Review Article

Access this article online
Quick Response Code:
Website:
http://www.braincirculation.org
DOI:
10.4103/bc.bc_8_23

A narrative review of retinal vascular parameters and the applications (Part I): Measuring methods

Yuan Gao[1,2], Lijun Xu[3], Ning He[4], Yuchuan Ding[5], Wenbo Zhao[6], Tingting Meng[2], Ming Li[6], Jiaqi Wu[1], Yazeed Haddad[5], Xuxiang Zhang[7], Xunming Ji[1,6]

[1]Department of Biomedical Engineering, School of Biological Science and Medical Engineering, Beihang University, Beijing, China, [2]Department of Ophthalmology, Xuanwu Hospital, Capital Medical University, Beijing, China, [3]School of Instrumentation and Optoelectronic Engineering, Beihang University, Beijing, China, [4]Engineering Research Center of Molecular and Neuro Imaging, Ministry of Education, School of Life Science and Technology, Xidian University, Xi'an, Shaanxi, China, [5]Department of Neurosurgery, Wayne State University School of Medicine, Detroit, Michigan, USA, [6]Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China, [7]Department of Ophthalmology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Address for correspondence:
Prof. Xunming Ji, Department of Biomedical Engineering, Beijing Advanced Innovation Center for Big Data-Based Precision Medicine, School of Biological Science and Medical Engineering, Beihang University, No.37, Xueyuan Road, Haidian District, Beijing 100191, China.
E-mail: jixm@ccmu.edu.cn

Submission: 03-02-2023
Revised: 11-05-2023
Accepted: 23-05-2023
Published: 27-09-2023

Abstract:

The retina is often used to evaluate the vascular health status of eyes and the whole body directly and noninvasively *in vivo*. Retinal vascular parameters included caliber, tortuosity and fractal dimension. These variables represent the density or geometric characteristics of the vascular network apart from reflecting structural changes in the retinal vessel system. Currently, these parameters are often used as indicators of retinal disease, cardiovascular and cerebrovascular disease. Advanced digital fundus photography apparatus and computer-assisted analysis techniques combined with artificial intelligence, make the quantitative calculation of these parameters easier, objective, and labor-saving.

Keywords:

Caliber, fractal dimension, retina, tortuosity

Introduction

Retinal vascular parameters including diameter, tortuosity, and fractal dimension (FD) can reflect the changes in the retinal vascular network structure. Several studies have reported that these parameters were associated with ocular and other diseases, such as glaucoma,[1] diabetic retinopathy,[2] hypertension,[3] cardiovascular[4] and cerebrovascular disease.[5] Particularly, carotid stenosis is a significant contribution to the risk of ischemic stroke, which can lead to neurological disability and death in adult.[6,7] The retinal vasculature can be regarded as a part of the cerebral vasculature, thus their associations may contribute to further studying the pathological mechanism of cerebrovascular diseases. However, there is no uniform measurement method for these parameters. Thus, it is very important to choose a simple and accurate calculation method for quantitative measurements of these parameters. Besides, it is difficult to quantify these parameters by manual methods. The development of computer-assisted analysis of digital fundus images enables retinal vascular parameters measurement in a timely, accurate, and reliable manner while reducing subjective human error.[8] Diameter is one of the most commonly used indexes to evaluate vascular characteristics.[9] Currently, several methods and formulas were put forward for retinal vascular caliber quantitative calculation, such as Full width half-maximum (FWHM) or half-height at full-width,[10,11] Gaussian fitting function,[12] Sobel edge detection,[13] sliding linear regression filter (SLRF),[14] Parr-Hubbard[15] and Revised Parr-Hubbard formulas.[16] Retinal vascular tortuosity, is another indicator of retinal vessel morphology.[3,17] Some techniques for assessing retinal vessel tortuosity can be divided to three major groups: based on ration of curve length over chord line,[18] curvature-based methods,[19] and based on slope chain code (SCC).[20] FD is often used

How to cite this article: Gao Y, Xu L, He N, Ding Y, Zhao W, Meng T, *et al*. A narrative review of retinal vascular parameters and the applications (Part I): Measuring methods. Brain Circ 2023;9:121-8.

to quantitative assessment of the complexity and branch self-similarity of retinal microvasculature pattern.[21] The box-counting technique is the most common approach to calculate FD.[22,23] In this review, we conclude the current methods of retinal caliber, tortuosity, and FD measurement, providing important reference values for using these metrics as an indicator of retinal disease and systemic disease in subsequent clinical studies.

Measuring Methods of Retinal Vasculature

Vascular caliber in retina

According to the dependence of the caliber calculation method on a particular value parameter, retinal caliber calculation methods include two principal groups: nonparametric and parametric methods.[24] In addition, the Parr-Hubbard formula, revised Parr-Hubbard formula and commercially available measurement software based on them were also usually used to measure retinal vascular caliber.

Nonparametric methods

The nonparametric method used the edge detection technique and consider the cross-sectional caliber to be the smallest Euclidean distance between the two places on the vascular edges.[24] The Sobel and "Canny," are the two most common edge detection methods to track or emphasize the boundaries between the two sides of a blood vessel. The Sobel operator is a two-dimensional spatial gradient detector, including horizontal and vertical edge detectors with a pair of three-dimensional convolution masks that is primarily applied to calculate the area of an image which has significant volume differences in both the X and Y directions.[13] It has the advantage of rapid detecting. However, it cannot detect thin and smooth edges accurately.[25] Canny operator is a classical detector on account of gradient and Laplacian methods.[26] The method has three characteristics of single detection edge response, good edge positioning, and low detection errors.[27] However, when the noise in an image is removed using the Canny method and Gaussian smoothing, it is unable to discern the somewhat hazy edges.[25] To address the problem, Rahman proposed an approach that relied on histogram processing as an image preprocessing step before the canny edge detector and confirmed promising outcomes.[25] Moreover, other detection techniques such as Edge pixel Grouping[28] and multi-scale segmentation methods.[29] These methods have some limitations such as difficulty for fine vessels measurement and sub-pixel accuracy. Besides, high-intensity central light reflexes in high-resolution fundus images cannot been accurately obtained as vessel boundaries.[24]

Parametric methods

Calculate a caliber by Gaussian function fitting intensity samples curve across a blood vessel cross-section.[24] Gang *et al.* have modeled retinal vessel cross-section using an amplitude-modified second-order Gaussian function.[30] The diameter is then calculated using FWHM method, which is introduced by Brinchmann-Hansen and defined as the distance along either of the vessel's edges between the center points of highest intensity variation.[10] The spreading factor (sigma) of the Gaussian best fit is linearly associated with caliber and thus can be applied to make diameter estimation simple. Nevertheless, it cannot capture the central light reflex of the vessels, also known as "specular reflection."[31] To cope with this problem, some researchers created twin and piecewise Gaussian functions to describe the gray level profile distributions over a vessel cross-section and quantify the vessel diameter.[32,33] SLRF was another method for caliber calculation. This method appears to be the most reproducible and consistent in measurements of retinal caliber diameter compared to the other two automated methods(Gaussian function and Sobel detection algorithm) (Sobel).[14] However, the approach grows unreliable when the total vessel width is <10 pixels.[13] For compensation, Aliahmad proposed a multi-step regression method for retinal vessel diameter calculation that a three-criteria function provides the best evaluation of the vessel profiles while reducing residual fitting errors brought on by uneven illumination and background noise to address the issue of sigma.[24]

Parr-hubbard and revised parr-hubbard formulas

Parr *et al*. put forward a method for computing the width of the central retinal artery (CRA) (the width of their single parent trunk) from the widths of all the retinal arteries measurement:[15]

$$\hat{W}= (0.87W_1^2 +1.01W_2^2-0.22W_1W_2-10.76)^{1/2} \quad (1)$$

W_1, represents the narrower branch; W_2, represents the wider branch; W, represents the parent trunk. The equivalent CRA width represents the general diameter of the retinal arteries and enables comparison of the arterial diameter between different eyes since it is unaffected by the number and pattern of the retinal larger arteries' branching.[15] Nevertheless, the Parr-Hubbard equation is influenced by the number of vessels measurement and sensitive to scale due to constant terms in the equations. Knudtson *et al*. proposed a revised equation for summarizing caliber measurement using the six largest arterioles and venules from fundus images:[16]

$$\text{Arterioles: } \hat{W} = 0.88^{*}(W_1^2+W_2^2)^{1/2} \quad (2)$$

$$\text{Venules: } \hat{W} = 0.95^{*}(W_1^2 + W_2^2)^{1/2}$$

W_1, the widths of the narrower branch; W_2, the wider branch; $\hat{W}$, the parent trunk arteriole or venule. The revised Parr-Hubbard formula is closely related to the previous Parr-Hubbard formula, but has the benefits of being independent of image scale, more resilient against the number of vessels variations, and thus being simpler to apply.[16] It has been used for caliber measurement by computer software in numerous studies.

Retinal vascular caliber can be calculated from the vessels inside the region of interest (ROI) in the computer software. The commonly used ROIs are concentric zones centered on the optic disc including the regions from the disc margin to ½ disc diameter from the disc (Zone A), ½ disc diameter from the disc to 1disc diameter from the disc (Zone B), and 2disc diameter from the disc (Zone C).[29,34-36] Among them, Zone B and Zone C are the two most popular regions for calculation[29] [Figure 1]. Although there are many methods for calculating retinal vessel diameter, the methods based on the Parr-Hubbard formula and the revised Parr-Hubbard formula in the ROI region were the two most commonly applied in clinical research, especially the latter.

With the rise of artificial intelligence and its application in the medical field, the measurement method of retinal vascular caliber has developed from the original semi-automatic to fully automatic. Cheung *et al*. developed and validated an automated measurement method of retinal vascular caliber based on deep-learning algorithm without the need for vessel segmentation (SIVA-DLS), which has high agreement with expert human graders.[37] Besides, fully automated software also included QUARTZ[38] and ALTAIR.[39]

Retinal vascular tortuosity

There is no uniform definition of retinal vascular tortuosity since it always varies with different measurement methods. Tortuosity is a degree of vascular curvature in a qualitative manner. Ophthalmologists in clinical practice usually divide tortuosity into a qualitative scale including mild, moderate, and severe according to a vessel segment's curvature, number of twists, frequency, and amplitude.[40,41] There are main four types of measurement methods: arc-length over chord length ratio methods, curvature-based, angle-based, and other domain-based methods.[42]

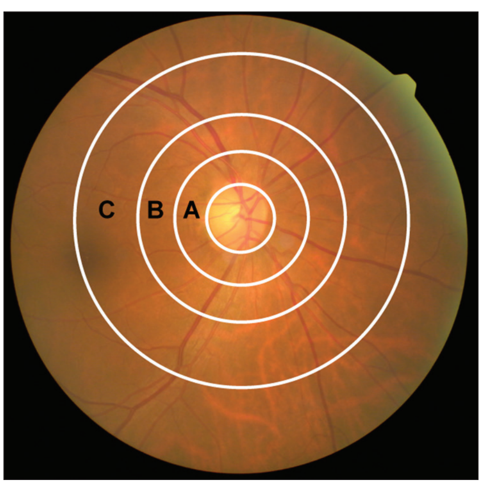

Figure 1: An image from fundus photography shows that the ROI are concentric zones centered on the optic disc including the regions from the disc margin to ½ disc diameter from the disc (Zone A), ½ disc diameter from the disc to 1 disc diameter from the disc (Zone B), and 2 disc diameter from the disc (Zone C). ROI: Region of interest

The arc length over chord length ratio, first reported by Lotmar *et al*., is the simplest and most commonly used method:[18,43]

$$\text{Tortuosity} = \text{Arc}_{\text{length}}/\text{Chord}_{\text{length}} \quad (3)$$

$$\text{Arc}_{\text{length}} = \sum_{i=0}^{n-1}\sqrt{\left(x_i+1-x_i\right)^2+\left(y_i+1-y_i\right)^2}$$

$$\text{Chord}_{\text{length}} = \sqrt{\left(x_n-x_1\right)^2+\left(y_n-y_1\right)^2}$$

However, this approach is deficient for the calculation of vessels with smooth curvature and variation in curvature direction.[42] Bullitt *et al*. noted that limitation, then proposed a method to calculate the number of inflection points (twists), which can calculate it caused by the blood vessel when the blood vessel changes direction, and then sum the total angle of each effective point on the curve, and normalize the result by the total curve length.[44] Grisan *et al*. raised the tortuosity density (TD) index to evaluate vascular tortuosity by calculating the contributions to tortuosity of uniformly convex or concave arcs:[45,46]

$$TD = \frac{n-1}{n}\frac{1}{Lc}\sum_{i=1}^{n}\left[\frac{Lcsi}{Lxsi}-1\right] \quad (4)$$

$$\tau = \frac{n-1}{Lc}\sum_{i=1}^{n}\left[\frac{Lcsi}{Lxsi}-1\right] \quad (5)$$

n represents the number of "turns;" L_{csi} represents the arc length of segment *I*; L_{xsi} represents the chord length of segment *i*; L_c represents the total length of the vessel's centerline; τ represents the tortuosity. Curvature-based measurement is a calculation of how curved a curve is at a certain coordinate and is an integral function of the estimated curvature along the vascular skeleton, usually a weighted sum of absolute or squared curvatures.[19,42] Hart *et al*. proposed two tortuosity measurement methods which are the integral of vessel centerline curvature and curvature squared, and they found that the squared curvature was the closest to the eye doctor' concept of curvature.[19] Trucco *et al*. pointed out that tortuosity can increase with thickness, thus its measurement not only needs skeleton curvature but also need vessel caliber.[47] Some researchers thought curvature-based tortuosity measurements are more reliable but more computationally intensive and particularly rely on the technique of tortuosity measurement in comparison with the method of the arc length over chord length ratio.[48,49] Besides, some methods measured tortuosity by calculating the direction variations of the vessel. Foracchia *et al*. assessed tortuosity by a simulated annealing optimization technique identifying the model parameter and computing the number of changes in direction more than a predetermined threshold.[50] Rodriguez introduced the fast Fourier transform of the vessel's curvature to compute tortuosity.[51] Bribiesca *et al*. put forward a new approach to tortuosity measurement based on SCC which is not affected by translation, rotation, or scaling.[20] "The accumulated slope A_{cc} of a chain is the sum of the slope changes *n* around the curve, is defined by:

$$A_{cc} = \sum_{i=1}^{n} ai \qquad (6)$$

"*n* is the number of slope changes; ai: "the slope change of the contiguous straight-line segments of the curve at that element position" and the tortuosity ꞇ "is the sum of all the absolute values of the chain elements, and is defined by

$$ꞇ = \sum_{i=1}^{n} |ai| \qquad (7)$$

Lisowska *et al*.[20] assessed five retinal vascular tortuosity measurement methods including distance measure, two curvature-based measures, TD, and slope chain coding with the retinal vessel tortuosity dataset public dataset.[46] They discovered that the curvature approach performs best to high-frequency resampling, but is the most sensitive to variations in sample number; the TD index has excellent performance as a whole, but not always the best; Slope-chain coding is most effective at low sampling rates, but need to choose the appropriate length of the linear elements.[46]

In addition, some methods calculated the curvature regions outside the space domain without vessel extraction such as the Hough transform.[52] Mustafa *et al*. proposed a fully automated retinal tortuosity measurement method based on nonlinear curvature that compares the mean tortuosity value with a computer threshold value, T, and if the average tortuosity value is less than the T, the retinal vascular network is considered normal, and vice versa.[53] Aras *et al*. calculated retinal vascular tortuosity using the relative length variation method, then used K nearest neighbor to classify the retinal images as normal, tortuosity, moderate, and severe tortuosity, and the best accuracy reached more than 90%.[54] Nowadays, based on the above calculation methods, a variety of commercial computer-assisted analysis software has been developed and will be introduced in the later section.

Fractal dimensions in retina

The geometric complexity and branch self-similarity of the vascular network, which have been employed to characterize physiological processes as well as various anatomical structures, were measured using the FD.[55] Retinal vascular FD reflects the overall branching pattern of retinal vessels and may aid in the detection of subclinical vascular abnormalities.[56] A mono-fractal or a multifractal method was used to evaluate FDs.[57,58] In mono-fractal method, classical FD estimation methods included box-counting methods, area measurement methods, and fractional Brownian motion methods. The box-counting technique is the most commonly used method to calculate FD and the mathematical formula is expressed as:

$$D = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{-\log \varepsilon} \qquad (8)$$

N (*ε*), the number of boxes; scales ε, the number of pixels.[59] Konatar *et al*. implemented three methods including standard nonoverlapping, gliding or overlapping box scanning, and random box scanning to measure FD.[59] By examining photographs of the retinal microvasculature from public databases and comparing the findings to those of Image J, they confirmed that the results of the three approaches were rather near to the anticipated theoretical values.[59] In addition, Zode *et al*. demonstrated that the results obtained using box-counting method were more accurate than using the mass-radius method.[60] However, although this method is simple, it is sensitive to box size and requires signal binarization and the segmentation of retinal vessels' images.[61] Fractional Brownian motion methods are based on the variogram (Gaussian modeling) or the Fourier transform algorithms of the image to compute the fractional dimension. Azemin *et al*. used the Fourier FD approach to quantify the grayscale images projected on to 3D fractal surface and compute the retinal FD.[62] The superiority of Fourier FD is that it calculates FD of grayscale images without image segmentation and relatively insensitive to noise and thus handles effectively data with low signal-to-noise ratio.[62] The Isarithm method, blanket method, and triangular prism method are the three algorithms commonly used in area measurement methods.[61] The retinal vascular tree, which may merge several mono-FD, is one example of a complicated spatial arrangement that multifractal approaches are thought to be better suited to characterize.[57] The measurement methods include box-counting methods such as generalized FD, multifractal spectrum, the "sandbox" or cumulative mass method, the large-deviation multifractal spectrum method and wavelets.[61] Among these methods, the generalized sandbox method is commonly used to compute the multifractal dimensions of the retinal vascular tree.[57] Employing a nonlinear SVM classifier, Ding *et al*. accomplished picture categorization into healthy group and pathological group according to the characteristics retrieved from multi-fractal and Fourier spectra of retinal arteries.[63] Relan *et al*. used the multi-fractal analysis quantified retinal FD in high-resolution fundus images to distinguish between two subgroups in images of healthy, diabetic retinopathy, and glaucoma patients through segmented and skeletonized images.[64] Bhat *et al*. showed how the Pixel-Based Multi Fractal Analysis method can aid in precisely locating the center of the optical disc and then tracing the entire disc.[65] In conclusion, there are many calculation methods and clinical applications of mono-fractal FD, while there are few studies on multi-FDs, and further research is needed in future.

Computer Software for Retinal Vascular Parameters

Currently, there are numerous developed semi-automatic and automatic software to measure retinal vascular parameters. For instance, the Retinal Analysis (RA; University of Wisconsin, Madison, WI),[66] the Integrative Vessel Analysis (IVAN; University of Wisconsin, Madison);[66,67] the Singapore I Vessel Assessment (SIVA1.0,2.0,3.0,4.0; National University of Singapore),[2,56,68-72] the Computer-Aided Image Analysis of the Retina program (CAIAR, Imperial College London, City University, London, UK);[73,74] Retinal Vessel Analyser (RVA; IMEDOS Systems UG, Jena, Germany);[75,76] VesselMap (ImedosSystems, Jena, Germany),[77-79] Vessel assessment and measurement platform for images of the retina (VAMPIRE),[80-82] QUantitative Analysis of Retinal vessel Topology and siZe (QUARTZ;UK)[38,83] Automatic Retinal Image Analysis (US Patent 8787638B2).[84,85]

Yip *et al*. found that the associations between retinal vessel calibers with systemic factors were similar by evaluating the consistency among three software (RA, SIVA, and IVAN), despite absolute measurement differences.[66] They proposed an algorithm that enabled RA and IVAN calculations to be converted to SIVA approximates, which is crucial for future data pooling and the definition of normative values.[66] French C and Heitmar R also compared the retinal vessel caliber measurement using the VesselMap and MONA REVA (VITO Health, Mol, Belgium), and confirmed the good consistency between the two platforms.[77] However, McGrory *et al*. found that the poor agreement in estimation results of these parameters employing the SIVA and the VAMPIRE software from fundus camera images.[68] To make retinal vascular parameters trustworthy and independent of different computer applications, they must be measured according to a standard,[68] which need further research.

Conclusion

There are many challenges in retinal vascular parameters measurement from fundus photographs. For example, there is no uniform calculation method and standardized protocol for retinal vascular parameters. A lack of consistent comparison between different methods and different software for retinal vascular parameters measurements in the same data set. In addition, many software is not free and expensive for user, which limits its clinical application. Furthermore, although artificial intelligence has been used widely to classify and identify the qualitative assessment of retinal diseases, few are applied to retinal arteriovenous segmentation and then retinal vascular parameters evaluation based on the segmented vessels in clinical studies. The reason may be related to short of public, large, annotated datasets which take lots of work by trained ophthalmologists. It is still very challenging to compare algorithms thoroughly and objectively because different cameras have varying luminous exposure, focus, quality, and pixels. Thus, simple, economic, fully automated software based on AI to measure retinal vascular parameters are needed to further develop and apply to the clinical studies and practice.

Financial support and sponsorship

This study was supported by the National Natural Science Foundation of China (No. 82071312 and 81971114).

Conflicts of interest

Prof. Xunming Ji is an Editor-in-Chief, Prof. Yuchuan Ding is an Editorial Board member of Brain Circulation. The article was subject to the journal's standard procedures, with peer review handled independently of them and their research groups.

References

1. Tham YC, Cheng CY, Zheng Y, Aung T, Wong TY, Cheung CY. Relationship between retinal vascular geometry with retinal nerve fiber layer and ganglion cell-inner plexiform layer in nonglaucomatous eyes. Invest Ophthalmol Vis Sci 2013;54:7309-16.
2. Torp TL, Kawasaki R, Wong TY, Peto T, Grauslund J. Temporal changes in retinal vascular parameters associated with successful panretinal photocoagulation in proliferative diabetic retinopathy: A prospective clinical interventional study. Acta Ophthalmol 2018;96:405-10.
3. Cheung CY, Zheng Y, Hsu W, Lee ML, Lau QP, Mitchell P, *et al.* Retinal vascular tortuosity, blood pressure, and cardiovascular risk factors. Ophthalmology 2011;118:812-8.
4. Liu M, Lycett K, Wake M, He M, Kerr JA, Saffery R, *et al.* Cardiovascular health and retinal microvascular geometry in Australian 11-12 year-olds. Microvasc Res 2020;129:103966.

5. Hilal S, Cheung CY, Wong TY, Schmetterer L, Chen C. Retinal parameters, cortical cerebral microinfarcts, and their interaction with cognitive impairment. Int J Stroke 2023;18:70-7.
6. Wolf VL, Ergul A. Progress and challenges in preclinical stroke recovery research. Brain Circ 2021;7:230-40.
7. Bir SC, Kelley RE. Carotid atherosclerotic disease: A systematic review of pathogenesis and management. Brain Circ 2022;8:127-36.
8. Fraz MM, Remagnino P, Hoppe A, Barman SA, Rudnicka A, Owen C, *et al*. A model based approach for vessel caliber measurement in retinal images. In: 2012 Eighth International Conference on Signal Image Technology and Internet Based Systems. Sorrento, Italy,2012, pp.129-36.
9. Farooq J, Lee JY. Vascular tortuosity in endovascular mechanical thrombectomy. Brain Circ 2021;7:3-7.
10. Brinchmann-Hansen O. The light reflex on retinal arteries and veins. A theoretical study and a new technique for measuring width and intensity profiles across retinal vessels. Acta Ophthalmol Suppl (1985) 1986;179:1-53.
11. Nasehi Tehrani J, Yan H, Zhu M, Jin C, McEwan AL. Measurement of retinal arteriolar diameters from auto scale phase congruency with fuzzy weighting and L1 regularization. Annu Int Conf IEEE Eng Med Biol Soc 2012;2012:1434-7.
12. Aliahmad B, Kumar DK, Janghorban S, Azemin MZ, Hao H, Kawasaki R. Automatic retinal vessel profiling using multi-step regression method. Annu Int Conf IEEE Eng Med Biol Soc 2011;2011:2606-9.
13. Tehrani JN, Wang CY, Jin C, McEwan AL, Gibbs O, Shanehsaz E, *et al*. Edge enhancement for retinal vasculature caliber evaluation in prediction of cardiovascular disease. In: 2011 4th International Conference on Biomedical Engineering and Informatics (BMEI). Shanghai, China, 2011, p. 210-3.
14. Chapman N, Witt N, Gao X, Bharath AA, Stanton AV, Thom SA, *et al.* Computer algorithms for the automated measurement of retinal arteriolar diameters. Br J Ophthalmol 2001;85:74-9.
15. Parr JC, Spears GF. General caliber of the retinal arteries expressed as the equivalent width of the central retinal artery. Am J Ophthalmol 1974;77:472-7.
16. Knudtson MD, Lee KE, Hubbard LD, Wong TY, Klein R, Klein BE. Revised formulas for summarizing retinal vessel diameters. Curr Eye Res 2003;27:143-9.
17. Turior R, Onkaew D, Kondo T, Uyyanonvara B. A Novel Approach for Quantification of Retinal Vessel Tortuosity Based on Principal Component Analysis. In: The 8th Electrical Engineering/ Electronics, Computer, Telecommunications and Information Technology (ECTI) Association of Thailand Conference 2011, Khon Kaen, Thailand, 2011, p.1023-6.
18. Lotmar W, Freiburghaus A, Bracher D. Measurement of vessel tortuosity on fundus photographs. Albrecht Von Graefes Arch Klin Exp Ophthalmol 1979;211:49-57.
19. Hart WE, Goldbaum M, Côté B, Kube P, Nelson MR. Measurement and classification of retinal vascular tortuosity. Int J Med Inform 1999;53:239-52.
20. Bribiesca E. A measure of tortuosity based on chain coding. Pattern Recognit 2013;46:716-24.
21. Aliahmad B, Kumar DK, Hao H, Unnikrishnan P, Che Azemin MZ, Kawasaki R, *et al.* Zone specific fractal dimension of retinal images as predictor of stroke incidence. ScientificWorldJournal 2014;2014:467462.
22. Tălu S. Fractal analysis of normal retinal vascular network. Oftalmologia 2011;55:11-6.
23. Wang H, Hu H, Gregori G, Zhang J, Jiang H, Wang J. The effect of software versions on the measurement of retinal vascular densities using optical coherence tomography angiography. Curr Eye Res 2021;46:341-9.
24. Aliahmad B, Kumar DK, Janghorban S, Azemin MZC, Hao H, Kawasaki R. Retinal Vessel Diameter Measurement Using Multi-Step Regression Method. In: 2012 ISSNIP Biosignals and Biorobotics Conference: Biosignals and Robotics for Better and Safer Living (BRC), Manaus, Brazil, 2012, p. 1-4.
25. Rahman MA, Amin MF, Hamada M. Edge Detection Technique by Histogram Processing with Canny Edge Detector. In: 2020 3rd IEEE International Conference on Knowledge Innovation and Invention (ICKII); 2020.
26. Canny J. A computational approach to edge detection. IEEE Trans Pattern Anal Mach Intell 1986;8:679-98.
27. Sidhu RK. Improved Canny Edge Detector in Various Color Spaces. In: Proceedings of 3rd International Conference on Reliability, Infocom Technologies and Optimization; 2014.
28. Bhuiyan A, Kawasaki R, Lamoureux E, Wong TY, Ramamohanarao K. Vessel Segmentation from Color Retinal Images with Varying Contrast and Central Reflex Properties. In: 2010 International Conference on Digital Image Computing: Techniques and Applications, Sydney, NSW, Australia, 2010, p. 184-9.
29. Moghimirad E, Rezatofighi H, Soltanian-Zadeh H. Multi-Scale Approach for Retinal Vessel Segmentation Using Medialness Function. In: 2010 IEEE International Symposium on Biomedical Imaging: From Nano to Macro; 2010.
30. Gang L, Chutatape O, Krishnan SM. Detection and measurement of retinal vessels in fundus images using amplitude modified second-order Gaussian filter. IEEE Trans Biomed Eng 2002;49:168-72.
31. Hammer M, Leistritz S, Leistritz L, Schweitzer D. Light paths in retinal vessel oxymetry. IEEE Trans Biomed Eng 2001;48:592-8.
32. Li H, Hsu W, Lee ML, Wang H. A Piecewise Gaussian Model for Profiling and Differentiating Retinal Vessels. In: Proceedings 2003 International Conference on Image Processing (Cat. No. 03CH37429); Barcelona, Spain, 2003, p. 1-1069.
33. Li H, Hsu W, Lee ML, Wong TY. Automatic grading of retinal vessel caliber. IEEE Trans Biomed Eng 2005;52:1352-5.
34. Benitez-Aguirre P, Craig ME, Sasongko MB, Jenkins AJ, Wong TY, Wang JJ, *et al.* Retinal vascular geometry predicts incident retinopathy in young people with type 1 diabetes: A prospective cohort study from adolescence. Diabetes Care 2011;34:1622-7.
35. Benitez-Aguirre PZ, Wong TY, Craig ME, Davis EA, Cotterill A, Couper JJ, *et al.* The adolescent cardio-renal intervention trial (AdDIT): Retinal vascular geometry and renal function in adolescents with type 1 diabetes. Diabetologia 2018;61:968-76.
36. Kannenkeril D, Frost S, Nolde JM, Kiuchi MG, Carnagarin R, Lugo-Gavidia LM, *et al.* Microvascular changes at different stages of chronic kidney disease. J Clin Hypertens (Greenwich) 2021;23:309-16.
37. Cheung CY, Xu D, Cheng CY, Sabanayagam C, Tham YC, Yu M, *et al.* A deep-learning system for the assessment of cardiovascular disease risk via the measurement of retinal-vessel calibre. Nat Biomed Eng 2021;5:498-508.
38. Welikala RA, Fraz MM, Foster PJ, Whincup PH, Rudnicka AR, Owen CG, *et al.* Automated retinal image quality assessment on the UK Biobank dataset for epidemiological studies. Comput Biol Med 2016;71:67-76.
39. Maderuelo-Fernandez JA, Garcia-Garcia A, Chamoso P, Recio-Rodríguez JI, Rodríguez-González S, Patino-Alonso MC, *et al.* Automatic image analyser to assess retinal vessel calibre (ALTAIR). A new tool to evaluate the thickness, area and length of the vessels of the retina. Int J Med Inform 2020;136:104090.
40. Nafia TD, Handayani A, Mengko TL. Evaluation of Retinal Vascular Tortuosity Indexes. In: 2017 5th International Conference on Instrumentation, Communications, Information Technology, and Biomedical Engineering (ICICI-BME); 2017.
41. Nafia TD, Handayani A. Quantification of Retinal Vascular Tortuosity: Evaluation on Different Numbers of Sampling Points. In: 2018 2nd International Conference on Biomedical

Engineering (IBIOMED); 2018.
42. Aghamohamadian-Sharbaf M, Pourreza HR, Banaee T. A novel curvature-based algorithm for automatic grading of retinal blood vessel tortuosity. IEEE J Biomed Health Inform 2016;20:586-95.
43. Mapayi T, Tapamo JR. Performance Comparison of Supervised Learning Methods for Retinal Vessel Tortuosity Characterisation. In: 2018 Conference on Information Communications Technology and Society (ICTAS); 2018.
44. Bullitt E, Gerig G, Pizer SM, Lin W, Aylward SR. Measuring tortuosity of the intracerebral vasculature from MRA images. IEEE Trans Med Imaging 2003;22:1163-71.
45. Grisan E, Foracchia M, Ruggeri A. A novel method for the automatic grading of retinal vessel tortuosity. IEEE Trans Med Imaging 2008;27:310-9.
46. Lisowska A, Annunziata R, Loh GK, Karl D, Trucco E. An experimental assessment of five indices of retinal vessel tortuosity with the RET-TORT public dataset. In: 2014 36th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Chicago, IL, USA, 2014, p. 5414-7.
47. Trucco E, Azegrouz H, Dhillon B. Modeling the tortuosity of retinal vessels: Does caliber play a role? IEEE Trans Biomed Eng 2010;57:2239-47.
48. Fan F, He J, Wang C, Zhang Z, Zhang Q. Multi-scale retinal vessel tortuosity measurement based on wavelet transform. In: 2021 11th International Conference on Information Technology in Medicine and Education (ITME). Wuyishan, Fujian, China, 2021, p. 404-8.
49. Crosby-Nwaobi R, Heng LZ, Sivaprasad S. Retinal vascular calibre, geometry and progression of diabetic retinopathy in type 2 diabetes mellitus. Ophthalmologica 2012;228:84-92.
50. Foracchia M, Grisan E, Ruggeri A. Detection of optic disc in retinal images by means of a geometrical model of vessel structure. IEEE Trans Med Imaging 2004;23:1189-95.
51. Martin Rodriguez Z, Kenny P, Gaynor L. Improved characterisation of aortic tortuosity. Med Eng Phys 2011;33:712-9.
52. Ghadiri F,Pourreza H,Banaee T,Delgir M. Retinal Vessel Tortuosity Evaluation Via Circular Hough Transform. In: 2011 18th Iranian Conference of Biomedical Engineering (ICBME); Tehran, Iran, 2011, p.181-4.
53. Mustafa NBA, Zaki WMDW, Hussain A, Che Hamzah J. A Study of Retinal Vascular Tortuosity in Diabetic Retinopathy. In: 2016 International Conference on Advances in Electrical, Electronic and Systems Engineering (ICAEES), Putrajaya, Malaysia, 2016, p.636-641.
54. Aras RA, Nugroho HA, Ardiyanto I. Measurement and Classification Retinal Blood Vessel Tortuosity in Digital Fundus Images. In: 2020 3rd International Conference on Information and Communications Technology (ICOIACT); 2020.
55. Mapayi T, Dada EG, Olaifa M, Owolawi PA. Retinal Vessel Tortuosity Characterisation Using Boosted Linear Discriminant Analysis. In: 2018 International Conference on Intelligent and Innovative Computing Applications (ICONIC);Mon Tresor,Mauritius, 2018, p.1-5.
56. Arnould L, Guillemin M, Seydou A, Gabrielle PH, Bourredjem A, Kawasaki R, *et al.* Association between the retinal vascular network and retinal nerve fiber layer in the elderly: The Montrachet study. PLoS One 2020;15:e0241055.
57. Stosić T, Stosić BD. Multifractal analysis of human retinal vessels. IEEE Trans Med Imaging 2006;25:1101-7.
58. Doubal FN, MacGillivray TJ, Patton N, Dhillon B, Dennis MS, Wardlaw JM. Fractal analysis of retinal vessels suggests that a distinct vasculopathy causes lacunar stroke. Neurology 2010;74:1102-7.
59. Konatar I, Popovic T, Popovic N. Box-Counting Method in Python for Fractal Analysis of Biomedical Images. In: 2020 24th International Conference on Information Technology (IT); 2020.
60. Zode JJ, Choudhari PC, Uparkar M. Methods to Determine Fractal Dimension to Detect Branch Retinal Vein Occlusion. In: 2017 International Conference on Wireless Communications, Signal Processing and Networking (WiSPNET); 2017.
61. Lopes R, Betrouni N. Fractal and multifractal analysis: A review. Med Image Anal 2009;13:634-49.
62. Azemin MZ, Kumar DK, Wong TY, Kawasaki R, Mitchell P, Wang JJ. Robust methodology for fractal analysis of the retinal vasculature. IEEE Trans Med Imaging 2011;30:243-50.
63. Ding Y, Ward WO, Duan J, Auer DP, Gowland P, Bai L. Retinal vasculature classification using novel multifractal features. Phys Med Biol 2015;60:8365-79.
64. Relan D, Khatter K. Effectiveness of Multi-fractal Analysis in Differentiating Subgroups of Retinal Images. In: 2020 IEEE 17th India Council International Conference (INDICON); 2020.
65. Bhat M, Trun Patil MS, Shrinivas MP, Geetha KS. Automated Retinal Optic Disc Detection Using Pixel Based Multi Fractal Analysis. In: 2017 International Conference on Computer, Communication and Signal Processing (ICCCSP), Chennai, India. 2017. p.1-8.
66. Yip W, Tham YC, Hsu W, Lee ML, Klein R, Klein B, *et al.* Comparison of common retinal vessel caliber measurement software and a conversion algorithm. Transl Vis Sci Technol 2016;5:11.
67. Ajaz A, Aliahmad B, Kumar H, Sarossy M, Kumar DK. Agreement study between color and IR retinal images based on retinal vasculature morphological parameters. BMC Ophthalmol 2019;19:27.
68. McGrory S, Taylor AM, Pellegrini E, Ballerini L, Kirin M, Doubal FN, *et al.* Towards standardization of quantitative retinal vascular parameters: Comparison of SIVA and VAMPIRE measurements in the Lothian birth cohort 1936. Transl Vis Sci Technol 2018;7:12.
69. Arnould L, Binquet C, Guenancia C, Alassane S, Kawasaki R, Daien V, *et al.* Association between the retinal vascular network with Singapore "I" vessel assessment (SIVA) software, cardiovascular history and risk factors in the elderly: The Montrachet study, population-based study. PLoS One 2018;13:e0194694.
70. Adiarti R, Ekantini R, Agni AN, Wong TY, Sasongko MB. Retinal arteriolar narrowing in young adults with glaucomatous optic disc. J Glaucoma 2018;27:699-702.
71. Arnould L, Guenancia C, Binquet C, Delcourt C, Chiquet C, Daien V, *et al.* Retinal vascular network: Changes with aging and systemic vascular disease (cardiac and cerebral). J Fr Ophtalmol 2022;45:104-18.
72. Thomas GN, Ong SY, Tham YC, Hsu W, Lee ML, Lau QP, *et al.* Measurement of macular fractal dimension using a computer-assisted program. Invest Ophthalmol Vis Sci 2014;55:2237-43.
73. Shah DN, Wilson CM, Ying GS, Karp KA, Cocker KD, Ng J, *et al.* Comparison of expert graders to computer-assisted image analysis of the retina in retinopathy of prematurity. Br J Ophthalmol 2011;95:1442-5.
74. Wilson CM, Cocker KD, Moseley MJ, Paterson C, Clay ST, Schulenburg WE, *et al.* Computerized analysis of retinal vessel width and tortuosity in premature infants. Invest Ophthalmol Vis Sci 2008;49:3577-85.
75. Marincowitz C, Webster I, Westcott C, Goswami N, de Boever P, Seidel G, *et al.* Vascular health assessment with flow-mediated dilatation and retinal image analysis: A pilot study in an adult population from Cape Town. Cardiovasc J Afr 2021;32:133-40.
76. Türksever C, López Torres LT, Valmaggia C, Todorova MG. Retinal oxygenation in inherited diseases of the retina. Genes (Basel) 2021;12:272.
77. French C, Heitmar R. Comparison of static retinal vessel caliber measurements by different commercially available platforms. Optom Vis Sci 2021;98:1104-12.

78. Forés R, Manresa JM, López-Lifante VM, Heras A, Delgado P, Vázquez X, *et al.* Relationship between retinal microvasculature, cardiovascular risk and silent brain infarction in hypertensive patients. Diagnostics (Basel) 2021;11:937.
79. Heitmar R, Kalitzeos AA, Patel SR, Prabhu-Das D, Cubbidge RP. Comparison of subjective and objective methods to determine the retinal arterio-venous ratio using fundus photography. J Optom 2015;8:252-7.
80. Cirla A, Drigo M, Ballerini L, Trucco E, Barsotti G. VAMPIRE(®) fundus image analysis algorithms: Validation and diagnostic relevance in hypertensive cats. Vet Ophthalmol 2019;22:819-27.
81. Perez-Rovira A, MacGillivray T, Trucco E, Chin KS, Zutis K, Lupascu C, *et al.* VAMPIRE: Vessel assessment and measurement platform for images of the REtina. Annu Int Conf IEEE Eng Med Biol Soc 2011;2011:3391-4.
82. INSPIRED Project. On the quantitative effects of compression of retinal fundus images on morphometric vascular measurements in VAMPIRE. Comput Methods Programs Biomed 2021;202:105969.
83. Tapp RJ, Owen CG, Barman SA, Welikala RA, Foster PJ, Whincup PH, *et al.* Retinal vascular tortuosity and diameter associations with adiposity and components of body composition. Obesity (Silver Spring) 2020;28:1750-60.
84. Lau AY, Mok V, Lee J, Fan Y, Zeng J, Lam B, *et al.* Retinal image analytics detects white matter hyperintensities in healthy adults. Ann Clin Transl Neurol 2019;6:98-105.
85. Lai M, Lee J, Chiu S, Charm J, So WY, Yuen FP, *et al.* A machine learning approach for retinal images analysis as an objective screening method for children with autism spectrum disorder. EClinicalMedicine 2020;28:100588.