Quantification of Vessel Density in Retinal Optical Coherence Tomography Angiography Images Using Local Fractal Dimension

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Quantification of vessel density in retinal optical coherence tomography angiography images using local fractal dimension

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ABSTRACT

Purpose: To evaluate a fully automated local fractal dimension method to quantify vessel density and foveal avascular zone (FAZ) area in optical coherence tomography angiography (OCTA) images

Methods: 52 Asian Indian normal eyes underwent imaging prospectively with OCTA system (Optovue Inc., Fremont, USA). Superficial and deep retinal vascular plexus was imaged. Local fractal analysis was applied to the OCTA images. A scan area of 3 mm × 3 mm was selected in the superficial and deep retinal layers. FAZ area and vessel density were quantified in circular and sectoral zones around the fovea. A unique contour map of vessel density and dropout zones was developed to perform regional comparisons.

Results: FAZ of superficial (0.35±0.013 mm²) and deep (0.49±0.012 mm²) retinal vascular plexus was segmented. The agreement between the manually segmented and local fractal dimension segmented FAZ area was 0.97 (95% CI: 0.94-0.98) and did not change significantly with age (p=0.94 and 0.21, respectively). The vessel density was greater in the deep than the superficial retinal vascular plexus (p<0.0001). When the image was subdivided into sectors around the FAZ, inferior sector had greater vessel density than the others (temporal, superior, and nasal) in both superficial and deep retinal vascular plexus (p<0.05). These observations were similar to recent studies on animal retinal vasculature map.

Conclusions: A novel implementation of local fractal dimension to calculate vessel density and FAZ area was demonstrated. Age did not impact vessel density but sectoral analyses showed greater vessel density in the inferior zone.
Introduction

Diagnosis and treatment of diseases that involve vessel abnormalities may require detailed analysis of the retinal vasculature to understand its role in disease pathophysiology. Fundus fluorescein angiography (FFA) and indocyanine green angiography (ICGA) are both invasive techniques that require intravenous injection of dye to obtain two dimensional high contrast images of the retinal circulation. Confocal scanning laser ophthalmoscopy was developed to provide depth resolved images of the retina but the depth resolution is inadequate to delineate the individual retinal layers and capillaries. Optical coherence tomography angiography (OCTA) is a recent, non-invasive and dye less imaging technique for evaluating the vessels by capturing the dynamic motion of the erythrocytes. It has been shown to be a useful imaging modality for evaluation of ophthalmologic diseases such as age related macular degeneration, diabetic retinopathy, arteriovenous occlusions and glaucoma.

A few previous studies on OCTA have been qualitative in nature. There have been quantitative studies on the microstructure and microvasculature organization in the human retina using confocal scanning laser microscopy. However, more quantitative studies on OCTA are needed. Therefore, this study was aimed at automated segmentation and quantification of the foveal avascular zone (FAZ) and the vessel density in the superficial and deep retinal vascular plexuses in a normal population of Asian Indian eyes. This study used local fractal analysis, which quantified the complexity of the vascular network. The method was refined further by using local fractal dimension, which is a technique used to identify local or regional variations in the complexity of an image and was used for evaluating the vessel distribution in the OCTA scans. Statistical analysis was performed to assess the distribution of FAZ area and
vessel density across different age groups in superficial and deep retinal vascular plexus of normal subjects.

**Methods**

**Study population**

This study was approved by the institutional ethics committee of Narayana Nethralaya Multi-specialty eye Hospital, Bangalore, India. The research followed the tenets of the Declaration of Helsinki. Written informed consent was obtained from all the subjects before imaging. 52 eyes of normal Asian Indian subjects were included in this study. The subjects were between 20 to 67 years of age. Subjects after normal ocular examination with 6/6, N6 vision and refractive error range -1D to +1D were included in the study. Subjects with systemic history of diabetes, hypertension and other vascular pathologies, refractive error beyond the specified limits, pathological ocular conditions including glaucoma, inflammatory conditions, history of ocular trauma and prior ocular surgery were excluded from the study.

**Study Design**

All subjects underwent imaging on AngioVue OCTA system (Optovue Inc., Fremont, CA) by a single operator using the AngioVue software of the RTVue XR Avanti Spectral Domain OCT (SD-OCT) (Optovue Inc., Fremont, CA). The device has a high acquisition speed of 70,000 A-scans per second. The scan area was 3 mm x 3 mm for all the subjects. Analyses were performed on the OCTA images generated from the superficial and deep retinal vascular plexuses.
Study Endpoints

OCTA scans of superficial and deep retinal vascular plexuses have a dense vascular network and local fractal dimensions$^{17,18}$ were calculated to indicate presence of vessels. Box counting method was used to calculate the fractal dimension given by eq. (1):

$$\text{FractalDimension} = \frac{\log(N_s)}{\log(s)}$$ .................................................................(1)

In eq. (1), $N_s$ was the number of boxes of magnification (s) needed to enclose the structure. From eq. (1), a moving window of size $(2w+1) \times (2w-1)$ was used to calculate the local fractal dimension of the OCTA scans using the following equation$^{18}$:

$$R(i, j) = \text{LocalFractalDimension}[I(i + k, j + k); -w < k < w] .............(2)$$

In eq. (2), $I$ was the original image and $R$ was the image formed after replacing the value of centre pixel of each window with the fractal dimension of the window.$^{18}$ Window sizes (in pixels) of $5 \times 5, 7 \times 7, 9 \times 9, 11 \times 11$ and $13 \times 13$ were used to calculate the local fractal dimension. Then FAZ was segmented by first binarizing the image followed by connected component labeling (MathWorks Inc., Natick, MA). The area of FAZ was calculated from the binary image.

Each pixel in the image had a local fractal dimension number, which was calculated using the moving window as described in eq. (2). The fractal dimension value varied with the distribution of vessels around the pixel in the image. Thus, the local fractal dimension of a pixel present in a larger vessel was higher as compared to that of a pixel present in a smaller vessel or in a non-vessel region of the image. The ratio of local fractal dimension of each pixel in an OCTA image to its' maximum fractal dimension was calculated. This normalized ratio was then used to plot a contour map. The contour map of the normalized ratio essentially provided a
pictorial representation of an apparent probability index of presence of vessel of certain size at each pixel, with an index closer to 1 indicating large vessels and an index closer to 0 indicating non-vessel regions. Figures 1A and 1C show the OCTA image of the superficial and deep retinal vascular plexus of an eye. Figures 1B and 1D show their respective contour maps of the normalized ratio of local fractal dimension at a pixel to maximum value of local fractal dimension in the same image. By visual examination, the small vessels and capillaries were observed to lie between a ratio value of 0.7 and 0.9. The non-vascular regions were observed to lie between a ratio value of 0.0 and 0.3. The smaller gaps between vessels were observed to lie between a ratio value of 0.3 and 0.7. The larger vessels had the highest magnitude of local fractal dimension and thus, the corresponding pixels within the larger vessels had a normalized ratio close to or equal to 1. Figure 2 shows the contour map of OCTA image of the superficial retinal vascular plexus with the marked FAZ, examples of large and small vessels, along with the gaps between the vessels.

Then vessel density was expressed in percentage by taking the ratio of the total vessel area (all pixels with a ratio value between 0.7 and 1.0) to the total area of analyzed region (size of the image in pixels). Two forms of analyzed regions were used. First vessel density was calculated in three circular regions after excluding the FAZ area from each region viz. C1 (diameter = 1.5 mm), C2 (diameter = 2 mm) and C3 (diameter = 2.5 mm). Figure 3A shows the OCTA image of a superficial retinal vascular plexus and a schematic representation of the three circular region C1, C2 and C3. Vessel density was also calculated in the parafoveal sectors after excluding the FAZ area from the sectors viz. temporal (T), superior (S), nasal (N) and inferior (I) sector of a circular zone of diameter 2.5 mm only. Figure 3B shows the OCTA image of a
superficial retinal vascular plexus and the schematic four parafoveal sectors (T, S, N, I). All the above methods were implemented using MATLAB v7.10 (Mathworks Inc., Massachusetts, USA).

Statistical Analysis

All analyzed variables were reported as mean ± standard error of the mean after confirming normality of distribution with the Kolmogorov-Smirnov test. The analyzed variables were FAZ area (mm²) and vessel density (%). The agreement between the manual and automated segmentation of FAZ areas for different window sizes was assessed using intra-class correlation coefficient (ICC). Manual segmentation of the FAZ was performed by a single observer using ImageJ (v1.49, National Institute of Health, USA). The subjects were divided into three age groups: Group A=20 -30 years, Group B=31-45 years and Group C=46 to 67 years. Repeated measures analysis of variance (rANOVA) was performed among the age groups for each circular zone and sector. A p-value < 0.05 was considered statistically significant. All p-values were 2-sided and Bonferroni corrected. All statistical analyses were performed in MedCalc v15.8 (MedCalc Inc., Ostend, Belgium).

Results

Number of eyes was 17, 17 and 18 in age group A, B and C, respectively. Table 1 shows the intra-class correlation coefficient (ICC) between manually segmented FAZ and segmentation of the FAZ using normalized ratio of local fractal dimension. The window size of 5×5 had the best ICC of 0.97 (95% confidence interval: 0.94 to 0.98). As the window size was increased, ICC decreased in magnitude. Therefore, only a window size of 5×5 was used for further analyses. The
mean area of the FAZ by manual segmentation and segmentation using the normalized ratio in the superficial retinal plexus was 0.36±0.013 mm² and 0.35±0.013 mm², respectively (p<0.0001). Similarly the mean area of FAZ in the deep retinal plexus was 0.49±0.012 mm² and 0.48±0.013 mm², respectively (p<0.0001). The FAZ area in the superficial (p=0.94) and deep retinal vascular (p=0.21) plexus did not change significantly with age of the subject.

Table 2 shows the mean vessel density of the superficial retinal plexus in circular zones (C1, C2, C3) and parafoveal sectors (T,S,N,I) among the age groups. In zones C1, C2 and C3, age did not affect the vessel density (p=0.33, 0.31 and 0.29, respectively). Vessel density in a given age group was similar among the three circular zones (p>0.05). In the parafoveal sectors, the vessel density in the inferior sector was significantly different from the others (p<0.001), e.g., in age group A, mean vessel density in the inferior sector (53.61%) was greater than the same in other sectors (48.75% in temporal, 47.57% in superior and 47.04% in nasal). However, this difference in vessel density between sectors was independent of age (p=0.92). Table 3 shows the mean vessel density of deep retinal vascular plexus in circular zones (C1,C2,C3) and parafoveal sectors (T,S,N,I) among the age groups. In zones C1, C2 and C3, age did not affect the vessel density (p=0.72, 0.29 and 0.54, respectively). Also, vessel density in a given age group was similar in all circular zones (p>0.05). The vessel density in all the parafoveal sectors was unaffected by age (p>0.05). However in a given age group, vessel density in superior and inferior sector were similar (p>0.05) but significantly greater than vessel density in the temporal and nasal sector (p=0.02), e.g., in age group A, mean vessel density in superior (54.04%) and inferior (56.56%) sector was greater than mean vessel density in temporal (50.44%) and nasal sector.
(49.68%). A bar plot of the observations of the parafoveal sectors from table 2 and 3 are plotted in Figure 4A and B, respectively.

Since only sector (T,S,N,I) based analyses achieved statistical analyses independent of age, the proportion of pixels within a range of values of the normalized ratio of local fractal dimension in the superficial and deep (Table 4) retinal vascular plexus of age group A was further analyzed. The proportion of pixels within a range of values of normalized ratio of local fractal dimension was calculated as the ratio of total number of pixels within the range of normalized ratio of local fractal dimension to the total number of pixels in the image. In the superficial vascular plexus (Table 4), the greater vessel density in the inferior sector was accompanied by reduction in the smaller gaps between the vessels in the same sector relative to others, e.g., mean proportion of pixels within the range of 0.3-0.7 was lower in the inferior sector than the others (Inferior vs. Superior: p=0.004; Inferior vs. Temporal: p=0.008; Inferior vs. Nasal: p<0.0001). Similarly (Table 4), reduction in the smaller gaps between vessels was found in the inferior sector of the deep retinal vascular plexus compared to its’ temporal and nasal sector (p=0.02 and 0.001, respectively). The proportion of pixels devoid of any vessels (normalized ratio of local fractal index less than 0.3) remained unchanged (p>0.05) among the sectors in both superficial and deep (1st column of table 4) retinal vascular plexus. From all the tabular data, it was evident that vessel density was always lower in the superficial than in the deep retinal vascular plexus (p<0.0001).
Discussion

In this study, local fractal dimension was used for segmenting and quantifying FAZ area and vessel density. The aim of this study was not just the quantification of retinal parameters but also to demonstrate the application and versatility of local fractal dimension in the analyses of OCT angiography images. Fractal objects show details at an arbitrarily small scale and can be used for capturing the complex and detailed microvascular network on OCT angiograms.\(^{17}\) Because of this complexity, conventional pre-processing techniques related to smoothening or filtering of images were not applied on the OCTA images prior to local fractal dimension calculation as this may have resulted in the loss of smaller vessels and capillaries. Only the motion adjusted (performed by the scanner) OCTA images from the device were analyzed. Fractal analysis has been used previously for measuring the retinal vessel density in fluorescein angiograms and retinal fundus photographs to evaluate the normal and abnormal vascular pattern but the results were inconclusive due to variations in imaging technique and method of fractal analysis used.\(^{19-23}\) Also, the methods calculated the fractal dimension for the entire image or in fixed regions of the image as compared to the method of moving window local fractal dimension used in this study, in order to provide better resolution of the retinal vasculature.

A study on OCTA FAZ area in normal subjects reported a mean superficial FAZ area equal to \(0.358\pm0.084\, \text{mm}^2\) and mean deep FAZ area equal to \(0.584\pm0.15\, \text{mm}\),\(^{26}\) which were comparable to the results of this study. The FAZ area did not vary significantly with age. Vessel density was similar among the circular zones (C1, C2 and C3 in figure 3A). However, the inferior sector had significantly higher vessel density than both temporal and nasal sector with concomitant drop in the gaps between the smaller vessels. This highlights that vessel density
analyses in the superficial and deep retinal vascular plexus needs to be a local rather than an average of the whole image. In recent studies on the retina and choroid, the superior and inferior retinal and choroidal zones were thicker than the nasal and temporal zones. This trend is similar to the superficial and deep retinal vascular plexus vessel density mapping across these zones in this study. This indicated a physiological correlation among vessel density, retina and choroid thickness, which needs to be evaluated further. This trend has also been observed in mice in vivo. The study showed that: (a) vessel density in superficial layer (31.49%) was lower than the deeper layer (45.96%) (b) vessel density was similar among the sectors in the superficial layer but not in the deeper layer. In mice, a small but significant reduction in vessel density was noted in the deep retinal plexus but not in the superficial retinal plexus with age. However, in this study, age did not affect the vessel density in both superficial and deep retinal vascular plexus irrespective of the region (circular or sector) of analyses. In another study on fluorescein angiography of the entire human retina, superior and inferior regions had higher vessel density than nasal temporal, which was similar to the data shown in Figure 4. Further, inferior retina had higher vessel density than the temporal retina by ~4%. In this study, the number was ~9.9% in both the superficial and deep retinal vascular plexus.

A study on capillary network in the perifovea has demonstrated variation in the capillary density and loop area in different retinal layers, with the deeper layers having larger capillary density and smaller loop area as compared to the superficial layer. These findings were similar to the findings of the current study, where an increase in vessel density and decrease in smaller vessel gaps were shown in the deep retinal vascular plexus as compared to the superficial retinal vascular plexus. Metabolic demands within the retina are heterogeneous and are met by
the capillary network, which essentially function to increase the absorption of nutrients and removal of waste. Therefore, it was speculated that the distinct metabolic demands of the superficial and deep retinal vascular plexus may be a reason for the difference in the vessel density in these two layers. Similar findings have also been reported in the brain where the microcirculation in the human cerebral cortex is adapted according to the neuronal demands. The higher vessel density in the deep retinal vascular plexus may also be attributed to the apparent decrease in the presence of large vessels and relatively denser presence of smaller vessels compared to the superficial layer, which in turn compensates for the lower flow per vessel in the deep retinal vascular plexus.

OCTA has a potentially wide applicability in retinal vascular diseases and a study showed that OCT angiograms were similar to fluorescein angiography images in showing important vascular detail. Currently, fluorescein angiography is the gold standard for retinal imaging but it may miss capillary microvasculature information, which may be measured by OCTA due to better resolution. A study also described the characteristics, sensitivity and specificity of detection of choroidal neovascularization on OCTA, which highlighted the versatility of OCTA in retinal and choroid disease diagnosis. Local fractal analysis can be used to detect such abnormalities in the superficial and deep retinal plexuses as well as in the outer retina and choroid layers. At present, use of OCTA is limited by small field of view unlike fluorescein angiography. However, it provided improved visualization of the radial peripapillary and deep capillary networks, which were not well distinguished in fluorescein angiography in normal eyes. In summary, this study described a novel method for automatic segmentation and quantification of FAZ area and vessel density in superficial and deep retinal vascular plexuses of
normal eyes. Age did not have a significant effect on the FAZ area and vessel density but local analyses of vessel density could be important for evaluation of longitudinal data based on OCTA images of retinal disorders.
Reference


Figure legends

Figure 1: (Top left) Optical coherence tomography angiography image of the superficial retinal vascular plexus of size 3mm × 3mm. (Top Right) Corresponding contour map created with normalized value of local fractal dimension values. (Bottom Left) Optical coherence tomography angiography image of the deep retinal vascular plexus of size 3mm × 3mm. (Bottom Right) Corresponding contour map created with normalized value of local fractal dimension.

Figure 2: Contour map created from optical coherence tomography angiography of superficial retinal vascular plexus with marked FAZ, examples of large and small vessels and vessel gaps.

Figure 3: (Left figure) Analyses of optical coherence tomography angiography of superficial retinal vascular plexus in three circular zones (C1,C2,C3), where d is the diameter. (Right Figure) Analyses of optical coherence tomography angiography of superficial retinal vascular plexus in four parafoveal sectors: Temporal (T), Superior (S), Nasal (N), Inferior (I).

Figure 4: (Top Figure) Mean vessel density in temporal (T), superior (S), nasal (N) and inferior (I) sectors of the superficial retinal vascular plexus in age group A (20-30 years of age). (Bottom Figure) Mean vessel density in temporal (T), superior (S), nasal (N) and inferior (I) sectors of the deep retinal vascular plexus. Error bars show the standard error of the mean. Symbols indicate statistically significant difference (p<0.05) between the means.
C1: \(d=1.5\) mm

C2: \(d=2\) mm

C3: \(d=2.5\) mm

S

N

I

I
Table 1: Intra-class correlation coefficient to assess agreement between foveal avascular area calculated by manual segmentation and foveal avascular area calculated by segmentation with the normalized ratio of local fractal dimension. CI is the confidence interval of the coefficient.

<table>
<thead>
<tr>
<th>Window size (in pixels)</th>
<th>Intra-class correlation coefficient</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 × 5</td>
<td>0.97</td>
<td>0.94 to 0.98</td>
</tr>
<tr>
<td>7 × 7</td>
<td>0.75</td>
<td>0.56 to 0.85</td>
</tr>
<tr>
<td>9 × 9</td>
<td>0.55</td>
<td>0.24 to 0.74</td>
</tr>
<tr>
<td>11 × 11</td>
<td>0.33</td>
<td>-0.15 to 0.61</td>
</tr>
<tr>
<td>13 × 13</td>
<td>0.43</td>
<td>0.01 to 0.66</td>
</tr>
</tbody>
</table>
Table 2: Mean ± standard error of the vessel density in superficial retinal vascular plexus in the three circular zones (Figure 3A) and four sectors (Figure 3B) among the age groups (A, B and C) ranging from 20 to 67 years. Vessel density was defined as the ratio of total number pixels with normalized ratio of local fractal dimension between 0.7 and 1.0 to the total number of pixels in the image.

<table>
<thead>
<tr>
<th>Vessel Density (%)</th>
<th>A*</th>
<th>B*</th>
<th>C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1#</td>
<td>45.04±1.19</td>
<td>45.19±1.40</td>
<td>49.08±2.99</td>
</tr>
<tr>
<td>C2#</td>
<td>47.85±1.26</td>
<td>47.39±1.33</td>
<td>52.10±3.36</td>
</tr>
<tr>
<td>C3#</td>
<td>49.19±1.36</td>
<td>48.61±1.21</td>
<td>53.62±3.46</td>
</tr>
<tr>
<td>Temporal</td>
<td>48.75±1.41</td>
<td>47.68±1.10</td>
<td>47.98±1.74</td>
</tr>
<tr>
<td>Superior</td>
<td>47.57±1.49</td>
<td>48.39±1.39</td>
<td>47.41±1.19</td>
</tr>
<tr>
<td>Nasal</td>
<td>47.04±1.56</td>
<td>47.43±1.29</td>
<td>48.27±1.43</td>
</tr>
<tr>
<td>Inferior</td>
<td>53.61±1.57</td>
<td>50.97±1.71</td>
<td>53.61±1.56</td>
</tr>
</tbody>
</table>

*Age groups: A = 20-30 years, B = 31-45 years, C = 46 to 67 years
#C1=diameter 1.5 mm, C2=diameter 2 mm, C3=diameter 2.5 mm
Table 3: Mean ± standard error of the vessel density in deep retinal vascular plexus in the three circular zones (Figure 3A) and four sectors (Figure 3B) among the age groups (A, B and C) ranging from 20 to 67 years. Vessel density was defined as the ratio of total number pixels with normalized ratio of local fractal dimension between 0.7 and 1.0 to the total number of pixels in the image.

<table>
<thead>
<tr>
<th>Vessel Density (%)</th>
<th>A*</th>
<th>B*</th>
<th>C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1†</td>
<td>48.81±1.46</td>
<td>52.08±2.77</td>
<td>47.21±1.15</td>
</tr>
<tr>
<td>C2†</td>
<td>52.78±1.40</td>
<td>56.43±2.47</td>
<td>51.18±1.24</td>
</tr>
<tr>
<td>C3†</td>
<td>54.16±1.32</td>
<td>57.83±2.28</td>
<td>52.71±1.13</td>
</tr>
<tr>
<td>Temporal</td>
<td>50.44±1.57</td>
<td>54.19±2.32</td>
<td>49.52±1.79</td>
</tr>
<tr>
<td>Superior</td>
<td>54.04±1.29</td>
<td>56.28±2.13</td>
<td>53.77±1.34</td>
</tr>
<tr>
<td>Nasal</td>
<td>49.68±1.58</td>
<td>50.47±2.55</td>
<td>50.35±1.66</td>
</tr>
<tr>
<td>Inferior</td>
<td>56.56±1.43</td>
<td>57.59±1.94</td>
<td>55.21±1.65</td>
</tr>
</tbody>
</table>

*Age groups: A = 20-30 years, B = 31-45 years, C = 46 to 67 years
†C1=diameter 1.5 mm, C2=diameter 2 mm, C3=diameter 2.5 mm
Table 4: Mean ± standard error of proportion of pixels within a range of normalized ratio of local fractal dimension in the superficial and deep retinal vascular plexus of age group A.

<table>
<thead>
<tr>
<th>Proportion (%)</th>
<th>Superficial retinal vascular plexus</th>
<th>Deep retinal vascular plexus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0-0.3</td>
<td>0.3-0.7</td>
</tr>
<tr>
<td>Temporal</td>
<td>41.64±0.50</td>
<td>9.61±1.53</td>
</tr>
<tr>
<td>Superior</td>
<td>42.07±0.67</td>
<td>10.36±1.85</td>
</tr>
<tr>
<td>Nasal</td>
<td>39.49±0.67</td>
<td>13.47±1.95</td>
</tr>
<tr>
<td>Inferior</td>
<td>42.52±1.43</td>
<td>3.87±1.27</td>
</tr>
</tbody>
</table>