field of the TP device there are typically no large arteries or veins, and therefore, this technique provides an entoptic view of smaller retinal vessels. Similar to a prior report [23], several but not all subjects in our study reported seeing an approximately circular or elliptical FAZ surrounding the point of fixation (Fig. 3). In addition to the capillary arcade forming the FAZ, subjects report seeing numerous vessels radiating from the FAZ and several examples of the drawings can be seen in Figs. 3 and 4.

Eight sample drawings obtained with the TP entoptic technique are shown in Fig. 4 from the right and left eyes of four subjects (HZ, PM, SM and AB) who report atypical foveola vessel patterns. Panels A and B show two subjects (HZ and PM) with an FAZ in both eyes, but instead of having a simple elliptical shape [24], the FAZ boundary is highly vesiculated. The two subjects (SM and AB) shown in panels C and D have atypical foveola vessels. Subject SM (panel C) has an identifiable FAZ that clearly has one (left eye) or two (right eye) vessels traversing it. Subject AB (Fig. 4, panel D) has no identifiable FAZ.

Unlike subject SM, who drew a fine meshwork of vessels surrounding the central fovea, subject AB was unable to identify and draw the smaller individual vessels surrounding the foveola. However, as reported by other studies [30,31] subject AB saw a fine mesh of

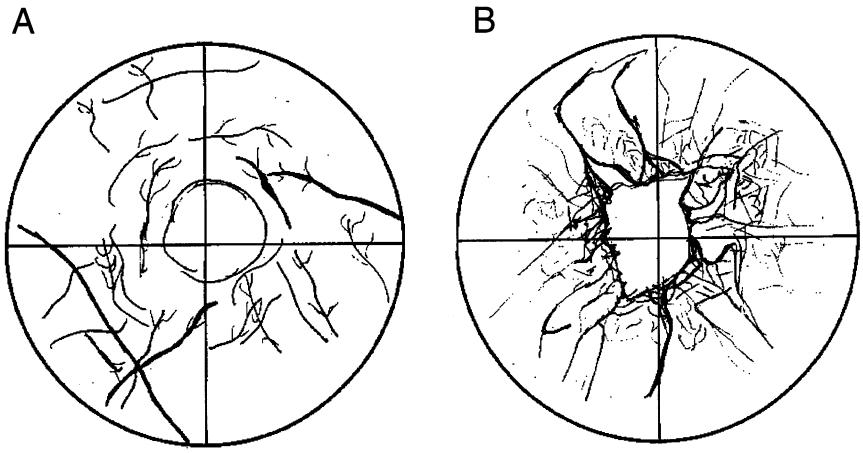

Fig. 3. Digitized entoptic drawings from the RE of two subjects (JH and LL) made with the trans-pupil (TP) method. This method illuminates a 7.7-degree circular field of view. Subjects either made their drawings with the aid of a circle and cross-hairs to help hold fixation and indicate the fixation point, with the aid of a simple circle (Fig. 4A) or with no aid (Fig. 4B).

vessels surrounding the central foveola between the larger venules and arterioles. That is, although the drawings of AB and SM seem to reflect an opposite trend in terms of number of vessels surrounding the foveola, both subjects verbally report the same trend: vessel density seems to increase just beyond the foveola. Most subjects report seeing a fine mesh of vessels outside of the foveola center in which individual vessels could not be located and drawn accurately, but the mesh was clearly visible.

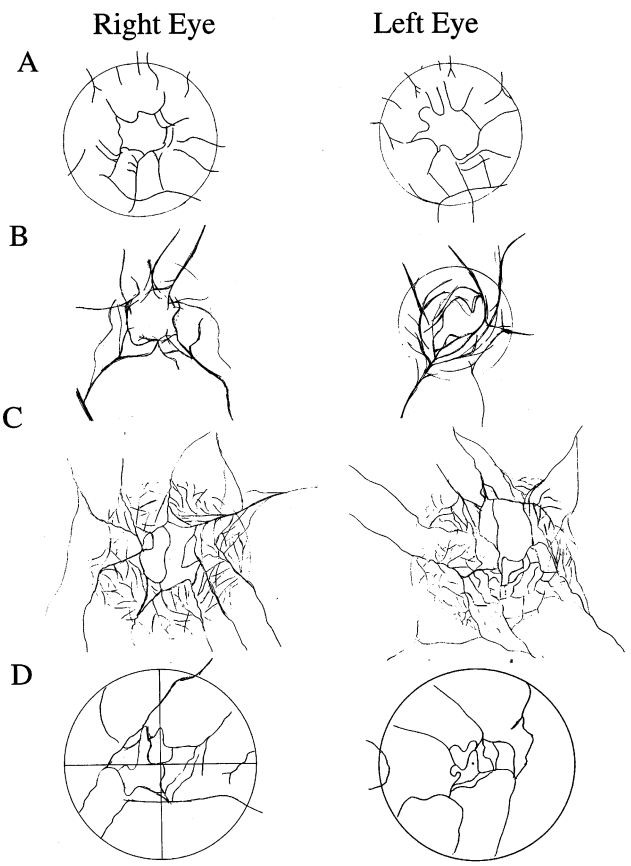

Fig. 4. Right and left eye drawings made with the TP method. Two subjects (HZ (A) and AB (D)) used a circle as a drawing aid so that they could scale their drawing to the Maxwellian-view field, but the other two subjects (PM (B) and SM (C)) made their drawings without the circle. Subject AB used cross-hairs to aid fixation with the right eye.

4.2. Comparison of entoptic and photographic images

In Fig. 5, we present an example of an original RE drawing (Fig. 5A) and its high contrast enhancement (Fig. 5C) is shown along with an original photographic image (Fig. 5B) and its high contrast enhancement (Fig. 5D). It is the enhanced images (Fig. 5C and D) that we overlay and compare (Fig. 6A).

The TS superposition of the traced fundus photographs (white) and the traced entoptic drawings (black) are shown in Fig. 6. There are obvious similarities between the subjective (entoptic) and objective (photographic) images, and in every case there are numerous vascular features in the photographic image that are clearly seen entoptically. For example, in every case, the general shape of the entoptic patterns match the corresponding photographic images. Vessel branching in the large superior and inferior temporal arteries and veins are often seen entoptically, e.g. the two large branches in the superior retina of the left eye of the subject AB (Fig. 6A), and the crossing over and branching of the superior and inferior temporal arteries and veins in the left eye of the subject LT (Fig. 6B). Also, large vessels traversing the macula are always observed, e.g. the right and left eyes of LT (Fig. 6B), and the left eyes of the subjects SM, HZ and PM (Fig. 6C, D and E, respectively). The large secondary vessels approaching and radiating from the central macula are quite accurately represented in the entoptic drawings of all sub-

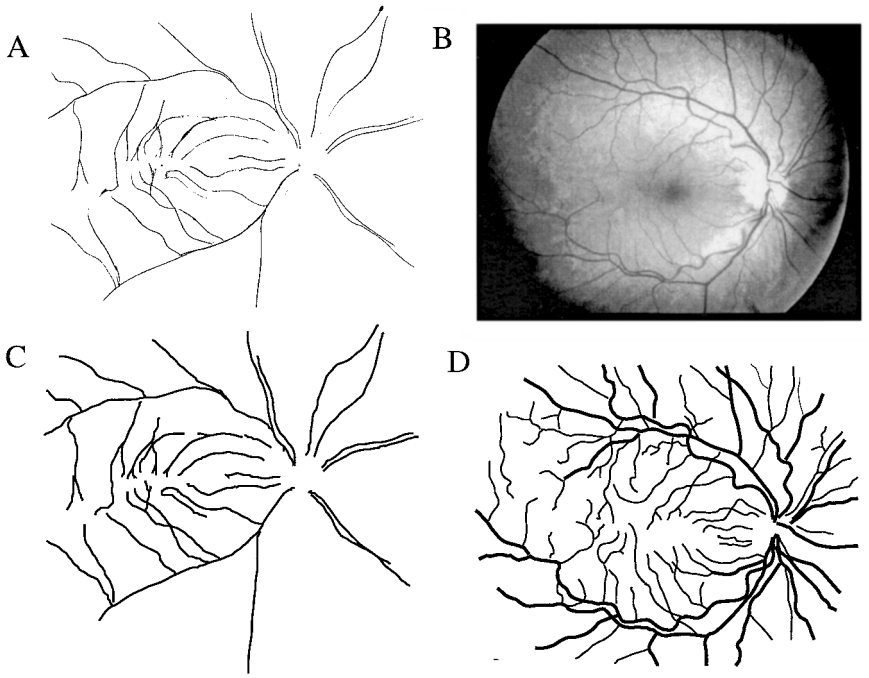

Fig. 5. Examples of the process by which original entoptic (A) and photographic (B) images were converted to digitized high contrast entoptic (C) and photographic (D) images from the RE of subject AB. It is important to note that, as explained in the text, the entoptic images have been top–bottom inverted to conform with the typical photographic view of the retina.

jects. Careful scrutiny of these superimposed images (Fig. 6) shows that major vessels within the central 30–50° that are visible in the standard fundus photograph of the retina are clearly seen entoptically. Although not typically drawn in the correct location, most major vascular branches and bends are recorded in the drawings. Not surprisingly, many of the smaller tertiary branches seen entoptically in the macula are not visible on the fundus photographs which characteristically lack any vascular details in the fovea. Conversely, many of the branches in the peripheral retina recorded photographically are not drawn entoptically.

Traced and scanned drawings made with the TP entoptic method are superimposed onto the traced and scanned FAs in Figs. 7 and 8. The entoptic drawings were scaled, rotated and translated to achieve maximum correspondence in the central fovea where subjects report the most confidence in their drawings. Data from the REs of two individuals (JH and LL) with typical FAZs are shown in Fig. 7. Several features of these two figures are of interest. First, the FAs from these two eyes, although taken by the same equipment and operator, provide very different levels of foveal vascular detail. The FA shown in the left panel clearly shows the capillary arcade forming the FAZ and numerous smaller capillaries running tangentially to the FAZ, but the FA of subject (LL) shown in the right hand panel lacks any capillary detail, including the FAZ, but it does include the larger radially organized vessels.

Several features seen entoptically can easily be identified in the angiographic images. For example, the large tangentially oriented vessel in the superior-temporal fovea and the shape of the FAZ correspond well in both images in the left panel of Fig. 7. In the right panel of Fig. 7, the two larger radially oriented vessels that emerge traveling inferior-temporally but change orientation by 90° to inferior-nasal are easily seen in both images. There are some similarities between the entoptic and angiographic image in the left panel, both showing radial and tangential vessels, but a precise match-up is not seen. The same comparison cannot be made in the right panel because the angiographic image lacks sufficient detail. However, most of the radially-oriented vessels seen angiographically have corresponding vessels in the entoptic image.

The entoptic images from two subjects with atypical or absent FAZs are shown in Fig. 8. Fig. 8A shows data from the left and right eyes of subject AB who reports seeing no FAZ surrounding the fixation point. Several striking similarities between the entoptic (black) and the FA (white) images can be readily observed. In the central fovea, where the capillaries are only marginally visible in the original FAs, almost every twist and turn visible in the FA is matched almost perfectly in the entoptic image. Larger radially-oriented vessels easily seen in the FAs correspond almost perfectly with the vessels seen entoptically radiating from the central fovea. However, as was seen in the TS method, these extra-foveola vessels are often drawn in slightly differ-

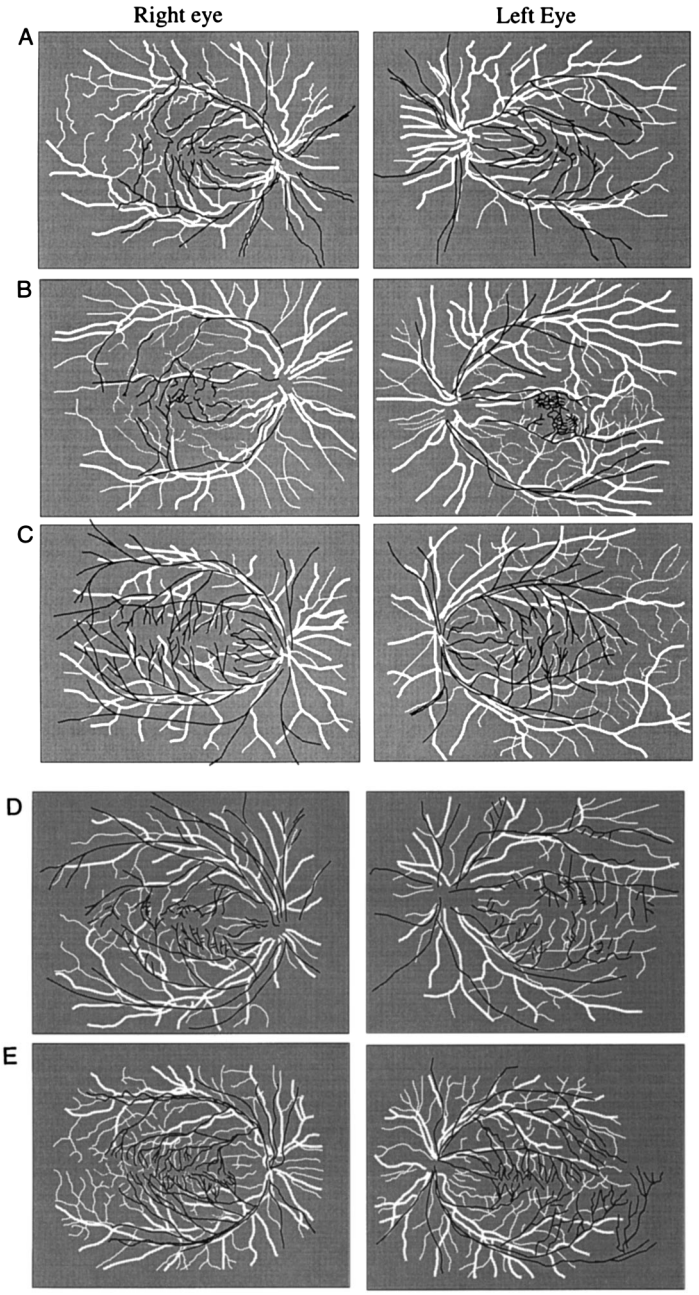

Fig. 6. Superimposed entoptic images (TS method) with photographic images for the right and left eyes of five subjects (AB, LT, PM, HZ and SM, in A, B, C, D and E, respectively). The white lines are the high contrast photographic images and the black lines are the high contrast entoptic images, which have been top–bottom inverted to conform to the clinician's view represented in the photographic images.

ent locations. Outside of the central fovea, no individual capillaries are visible in the FAs or within the entoptic image of this subject.

An atypical FAZ was also seen in the right eye of subject LT (Fig. 8B). Instead of the standard symmetric FAZ (as seen in the left eye of this subject), a small central vertical capillary runs through the fixation point. The resulting central foveola capillary pattern is asymmetric and can be seen in both FA and entoptic images. In the right eye, the subject sees three major vessels supplying the central foveola, one superior, one inferior-nasal and one inferior-temporal. Each of these seen entoptically correspond to the three larger vessels seen angiographically. Multiple branchings are seen from all three vessels in both images. The left eye of this subject reveals a typical FAZ with the capillary arcade forming a vertically oriented diamond pattern. Six of the eight major vessels supplying the arcade are seen entoptically.

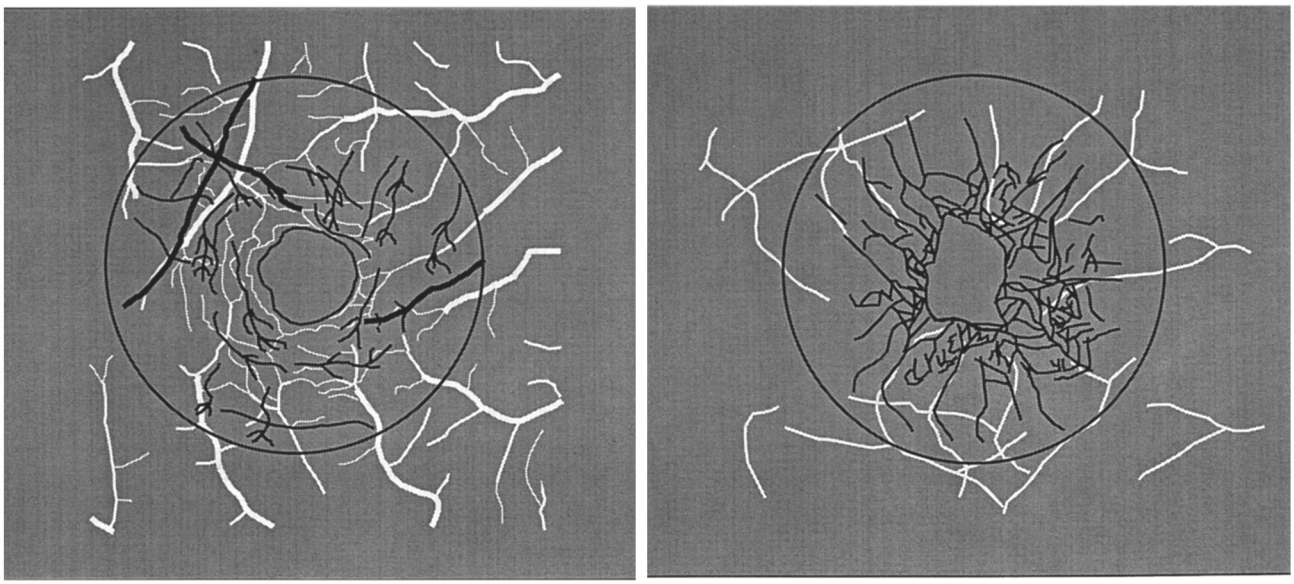

Fig. 7. Super-position of high contrast renditions of the entoptic (TP method) and angiographic foveal images from the right eyes of two subjects (JH: left panel, and LL: right panel). As in Fig. 6, the black and white lines represent the entoptic and angiographic images and the entoptic images have been top–bottom inverted.

4.3. Quantification of the entoptic image

A common trend can be seen in all of the data presented in Figs. 1–8: the very smallest capillaries can be seen entoptically at the center of the fovea, but with increasing eccentricity, only larger and larger vessels can be seen. This pattern was observed in the data from every eye that was tested, and the following section describes this trend quantitatively.

Once the super-position data analysis was completed, we identified the most striking examples of vessels seen in both the photographic and entoptic images. We then examined the location and width of these vessels in the digitized photographic images. Using a model eye to scale the photographic images (see Methods), we were able to plot the vessel width as a function of eccentricity, and data from two eyes are shown in Fig. 9. The filled circles represent vessels seen entoptically with the TS method, and open squares identify vessels seen with the TP method. The pattern of results is virtually identical for these two subjects. The largest vessels are seen at eccentricities between 15 and 20° with diameters between 150 and 200 microns. These entoptic data correspond well with the histological data showing large arteries and veins around the disk of about the same diameter [2]. The smallest vessels are seen entoptically in the central foveola at eccentricities of 0–1°. The measured diameter of these capillaries was between 15 and 20 microns, which is larger than expected from histological data [2] and probably reflects optical blurring in the photographic images.

In addition to quantifying the diameter of entoptically visible vessels, we were interested in the subjects' ability to localize entoptically visible features. After careful scrutiny of the super-position data, and with the cooperation of the subjects, we identified a series of vascular features that were visible entopti-

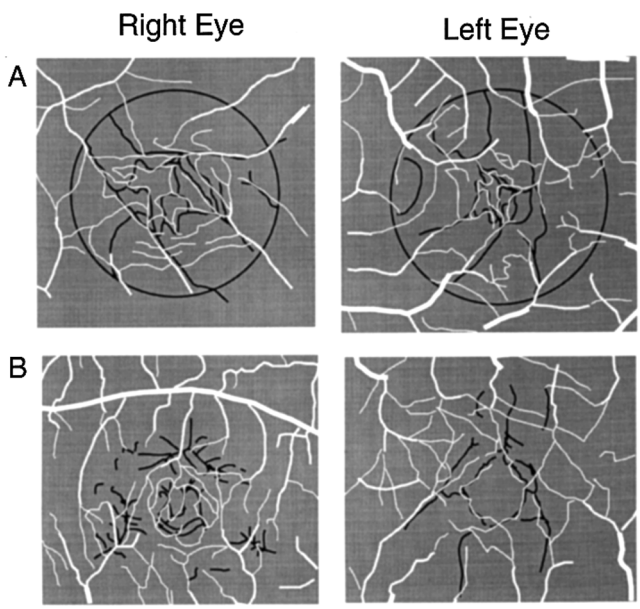

Fig. 8. Super-position of TP entoptic images with the contrast enhanced angiographic images of the right and left eyes of subjects AB (A) and LT (B). The white lines are the high contrast photographic images and the black lines are the high contrast entoptic images, which have been top–bottom inverted to conform to the clinician's view represented in the photographic images.

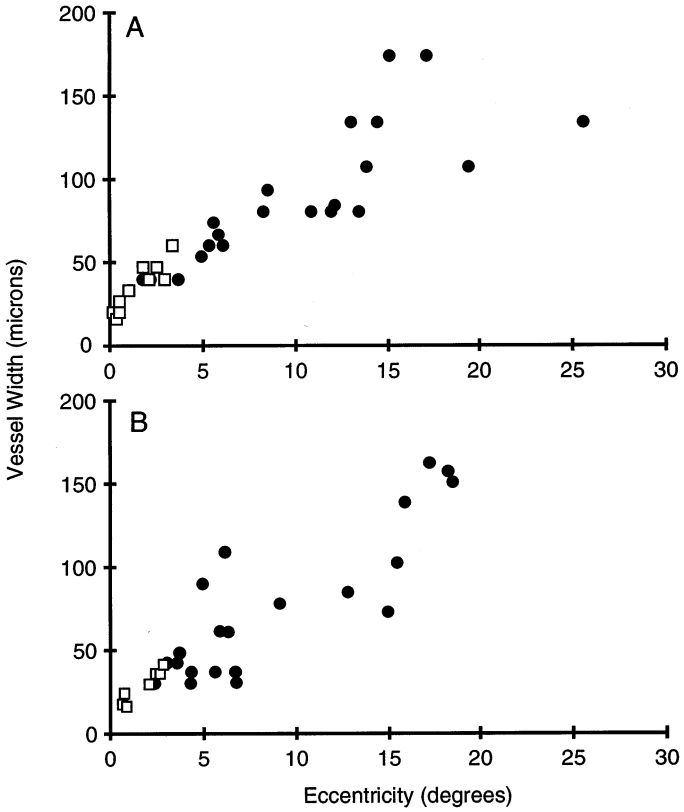

Fig. 9. Measured width in microns of entoptically visible vessels as a function of eccentricity in degrees from the foveal center for the left eye of subject AB (A) and the right eye of subject LT (B).

cally and clearly identifiable in the photographic images. Once a series of such features were identified, we used the LED wand to entoptically mark the location of each feature using the TS method. A sample data set is shown in Fig. 10A. The circle represents a photographic negative from the angiogram of the central 10 degree field of the left eye of subject AB. The 11 arrows point to the series of features that were visible entoptically. All but one (the bend in the vessel in the inferior nasal retina) were vascular branchings. The 11 points in the LED tracing after scaling are shown as white circles. Although not perfect, the relative locations of the entoptically seen features were quite accurate.

The accuracy was evaluated by measuring the eccentricity in Cartesian coordinates (x and y) for each feature in the photographic and entoptic image. These two coordinates are plotted as a function of each other in Fig. 10B from the right eyes of three subjects (AB, LT and HZ). The accuracy of the entoptic localization is reflected by the high correlation between entoptic and photographic eccentricity, and by the fact that the best fitting line has a slope of 1 and an intercept of approximately zero. Contrary to our expectations, localization error did not increase dramatically with increasing eccentricity. Error in the fovea was on average about 0.5°, and this increased to about 1° outside of the fovea.

5. Discussion

Although entoptic images of the retinal vasculature have been documented for over 100 years [16], few attempts have been made to quantify and provide a detailed description of the entoptic image [16,17,19,21,24,32,33]. The present study is the first to quantify the entoptic image of normal eyes and compare entoptic and photographic images. A previous study compared entoptic and angiographic images of diabetic retinopathy [29].

In many ways, the entoptic image is elusive. Although it may appear perfectly clear to the subject, only the subject can see it. For some of our more naive observers, this concept was difficult to appreciate. If they could see it so clearly, surely we could also. This very personal nature of the entoptic image has made it difficult to study. The most successful previous attempt to quantify entoptic images employed a computer simulation of the blue field entoptoscopic image, the flying corpuscles [26] in which the subjects matched the entoptic image with a computer simulated blue field entoptoscopic image in order to examine the dynamic nature of the flying corpuscles. Other studies have had subjects trace their FAZ [23,24] or match the FAZ to a circle of a variable size [28,33]. Also, one study had subjects

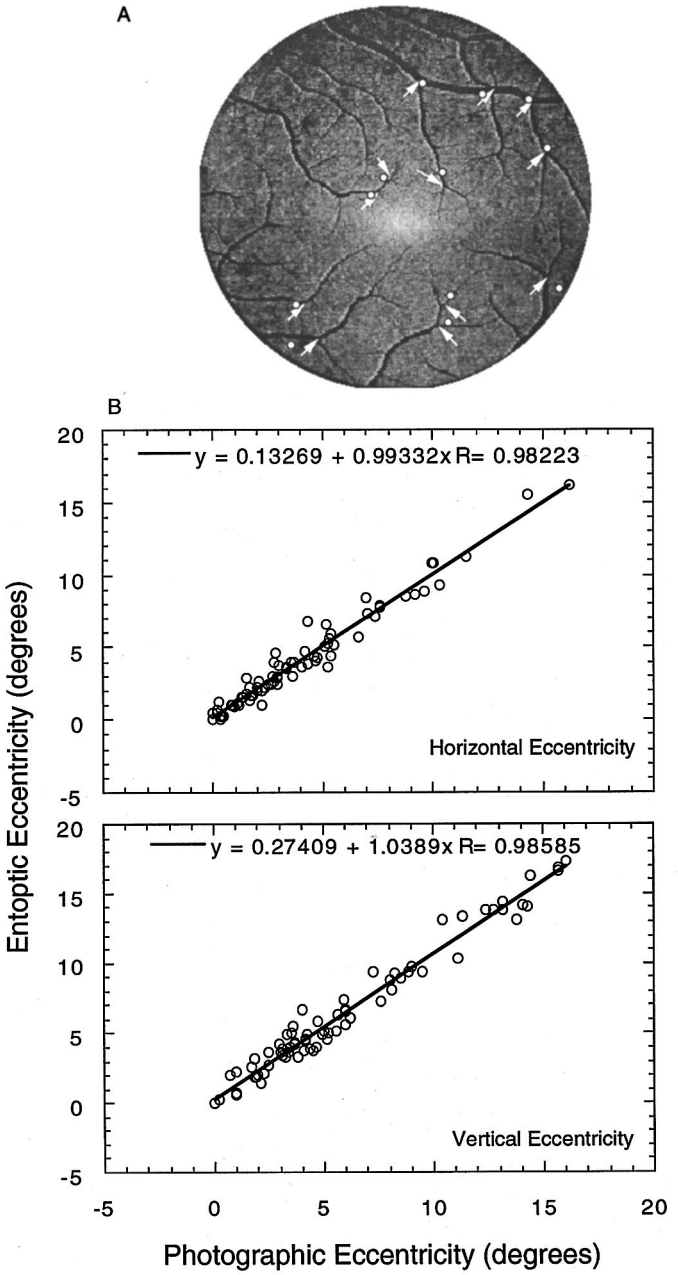

Fig. 10. Data from the feature location experiment. (A) Shows an example of the features selected that were visible in the entoptic and angiographic or photographic images. White arrows identify the locations of the features in this angiographic image, and the white circles show the entoptically determined locations. The angular eccentricities relative to the foveola center of each feature (arrows and circles) were determined for six eyes (right and left eyes of subjects AB, LT and HZ) and plotted as a function of each other in (B). Horizontal and vertical eccentricity in degrees of the entoptically located feature is plotted as a function of eccentricity of the photographically located feature. The lines are represent the best fitting linear regression, and the equations of each line are given.

match the entoptically visible capillary mesh seen parafoveally to simulated meshes of variable density [31].

The present study had three intertwined goals: (i) to compare two different methods for entoptic visualization (TS vs. TP); (ii) to compare the entoptic images to standard clinical photographic images; and (iii) to quantify the entoptic images. As predicted [22], the TP method provided a highly detailed shadow pattern sufficient to see capillary details in the central fovea of every subject tested (Figs. 3 and 4). Whereas, the TS method provided a large field of view but did not consistently provide a view of the foveal capillary detail. Although our particular Maxwellian-view instrumentation only illuminated a 7.7-degree area of the retina, a Maxwellian view system with a considerably

larger (20–30°) would be easy to construct. The TS method, probably due to the optical scattering of the beam as it passed through the sclera, illuminates between 30 and 50 degree area of the eye's posterior pole.

There are some interesting methodological limitations faced by the TP and TS entoptic methods. First, the TP method can readily take advantage of the wavelength-specific absorption of hemoglobin, and create higher contrast shadows by using short wavelength light [22]. The short wavelength approach cannot easily be employed by the TS method. Unlike the TP method, light illuminating the posterior pole in the TS method must first pass through the sclera, choroid and retina. Since the choroid is full of blood and the pigment epithelium has melanin, sufficient short wavelengths may not reach the posterior pole of the eye. Therefore, in addition to the optical scattering of the sclera, the short wavelength absorption by the choroid may limit the visibility provided by the TS method.

In spite of the differences between the entoptic and photographic images compared in Fig. 6, we are struck by their similarity and conclude that the TS method provides an entoptic image of the central 40 or 50° of similar spatial detail to that see in color photography. The comparison between the TP entoptic method and the angiographic images shown in Figs. 7 and 8 suggests that these two methods are similar in their ability to image the foveal vessels. We suspect that the very best angiograms such as those shown in the following references [3–8,10,11] will probably provide a more detailed but imperfect [11] view of the foveal capillaries. However, our study confirms the suggestion made by Bird and Weale [17], that typical quality angiograms will likely provide an image of the foveal vessels which is inferior to that provided by the entoptic method.

We speculated in the introduction that entoptic visualization will fundamentally be limited by the decline in neural resolution of the retina with increasing retinal eccentricity. The correlation between visible vessel width and eccentricity (Fig. 9) illustrates a high degree of correlation between the eccentricity and width of the entoptically visible vessels. The data are well fit by a simple linear function having a slope of 7.5 microns per degree. Increasing eccentricity from 0 to 20° produced an increase in the visible vessel width of about a factor of 8, which is similar to the increase in MAR from the fovea to 20° [34]. The upper bound of the data shown in Fig. 9 represents the largest vessels at a given eccentricity. An interesting detail of the data in Fig. 9B is the two data points indicating vessels of 100 micron diameter only 5° from the foveola. This subject (LT) is one of the few who had such large vessels traversing the macula (Fig. 2 and Fig. 6B). Although the entoptic image shows a clear linear relationship, this does not exist in the vessel pattern since small capillaries are present throughout the retina [2,11,35].

The quantitative localization data shown in Fig. 10 suggests that, as long as the vascular detail is visible, it can be located with considerable accuracy. Interestingly, the failure to observe a large increase in localization error with increasing eccentricity seems to contradict studies of vernier acuity which show that localization accuracy declines rapidly with increasing eccentricity [36,37]. This contradiction suggests that some other, retinal locus-independent variable is the major source of these localization errors. Eye movements, which would move all points in the entoptic image equally with respect to the non-entoptic tracing, are a likely candidate to explain this discrepancy.

Many of the subjects (AB, LT, HZ, JH, PM and NS) used in this study were familiar with entoptic imagery and retinal vascular patterns. These knowledgeable subjects, plus the other two subjects (SM, LL) were all highly motivated to make a special effort to capture these entoptic images. It is not clear how important this prior knowledge was in determining the success of this method. This question becomes significant when applying entoptic viewing to the study of clinical problems. It is our experience with normals that, with the TP method, virtually everyone can see their foveal vascular pattern including the capillary arcade forming the FAZ, an informal count from our two labs (AB and RA) suggests that less than ten normals in about 500 could not see their foveal vascular patterns with the TP method. Consistent with our informal observation, a study of patients using the TS method [38] found that virtually all patients, even those with cataracts and retinal pathology, could see their vascular pattern. If these numbers are correct, we might expect that a large number of patients with retinal/vascular defects might be able to entoptically visualize, draw and localize these defects within their own retinal vascular pattern as demonstrated by Kluxen and Wilden [18] with the TS method. Using the same TP entoptic method described in the present study, Applegate et al. found the sensitivity and specificity with which untrained diabetics detect their own parafoveal area defects are 51 and 82%, respectively [29].

Acknowledgements

We are particularly indebted to our subjects who worked hard to draw what they could only see in the peripheral field. This research effort was supported in part by Grant EY10097 and Supplement EY10097-A1S1 to RAA from the National Eye Institute, National Institutes of Health, Bethesda, MD; a grant from the San Antonio (TX) Area Foundation to R.A. Applegate; and an unrestricted research grant to the Department of Ophthalmology, The University of Texas Health Science Center at San Antonio from Research to Prevent Blindness, Inc., NY.

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