Human & Veterinary Medicine
International Journal of the Bioflux Society

OPEN ACCESS
Research Article

Image analysis of the normal human retinal vasculature using fractal geometry

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Abstract. The main objective of this study is to determine a global index of the geometric complexity of the human retinal vascular network using the fractal geometry. Material and Methods: The fractal analysis of digital retinal images was performed with the Image J fractal analysis software and the fractal dimensions were calculated using the standard box-counting method. Results: The mean fractal dimension of normal retinal vascular network was 1.6147 ± 0.0151 in segmented variant and 1.5554 ± 0.0239 in skeletonised variant. Also, the mean lacunarity parameter was 0.5210 ± 0.0343 in segmented version and 0.2916 ± 0.0218 in skeletonised version.

Key Words: retina, retinal microcirculation, fractal analysis, fractal dimension.

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Rezumat. Obiectivul principal al acestui studiu este de a determina un indice global al complexităţii geometrice a reţelei vasculare retiniene umane cu ajutorul geometriei fractale. Material si metoda: Analiza fractală a imaginilor digitale retiniene a fost efectuată cu software-ul de analiză fractală Image J şi dimensiunile fractale au fost calculate folosind metoda standard „box-counting”. Rezultate: Dimensiunea medie fractală a reţelei vasculare retiniene normale a fost 1,6147 ± 0,0151 în varianta segmentată şi 1,5554 ± 0,0239 în varianta skeletonizată. De asemenea, valoarea medie a parametrului „lacunaritate” a fost 0,5210 ± 0,0343 în varianta segmentată şi 0,2916 ± 0,0218 în varianta skeletonizată.

Cuvinte cheie: retina, microcirculaţie retiniană, analiză fractală, dimensiune fractală.

Introduction

Over the past few decades, various methods have been proposed in the image analysis of human retinal vascular network that offer new techniques to evaluate different aspects of retinal vascular topography (Kyriacos *et al* 1997; Saine & Tyler 2002; Ţălu 2005; Losa *et al* 2005; Patton *et al* 2006; Ţălu *et al* 2009; Jastrow 2010; Holz & Spaide 2010; Abramoff *et al* 2010; Ştefănuţ *et al* 2010; Ţălu *et al* 2011; Ţălu 2011b).

In the screening and monitoring the health status of the human eye, a key role plays the fractal and multifractal analysis of the retinal vasculature (Mainster 1990; Lakshminanarayanan *et al* 2003; Masters 2004; Stosic & Stosic 2006; Di Ieva *et al* 2008; Lopes & Betrouni 2009; Jelinek *et al* 2010; Wainwright *et al* 2010; Azemin *et al* 2011; Gould *et al* 2011; Ţălu & Giovanzana 2011; Ţălu 2011a).

Several fractal studies have established that the average values of the estimated fractal dimensions of normal human retinal vascular network were approximately 1.7. Also, experimental results have shown a complex dependence of the estimated fractal dimensions on a number of factors involved as: diversity of subjects, image acquisition, type of image, its processing, fractal analysis methods, including the algorithm and specific calculation used (Kyriacos *et al* 1997; Hoover *et al* 2000; Masters 2004; Niemeijer *et al* 2004; Staal *et al* 2004; MacGillivray *et al* 2007; Mendonça *et al* 2007).

In addition, the information on the eye fundus plays an important role in detection and diagnosing of many vascular and non-vascular diseases (Hubbard *et al* 1999; Hughes *et al* 2006; Kunicki *et al* 2009; Cheung *et al* 2010; Ţălu & Giovanzana 2011).

Fractal analysis of human retinal vasculature can be used as a non-invasive screening test for detection of early retinal vascular diseases. It is a more useful descriptor that offers a new language for examining the complex patterns found in ophthalmic practice.

Fractal analysis

In mathematical calculation, box-counting or box dimension is the most common method used to estimate the fractal dimension.

The lower and upper box-counting dimensions of a subset $F \subset R^n$ are respectively defined by (Falconer 2003):

$$\underline{\dim}_B(F) = \underline{\lim}_{\delta \to 0} \frac{\log N_\delta(F)}{-\log \delta}; \qquad \overline{\dim}_B(F) = \overline{\lim}_{\delta \to 0} \frac{\log N_\delta(F)}{-\log \delta} \quad (1)$$

If these are equal then the common value is referred to as the box-counting dimension of F and is denoted by (Falconer 2003):

$$\dim_B(F) = \lim_{\delta \to 0} \frac{\log N_\delta(F)}{-\log \delta} \quad (2)$$

(if this limit exists), where $N_\delta(F)$ is any of the following:
(i) the smallest number of closed balls of radius $\delta > 0$ that cover F;
(ii) the smallest number of cubes of side δ that cover F;
(iii) the number of δ-mesh cubes that intersect F;
(iv) the smallest number of sets of diameter at most δ that cover F;
(v) the largest number of disjoint balls of radius δ with centers in F.

In our study the box counting algorithm was performed using the Image J software (Wayne Rasband, National Institutes of Health, in Bethesda, Maryland, USA) (http://imagej.nih.gov/ij) together with the FracLac plug-in (A. Karperien – Charles Sturt University, Australia) (http://rsbweb.nih.gov/ij/plugins/fraclac/FLHelp/Introduction.htm).

The algorithm was applied with the following options: a) Grid positions – 10; b) Calculating of grid calibers – use default box sizes.

The fractal dimension was calculated as the slope of the regression line for the log-log plot of the scanning box size and the count from a box counting scan.

The "count" usually refers to the number of grid boxes that contained pixels in a box counting scan. For lacunarity, the number of pixels per box is counted.

The slope of the linear region of the plot is (−D), where D is the box-counting dimension that corresponds to the fractal dimension. The average results were expressed as mean value and standard deviation.

Lacunarity is complementary to the fractal dimension, as irregular shapes with the same fractal dimension may have different lacunarity values. This measure supplements fractal dimensions in characterizing patterns extracted from digital images. FracLac calculates lacunarity using different methods.
Lacunarity is generally based on the pixel distribution for an image, which is obtained from scans at different box sizes at different grid orientations. The most basic number for lacunarity, λ, is expressed as (http://imagej.nih.gov/ij):

$$\lambda = (\sigma / \mu)^2 \quad (3)$$

where σ is the standard deviation and μ is the mean for pixels per box at this size, ε, in a box count at this orientation, g. To put heterogeneity from one perspective and one series of grid sizes into an average, the mean ($\overline{\lambda}$ or Λ) from all ε sized boxes at a grid orientation, g, is calculated with relations:

$$F\lambda = 1 + \lambda \quad (4)$$

$$\Lambda = \left(\sum [F\lambda]\right) / n \quad (5)$$

Table 1. Results of the fractal dimensions, lacunarity parameters Λ and correlation coefficients of six analyzed images from the STARE database, in segmented and skeletonised variants.

File no.	Type	Value of fractal dimension (D)	Lacunarity parameter Λ	Correlation coefficient (R2)
im0077.ah	Seg.	1.6212	0.4990	0.9936
	Skl.	1.5597	0.2648	0.9864
im0081.ah	Seg.	1.5917	0.4703	0.9938
	Skl.	1.5270	0.2854	0.9868
im0082.ah	Seg.	1.6174	0.5683	0.9940
	Skl.	1.5617	0.2734	0.9874
im0162.ah	Seg.	1.6396	0.5146	0.9940
	Skl.	1.6017	0.2969	0.9895
im0163.ah	Seg.	1.6015	0.5618	0.9945
	Skl.	1.5377	0.3331	0.9878
im0235.ah	Seg.	1.6171	0.5124	0.9946
	Skl.	1.5451	0.2961	0.9871
Average	Seg.	1.6147 ± 0.0151	0.5210 ± 0.0343	
	Skl.	1.5554 ± 0.0239	0.2916 ± 0.0218	

where: n is the number of box sizes; and the F stands for "foreground pixels", that signifies lacunarity calculated using the count of foreground pixels independently of other considerations. In our case, the lacunarity parameter Λ in each digital retinal image provides the gaps in the retinal vascular network.

Materials and Methods

Let us consider a set of six digital retinal images corresponding to normal states of the retina, randomly selected from the STARE database (http://www.parl.clemson.edu/stare/probing/) (files: im0077.ah, im0081.ah, im0082.ah, im0162.ah, im0163.ah and im0235.ah), manually segmented by the observer Adam Hoover (referred here by initials AH). The slides were captured by a TopCon TRV-50 fundus camera at 35° field of view. Each slide was digitized to produce a 605 x 700 pixel image, 24-bits per pixel. The files are in portable pixmap format (ppm).

The fractal analysis was performed in two cases: a) for the segmented (seg.) and (b) the skeletonised (skl.) versions. The binary skeletal patterns (8-bit) were extracted using the morphologic operations from the original micrograph images with the structuring elements.

Statistical analysis

The statistical processing of the results obtained with Image J software (Table 1) was done using GraphPad InStat software program, version 3.20 (GraphPad, San Diego, CA, USA) (http://www.graphpad.com/instat/instat.htm).

Microsoft Office Excel 2003 statistical functions were used to determine (average ± standard deviation) of the fractal dimensions and lacunarity parameters obtained with Image J software. The fractal dimension of the vascular trees followed a normal distribution.

Results

For all analyzed cases, the coefficients of correlation (R^2) were more than 0.9850 representing good linear correlation. An (R^2) of 1.0 indicates that the regression line perfectly fits the data. The average of fractal dimensions and lacunarity parameters Λ for segmented versions is slightly higher than the corresponding values for skeletonised versions.

The results obtained for the mean fractal dimension are in good agreement with results obtained in some previous studies that suggested that the fractal dimension of the vascular network in the normal human retina is approximately 1.7 (Masters 2004; Ţălu & Giovanzana 2011). The experimental and methodological parameters involved in previous studies partly explain the variations in results encountered in the literature.

Conclusions

The human retinal vasculature network has complex branching structures with self-similarity over a range of magnifications. Retinal veins and arteries have similar branching patterns. Comparable vessels have a similar orientation and a similar order of branching.

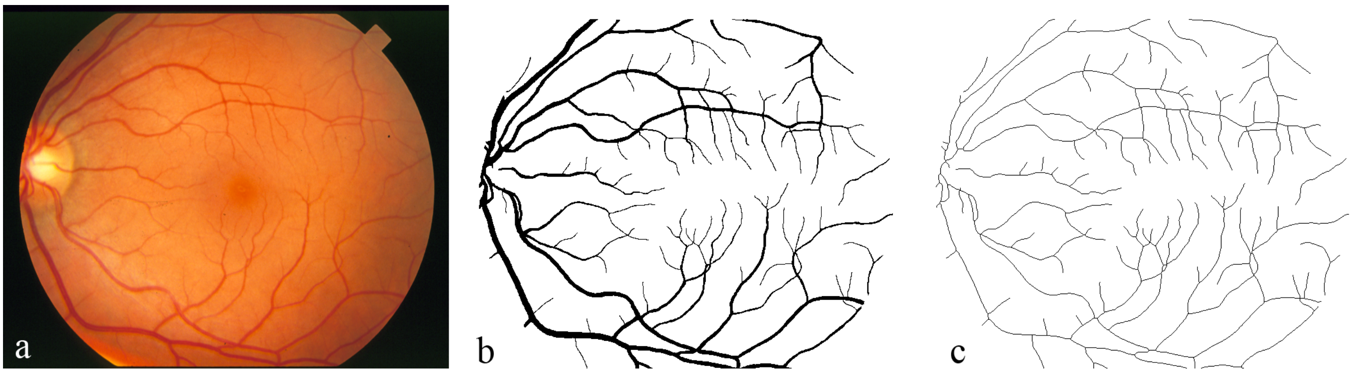

Figure 1. Image of a normal state retinal vessel network (file im0077.ah): (a) the color image version, (b) the segmented version and (c) the skeletonised version.

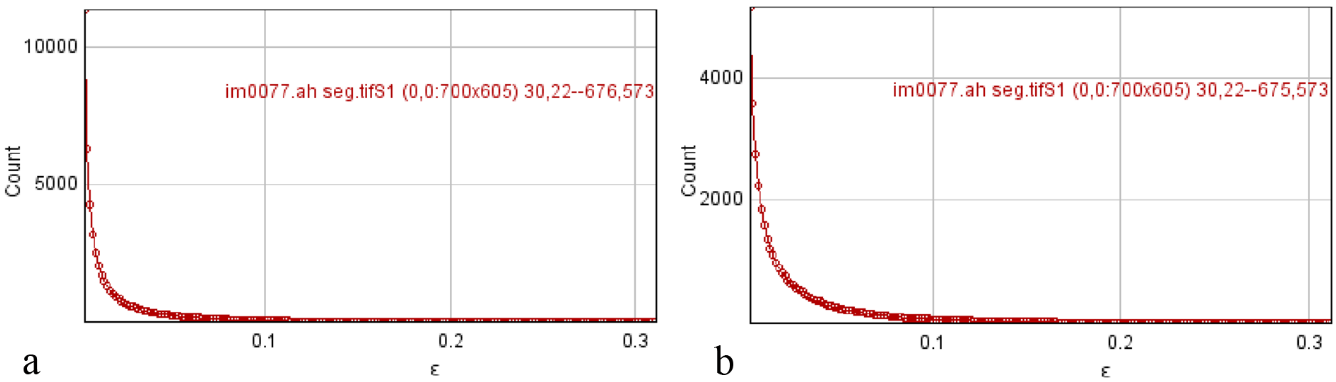

Figure 2. The (count) versus ε (file im0077.ah): (a) the segmented version and (b) the skeletonised version.

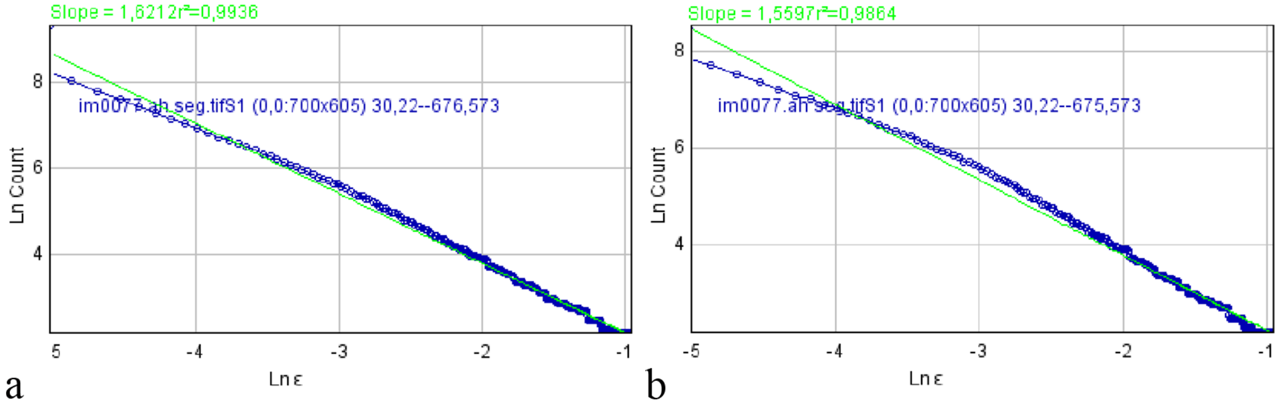

Figure 3. The Ln(count) versus Ln(ε) (file im0077.ah): (a) the segmented version and (b) the skeletonised version.

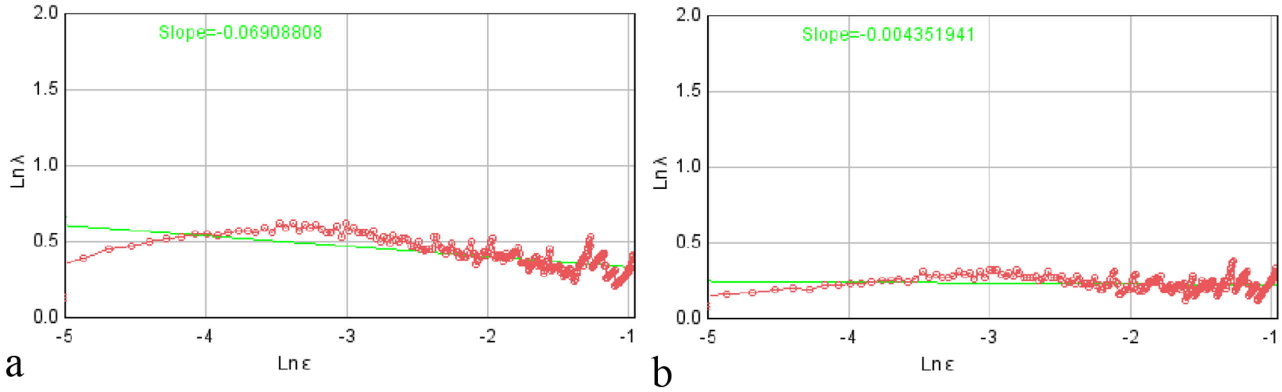

Figure 4. The Ln(λ) versus Ln(ε) (file im0077.ah): (a) the segmented version and (b) the skeletonised version.

The application of fractal analysis allows us to obtain a measure of complexity of the retinal vessel branching. The results obtained in our study show that the fractal estimation with the box-counting method is accurate and reproducible, with very small differences between initial and final average values.

The obtained skeletonised digital images can be analyzed mathematically using nonlinear methods in order to provide a numeric indicator of the extent of neovascularization. The evaluation of the skeleton could measure other characteristics of the retinal vessel pattern such as the presence of gaps and detection of branching points.

Fractal geometry provides a more accurate description of the human retina vascular network than Euclidian geometry and can be used as part of a screening tool for early detection of retinal diseases.

Acknowledgments

The author would like to thank the STARE project group for making their databases publicly available and for permission to use digital retinal images from the STARE database (http://www.parl.clemson.edu/stare/probing/). I would also like to thank Assoc. Prof. Mihai Ţălu, Ph.D. Eng. from Department of Applied Mechanics, Faculty of Mechanics, The University of Craiova, Romania, for his constructive comments.

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Citation	Ţălu, Ş., Giovanzana, S., 2012. Image analysis of the normal human retinal vasculature using fractal geometry. Human & Veterinary Medicine 4(1):14-18.
Editor	Ştefan C. Vesa
Received	10 January 2012
Accepted	26 February 2012
Published Online	15 April 2012
Funding	None Reported
Conflicts / Competing Interests	None Reported