

# RETINAL VESSEL DENSITY MEASUREMENT AS A TOOL FOR MICROVASCULAR RESEARCH

# BY

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A thesis submitted in fulfilment of the requirement for the degree of Master of Health Sciences (Optometry)

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#### ABSTRACT

This study aimed to evaluate the vessel density parameter as another measurement to study the morphological changes of retinal vessel. This study assessed the reliability of the vessel density parameter; provided the empirical evidence of fractal dimension (D<sub>F</sub>) as an indirect measure of the vessel density; investigated the effect of aging on the parameters and conducted a preliminary analysis of the ability of the parameters to distinguish between normal and diseased retinal states. A total of 200 coloured right eye fundus images were analyzed to obtain two quantitative parameters which were vessel density and D<sub>F</sub>. The coloured fundus images were transformed into black and white using MyVessel software. A circular area of approximately 2.6 optic disc radii surrounding the center of the optic disc was cropped using GNU Image Manipulation Program (GIMP) version 2.6.12 and the non-vessel fragments were deleted using the same software. The values of retinal vessel density and  $D_{\rm F}$  were obtained from the FracLac software version 2.5. The findings showed that the intragrader reliability of vessel density and D<sub>F</sub> as assessed using intraclass correlation coefficient (ICC) were 0.9975 (95% CI = 0.9954 - 0.9987) and 0.9645 (95% CI = 0.9340 - 0.9811), respectively. For intergrader reliability, the ICC were 0.9910 (95% CI = 0.9831 - 0.9831)0.9952) and 0.9725 (95% CI = 0.9484 - 0.9854) for vessel density and  $D_F$ , respectively. A strong correlation between vessel density and D<sub>F</sub> parameters was detected with Pearson's correlation coefficient, R of 0.942 (p<0.001). In response to increase in age, both vessel density and D<sub>F</sub> parameters showed declining trend, suggesting rarefaction of the retinal vessel. Preliminary analysis on the sensitivity of vessel density and D<sub>F</sub> parameters to differentiate between normal and diseased retinal states was obtained from the area under the receiver operating characteristic curve (AUC). The AUC of vessel density and  $D_F$  were 0.91 (p < 0.001) and 0.89 (p < 0.001), respectively. This study demonstrated the ability of the vessel density parameter to describe the difference between younger and older age groups. The vessel density parameter also showed high association with currently used geometrical parameter of retinal vessel, D<sub>F</sub>. Therefore, vessel density parameter may be considered as another parameter for quantitative analysis of retinal vessel.

### خلاصة البحث

تهدف هذه الدراسة إلى تقييم مؤشرات كثافةِ الأوعيةِ كمقياس آخرَ لدراسة التغيرات المورفولوجية في أوعية شبكيات العيون. قيمت هذه الدراسة صلاحيةً مؤشرٍ كثافةِ الأوعية؛ مع مراعاة الأدلةِ التحريبية للبعد الكُسُوري (D<sub>F</sub>) كمقياس غير مباشرٍ لكثافةِ الأوعية؛ وحققت في تأثير التشيُّخ على المؤشرات، وأجرت تحليلات أوليةً لمعرفة قدرة المؤشرات على التمييز بين الشبكيات السليمة والمصابة. تم تحليلُ 200 صورة مختلفةٍ لقاعة عين اليمني للحصول على مؤشراتٍ كميةٍ لكثافة الأوعية والبعد الكُسُوري. تم تحويل الصورِ الملونة لقيعانِ العيون الملونةِ إلى الأبيض والأسود باستخدام برنامج MyVessel. تم اقتصاصُ منطقةٍ دائريةٍ وسعها حوالي 2.6 نصف قطرٍ بصري حول مركز القرص البصري باستخدام برنامج معالجة الصور (GNU Image Manipulator Program - GIMP) نسخة 2.6.12 ، وتم حذف الشظايا الدخيلة باستخدام نفس البرنامج. تم الحصول على قِيمٍ كثافةِ الأوعية الشبكية والبعد الكُسُوري من برنامج FracLac نسخة 2.5. أظهرت النتائج أن صلاحية ما هو داخلَ المُقَيّمات (intergrader) لكثافة الأوعية والبعد الكُسُوري، كما قُيّمت باستخدام مُعامِل ارتباط ما هو داخل الطبقات ( interclass correlation coefficient, ICC)، وكانت النتائج كما يلي: 0.9975 و0.95% CI = 0.9954 – 0.9987 (0.9987 – 0.9987) لكثافة الأوعية و 0.9645 (95% ICC) للبعد الكُسُوري. أما لصلاحية ما هو داخل المُقَيِّمَات (intergrader) فكان الICC فكان الم 0.9910 ر0.9854 - 0.9831 CI %95) الكثافة الأوعية و 0.9725 ر0.975 (0.9854 - 0.9484 CI %95) للبعد الكُسُوري. تم الكشف عن علاقةٍ قوية بين مؤشراتِ كثافة الأوعية و البعد الكُسُوري مع معامل ارتباط بيرسون، R من 0.942 p) 0.942). استجابةً لزيادةِ العمر، أظهرت كلٌّ من مؤشراتِ كثافة الأوعية والبعد الكُسُوري نمطاً تراجعياً، مما أشار إلى خلخلةِ الأوعية في شبكية العين. تم الحصول على التحليل الأولي لحساسية مؤشرات كثافة الأوعية والبعد الكُسُوري للتمييز بين الشبكيات السليمة والمصابة من المنطقة التي تحتَ منحني التشغيل المُميَّزِ المستقبِل (AUC). كان AUC لكثافةِ الأوعية حوالي 0.91 (p<0.001) و 0.89 (p<0.001) للبعد الكُسُوري. أظهرت هذه الدراسة قدرةَ مؤشر كثافة الأوعية في وصف الفرقِ بين الفئات العمرية الأصغر والأكبر سناً. أظهرَ مؤشر كثافة الأوعية أيضاً ارتباطاً ملحوظاً مع المؤشِّر الهندسي المستخدَم حالياً مع أوعية الشبكية والبعد الكُسُوري. بناءً على ذلك، يمكن اعتبارُ مؤشر كثافة الأوعية كمؤشر آخرَ للتحليل الكمي لأوعية شبكية العين.

### **APPROVAL PAGE**

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Optometry).

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I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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## TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval page	iv
Declaration	V
Copyright Page	vi
Dedication	vii
Acknowledgements	viii
Table of Contents	ix
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xvii
List of Symbols	xix
CHAPTER ONE: INTRODUCTION	1
1.1 Background of the Study	1
1.2 Research Objectives	3
1.2.1 General Objective	3
1.2.2 Specific Objectives	3
1.3 Research Questions	3
1.4 Rationale and Hypotheses of the Study	4
1.5 Methodology Rationale	9
1.5.1 The Selection of Normal Retinal Samples and Determination	
of the Region of Interest	9
1.5.2 Quantitative Retinal Vessel Parameters Studied	11
1.5.3 Utilization of Public Retinal Image Databases: Digital Retinal	
Images for Vessel Extraction (DRIVE) Dataset and	
STructured Analysis of the Retina (STARE) Project Dataset	11
1.6 Organization of the Thesis	12
CHAPTER TWO: LITERATURE REVIEW	15
2.1 Embryologic Development of Retinal Vasculature	15
2.2 Anatomy of the Retinal Vascular Network	16
2.3 Geometrical Parameters of Retinal Vasculature	20
2.3.1 Retinal Vascular Calibre and Arteriovenous Ratio	21
2.3.2 Retinal Vascular Tortuosity	32
2.3.3 Retinal Vascular Bifurcation Angle	34
2.3.4 Arteriolar Length to Diameter Ratio (LDR)	36
2.4 Fractal Dimension as an Indirect Measure of Retinal Vasculature	
Complexity	36
2.4.1 Factors Affecting Retinal Vascular D <sub>F</sub> Measurement	39
2.5 Vessel Density Parameter as a Direct Measure of Retinal Vessel	
Morphology	42

3.1 Introduction	4
3.2 Methods	4
3.2.1 Research Design	4
3.2.2 Informed Consent	4
3.2.3 Material	4
3.2.3.1 Digitized Retinal Photograph	4
3.2.4 Research Tools	5
3.2.4.1 Navis-Lite Retinal Image Filing System	5
3.2.4.2 The Gnu Image Manipulation Program (GIN	MP)5
3.2.4.3 MyVessel Software	5
3.2.4.4 ImageJ Software and FracLac Version 2.5	5
3.2.5 Procedure of Data Collection	5
3.2.5.1 Reliability Analysis Procedure	5
3.2.5.2 Image Size Variation Analysis Procedure	e
3.2.6 Statistical Analysis	6
3.3 Results	6
3.3.1 Descriptive Analysis of the Dataset	6
3.3.1.1 Reliability Dataset	
3.3.1.2 Image Size Variation Dataset 3.3.2 Reliability Estimates of Vessel Density Param Aging Dataset	6 eter Using
<ul> <li>3.3.1.2 Image Size Variation Dataset</li> <li>3.3.2 Reliability Estimates of Vessel Density Param Aging Dataset</li> <li>3.3.3 Reliability Estimates of D<sub>F</sub> Parameter Using Aging I 3.3.4 Image Size Variation on Computed Vessel Density I 3.3.5 Image Size Variation on Computed D<sub>F</sub> Parameter</li> <li>3.4 Discussion</li></ul>	eter Using Dataset6 Parameter6
<ul> <li>3.3.1.2 Image Size Variation Dataset</li> <li>3.3.2 Reliability Estimates of Vessel Density Param Aging Dataset</li> <li>3.3.3 Reliability Estimates of D<sub>F</sub> Parameter Using Aging I 3.3.4 Image Size Variation on Computed Vessel Density I 3.3.5 Image Size Variation on Computed D<sub>F</sub> Parameter</li> <li>3.4 Discussion</li> <li>CHAPTER FOUR: RETINAL VASCULATURE FRACTAL DI WHAT DOES IT MEASURE?</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li> <li>3.3.2 Reliability Estimates of Vessel Density Param Aging Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset
<ul> <li>3.3.1.2 Image Size Variation Dataset</li></ul>	eter Using Dataset

ESSEL DENSITY AND $D_{\rm F}$	
5.1 Introduction	
5.2 Methods	
5.2.1 Procedure of Data Collection	89

5.2.2 Statistical Analysis	89
5.3 Results	89
5.3.1 Relationship between Vessel Density and Increasing Age	89
5.3.2 Relationship between D <sub>F</sub> and Increasing Age	92
5.3.3 Estimated Rate of Rarefaction in Vessel Density of Retinal	
Vessel	94
5.3.4 The Estimated Rate of Rarefaction in D <sub>F</sub> of Retinal Vessel	96
5.4 Discussion	97
CHAPTER SIX: PRELIMINARY STUDY: DIFFERENTIATION OF	
NORMAL AND DISEASED RETINAL STATE	100
6.1 Introduction	102
6.2 Methods	106
6.2.1 Collection of Retina Blood Vessel Segmentation	106
6.2.2 The Computation of Vessel Density and D <sub>F</sub>	107
6.2.3 Statistical Analysis	107
6.3 Results	108
6.3.1 Comparison of Vessel Density between Normal and Diseased	
Retinal Condition	108
6.3.2 Comparison of $D_F$ between Normal and Diseased Retinal	100
Condition	108
6.3.3 Assessment of the Sensitivity and Specificity of Vessel	
Density and $D_F$ Parameters in the Evaluation of Normal and	100
Diseased Retinal Condition	109
6.4 Discussion	110
CHAPTER SEVEN: GENERAL DISCUSSION	112
7.1 Limitations of the Study	112
7.2 Recommendations for Future Work	113
7.3 Summary	115
REFERENCES	116
ΔΡΡΕΝDΙΧ Δ. ΡΔΤΙΕΝΤ'S CONSENT FORM	128
APPENDIX R. FTHICAL APPROVAL	120
APPENDIX C: PERMISSION FOR COPYRIGHT	130
APPENDIX D: FRACLAC BOX COUNTING OPTIONS TO OBTAIN	
VESSEL DENSITY AND $D_F$ VALUE	131
APPENDIX E: OPHTHALMOLOGIST'S DIAGNOSIS OF STARE	
DATABASE RETINAL FUNDUS IMAGES	132
APPENDIX F: RELATED PUBLICATION	133
APPENDIX G: ABSTRACT FOR EXHIBITION & CONFERENCE	135

### LIST OF TABLES

Table I	<u>No.</u> <u>I</u>	Page No.
2.1	Computer-assisted Measurements of Retinal Vessel Calibre and Cardiovascular Risk Factors-related Studies.	26
2.2	Computer-assisted Measurements of Retinal Vessel Calibre and Brain Diseases-related Studies.	28
2.3	Computer-assisted Measurements of Retinal Vessel Calibre and Ocular Diseases-related Studies.	30
3.1	Reliability Estimates of Vessel Density Measurement.	65
3.2	Reliability Estimates of D <sub>F</sub> Measurement.	66
3.3	Reliability Estimate of Vessel Density Measurement between the Original and Enlarged Image Size.	68
3.4	Reliability Estimate of $D_F$ Measurement between Original and Enlarged Image Size.	69
4.1	Correlation between Vessel Density and D <sub>F</sub> .	79
4.2	Correlation between Vessel Density and $D_F$ of the DRIVE Dataset.	81
5.1	Polynomial Regression Analysis of Vessel Density Using Aging Dataset.	91
5.2	Polynomial Regression Analysis of D <sub>F</sub> Using Aging Dataset.	94
6.1	Comparison of Vessel Density between Normal and Diseased Retinal Condition from STARE Dataset.	108
6.2	Comparison of $D_F$ between Normal and Diseased Retinal Condition from STARE Dataset.	109

### LIST OF FIGURES

<u>Figur</u>	<u>e No.</u>	Page No.
1.1	Different Orientations of Grid Positions with Respect to an Image in a Box-counting Method. Source: Karperien (2013).	ı 4
2.1	Schematic Diagram of (A) Longitudinal Section of the Norma Capillary Structure Showed Pericytes Attached to the Basemen Membrane and (B) Cross-section of the Normal Capillary Structure Showed the Regular Arrangement of Endothelial Cells. Endothelia Cells Hold Together by Tight Junction which Formed Blood-retina Barrier.	l t l l 17
2.2	The Major Blood Vessel to the Eye Structure. Drawing by Dave Schumick. Source: Anand-Apte & Hollyfield (2010).	9 18
2.3	The Details of the Vascular Supply Through the Optic Nerve Head Drawing by Dave Schumick. Source: Anand-Apte & Hollyfield (2010).	I 19
2.4	The Measurement Zone of Retinal Vessel Calibre (Zone B) from a Digitized Retinal Image by Using Retinal Analysis Software (Department of Ophthalmology Visual Science, University of Wisconsin-Madison, WI). Source: Leung, Wang, Rochtchina, Tan Wong, Klein, Hubbard, & Mitchell (2003).	1 5 7 23
2.5	Example of A) Straight Venule with Lower Tortuosity Value (4.23x104) and B) Tortuous Venule with Higher Tortuosity Value (7.97 x 104) Measured Using SIVA Program. Source: Cheung Zheng, Hsu, Lee, Lau, Mitchell, Wang, Klein, & Wong (2011b).	, , , 33
2.6	The Measurement of the Bifurcation Angle between Two Daughter Veins of a 33 Year Old Female Sample.	35
2.7	Schematic Diagram of Self-similar Structure, the Small Magnified Structure Resembles the Largest Form. Source: Goldberger (1996).	l 37
2.8	Different Box Sizes Covering the Images of Interest and the Boxes that Contained Pixels are Counted. Source: Karperien (2013).	38
2.9	The Relationship Between Box Count Changes with the Box Scale Changes and the Determination of the $D_F$ Value from the Slope of the Linear Regression Line.	e f 39
2.10	Illustration of the Region of Interest (2.6 Optic Disc Radii) Foreground Pixels and Background Pixels. The Inner Bounding	,

Line is Called Hull Area.

3.1	Forty-five Degree Field of View of the Right Eye Digitized Retinal Image of a 35 Year Old Male Sample Captured by Non-Mydriatic Auto Fundus Camera Nidek AFC-230/210 (Nidek Co. Ltd., Aichi, Japan).	49
3.2	A) A Coloured Retinal Fundus Image of a 28 Year Old Male Sample, B) the Segmented Version of the Original Coloured Retinal Fundus from (A) Showed One and Half Arteriole and One Venule of the Inferior Nasal Region were not Segmented.	51
3.3	The Retinal Fundus Image of a 42 Year Old Malay Male Sample. This is the Example of Blurry Retinal Fundus due to Poor Focusing during the Image Capturing Process.	52
3.4	A Dark Retinal Fundus Background Image of a 41 Year Old Chinese Male Sample.	52
3.5	Myelinated Nerve Fiber in an 18 Year Old Malay Male Retinal Fundus which Obscures the Appearance of Retinal Blood Vessels in the Lower Retinal Fundus Region.	53
3.6	The Retinal Fundus Sample of a 17 Year Old Malay Male Sample. The Image Shows an Example of the Anisotropy Retinal Profile. The Optic Disc is Decentered.	53
3.7	The Step-by-step Procedure of Data Collection.	56
3.8	A) Forty-five Degree Field of View of the Right Eye Digitized Retinal Images as Captured by the Non-Mydriatic Auto Fundus Camera Nidek AFC-230/210 was Transformed by MyVessel Software into B) the Segmented Retinal Vessel, C) the Region of Interest (2.6 Optic Disc Radii) was Cropped and the Non-vessel Artifacts were Deleted Using GIMP software version 2.6.12 (www.gimp.org). D) The Magnified Region of Interest is Shown.	58
3.9	Computation of A) Vessel Density and B) $D_F$ Using FracLac Software Version 2.5.	59
3.10	Distributions of the A) Vessel Density, and B) $D_F$ as Obtained from the Intragrader for the First Time Grading Using the Reliability Dataset (n = 40).	61
3.11	Distributions of the A) Vessel Density, and B) $D_F$ as Obtained from the Intragrader for the Second Successive Grading Using the Reliability Dataset (n = 40).	62
3.12	Distributions of the A) Vessel Density, and B) $D_F$ as Obtained from the Intergrader.	63

43

3.13	Distributions of the A) Vessel Density, and B) $D_F$ of the Original Image Size (250 by 250 Pixels).	64
3.14	Distributions of the A) Vessel Density, and B) $D_F$ of the Resized Image Size (500 by 500 Pixels).	64
3.15	Intergrader Agreement of Vessel Density Using Bland-Altman Graphical Plot. The 95% CI of the Grading Difference Shows by the Dotted Lines.	65
3.16	Intergrader Agreement of $D_F$ Using Bland-Altman Graphical Plot. The 95% CI ( $\pm$ 1.96 SD) of the Grading Difference Shows by the Dotted Lines.	67
3.17	Bland-Altman Graphical Plot of the Agreement between Computed Vessel Density of 250 by 250 Pixel Images and 500 by 500 Pixel Images. The Region of Interest Remains the Same, but the Size of the Images was Enlarged.	68
3.18	Bland-Altman Graphical Plot of the Agreement between Computed $D_F$ of 250 by 250 Pixel Images and 500 by 500 Pixel Images. The Region of Interest Remains the Same, but the Size of the Images was Enlarged.	69
4.1	Distribution of the A) Vessel Density and B) D <sub>F</sub> of Retinal Vessel.	78-79
4.2	Relationship between Vessel Density and $D_F$ of Approximately 2.6 optic Disc Radii Cropping Region from the Aging Dataset.	80
4.3	Relationship between Vessel Density and $D_F$ of 40 Retinal Images Collected from the DRIVE Dataset.	81
5.1	A) The Complex Branching and Fractal-like Structure of Dendritic Arbor of the Giant Pyramidal Betz Cell of Motor Cortex in a Young Adult Man. B) Loss of Complexity in the Dendritic Arbor Structure in a 65 Year Old Man. Source: Lipsitz & Goldberger (1992).	86
5.2	The Heart Rate Time Series of, Top: a 22 Year Old Female Subject with the Mean Heart Beats per Minute of $64.7 \pm 3.9$ (Approximate Entropy was 1.09). Bottom: a 73 Year Old Male Subject with the Mean Heart Beats per Minute of $64.5 \pm 3.8$ (Approximate Entropy was 0.48). Approximate Entropy is a Measure of 'Nonlinear Complexity'. The Value Shows the 'Complexity' of the Signal from Older Subject is Markedly Reduced. Source: Lipsitz & Goldberger (1992).	87
5.3	Linear Regression Model of the Relationship between Vessel Density and Age of Aging Dataset ( $n = 200$ ).	90
5.4	Quadratic Regression Model of the Relationship between Vessel	

	Density and Age of Aging Dataset ( $n = 200$ ).	90
5.5	Cubic Regression Model of the Relationship between Vessel Density and Age of Aging Dataset ( $n = 200$ ).	91
5.6	Linear Regression Model of the Relationship between $D_F$ and Age of Aging Dataset (n = 200).	92
5.7	Quadratic Regression Model of the Relationship between $D_F$ and Age of Aging Dataset (n = 200).	93
5.8	Cubic Regression Model of the Relationship between $D_F$ and Age of Aging Dataset (n = 200).	93
5.9	Retinal Vessel Density Changes in the Study Samples with Age Ranged between 10 to 42 Years Old.	95
5.10	Retinal Vessel Density Changes in the Study Samples with Age Ranged between 43 to 73 Years Old.	95
5.11	Retinal $D_F$ Changes in the Study Samples with Age Ranged between 10 to 42 Years Old.	96
5.12	Retinal $D_F$ Changes in the Study Samples with Age Ranged between 43 to 73 Years Old.	97
6.1	Schematic Diagram of the Capillary System Connecting Arteries to Veins. Source: Encyclopaedia Britannica (By courtesy of Encyclopaedia Britannica Inc., copyright 2006; used with permission).	104
6.2	Comparison of Receiver Operating Characteristic (ROC) Curves of Vessel Density and $D_F$ Parameters. The Retinal Vessel Segmented Images were Taken from the STARE Database.	110

### LIST OF ABBREVIATIONS

a.	Artery
AMD	Age-related macular degeneration
ARIC	Atherosclerosis Risk in Communities
AUC	Area Under the ROC Curve
AV	Arteriovenous
AVR	Arteriovenous ratio
BMES	Blue Mountain Eye Study
BDES	Beaver Dam Eye Study
BH4	Tetrahydrobiopterin
BMI	Body mass index
CI	Confidence interval
CRAE	Central retinal artery equivalent
CRVE	Central retinal vein equivalent
d.f.	Degree of freedom
D	Diopter
$\mathbf{D}_{\mathrm{F}}$	Fractal dimension
DRIVE	Digital Retinal Images for Vessel Extraction
e.g.	( <i>exempli gratia</i> ): for example
et al.	( <i>et alia</i> ): and others
eNOS	Endothelial nitric oxide synthase
ETDRS	Early Treatment Diabetic Retinopathy Study
FAZ	Foveal avascular zone
σ	Gram
GIMP	Gnu Image Manipulation Program
HR	Hazard ratio
ie	( <i>id est</i> ): that is
ICC	Intraclass Correlation Coefficient
ID	Identification
IIIIM	International Islamic University Malaysia
IREC	IIIIM Research Ethics Committee
IRMA	Intraretinal microvascular abnormalities
I	Side length
	Length to diameter ratio
mm	Milimeter
MARP	Mean arterial blood pressure
MESA	Multi-Ethnic Study of Atherosclerosis
N/A	Not applicable
NIH	National Institutes of Health
NO	Nitric oxide
NTG	Normotensive glaucoma
OP	Odds ratio
DCE	Distributed growth factor
	Parifoval interconillary area
ГIА Deat	Performent and the performance of the performance o
	Posterior Reasivan Operating Characteristic
KUC	Receiver Operating Characteristic

ROI	Region of interest		
SD	Standard deviation		
SIVA	Singapore Eye Vessel Assessment		
SLM	Shape Language Modeling		
SPSS	Statistical Package for the Social Science		
STARE	STructured Analysis of the Retina		
TGF-β1	Transforming growth factor-β1		
TV	Television		
v.	Vein		
vs.	Versus		
VEGF	Vascular endothelial growth factor		
Wa	Calibre of the smaller branch vessel		
W <sub>b</sub>	Calibre of the larger branch vessel		
Wc	Calibre of the trunk vessel		
$W_1$	Width of the narrower branch		
<i>W</i> <sub>2</sub>	Width of the wider branch		
W	Width of the parent trunk		
Ŵ	Arteriole or venule parent trunk estimation		
WESDR	Wisconsin Epidemiologic Study of Diabetic Retinopathy		

## LIST OF SYMBOLS

β	Beta
К	Kappa
n	Sample size
р	Probability
R	Pearson product moment correlation
$R^2$	Coefficient of determination
μm	Micrometer
0	Degree
%	Percent
-	То
>	More than
<	Less than
$\geq$	Greater than or equal to
$\leq$	Less than or equal to
±	Plus-minus
Х	Times
=	Equal to
*	Statistical significance denotation

#### **CHAPTER ONE**

#### **INTRODUCTION**

Investigating retinal images using a computer analysis method is both of scientific and clinical importance as understanding the retinal vascular network would be useful in the detection of several systemic diseases and improving specific treatments of retinal disorders.

#### **1.1 BACKGROUND OF THE STUDY**

Image analysis has widely been used in medical field to aid in the diagnosis of medical images and improving clinician's confidence in the analysis of retinal images (Abràmoff, Garvin, & Sonka, 2010; Sharma, Sample, Zangwill, & Schuman, 2008; Patton, Aslam, Macgillivray, Deary, Dhillon, Eikelboom, Yogesan, & Constable, 2006). Recently, studies have shown a link between various retinal microvascular signs and both clinical and subclinical cerebrovascular (Kawasaki, Azemin, Kumar, Tan, Liew, Wong, Mitchell, & Wang, 2011; Ikram, de Jong, Bos, Vingerling, Hofman, Koudstaal, de Jong, & Breteler, 2006), cardiovascular (Wang, Liew, Wong, Smith, Klein, Leeder, & Mitchell, 2006; Witt, Wong, Hughes, Chaturvedi, Klein, Evans, McNamara, Thom, & Klein, 2006; Wong, Klein, Sharrett, Duncan, Couper, Tielsch, Klein, & Hubbard, 2002a) and metabolic outcomes (Grauslund, Hodgson, Kawasaki, Green, Sjølie, & Wong, 2009; Ikram, Janssen, Roos, Rietveld, Witteman, Breteler, Hofman, van Duijn, & de Jong, 2006; Witt et al., 2006).

Human retina has a unique property due to the direct accessibility of the circulatory system *in vivo*. Thus, offers an opportunity for researchers to study the structural features of the microcirculation. Advances in the retinal imaging technology

enable the image to be photographed, visualized and accessed at any time, without any need for mydriatic agent. The process of capturing the retinal image is easy and requires short time. Despite limited viewing of retinal blood vessel through the subjective clinical assessment, the revolution of digital imaging systems enables the retinal photographs to be captured with a larger field of view. Furthermore, the vascular network in terms of its branching, tortuosity as well as the abnormalities can be easily documented.

Several review papers have reported that the alteration in the retinal microvasculature possibly reflects similar changes in the cerebral and the coronary microvasculature due to the similar anatomical and physiological characteristics shared by these microcirculation systems (MacGillivray, Trucco, Cameron, Dhillon, Houston, & Van Beek, 2014; Cheung, Ikram, Sabanayagam, & Wong, 2012b; Ikram, Cheung, Wong, & Chen, 2012; Patton, Aslam, Macgillivray, Pattie, Deary, & Dhillon, 2005). Therefore, the findings from retinal vessel analysis may provide additional insight into early microvascular abnormalities before the appearance of clinical disease. Retinal arteries and veins represent the complexity of the retinal vessel branching system. Various geometric parameters have been employed by researchers in microvessel analysis, including vascular calibre, arteriovenous ratio, bifurcation angle, tortuosity, and fractal dimension.

Cheung, Tay, Mitchell, Wang, Hsu, Lee, Lau, Zhu, Klein, Saw, & Wong (2011a) reported that the quantitative assessment of retinal vascular structure is a precise and reliable method in investigating the relationship between microcirculation and cardiovascular disease. Recent studies focused on the geometrical analysis of retinal vascular branching pattern known as fractal dimension ( $D_F$ ).

#### **1.2 RESEARCH OBJECTIVES**

This study was aimed to achieve the following objectives:

#### **1.2.1 General Objective**

The goal of this study was to examine the vessel density parameter as another measurement to study the morphological changes of retinal vessel.

#### **1.2.2 Specific Objectives**

- i. To assess the intragrader and intergrader reliability of computer-assisted measurement of retinal vessel, as derived from computed vessel density and  $D_{\rm F}$ .
- ii. To provide the empirical evidence of  $D_F$  as an indirect measure of retinal vasculature density.
- iii. To investigate the mathematical model of vessel density and  $D_F$ , their trend and rate of changes in response to increase in age.
- iv. To conduct a preliminary analysis on the ability of vessel density and  $D_F$  parameters to distinguish between normal and pathological retinal condition.

#### **1.3 RESEARCH QUESTIONS**

- i. How reliable are the results from the quantification of the retinal vessel density and  $D_F$  measurements using computer-assisted method?
- ii. How does the vessel density measurement correlate with the  $D_F$  measurement of retinal vessel?

- iii. Does the correlation between vessel density and D<sub>F</sub> measurements will be affected when a different region of interest was used for retinal vessel analysis?
- iv. How does the vessel density measurement of retinal vessel structure affected when tested for the increase in age?
- v. How sensitive are the vessel density and  $D_F$  measurements to differentiate between normal and diseased retinal condition?

#### 1.4 RATIONALE AND HYPOTHESES OF THE STUDY

Fractal dimension  $(D_F)$  estimation employed a box-counting method which incorporates four different grids orientation in the computation. The illustration of the different orientation is depicted in Figure 1.1.



Figure 1.1 Different orientations of grid positions with respect to an image in a boxcounting method. Source: Karperien (2013).

Many studies focused on the D<sub>F</sub> to estimate the morphological changes in retinal vascular structure (Azemin, Kant, Wong, Jin, Mitchell, Kawasaki, & Wu, 2012; Li, Mitchell, Liew, Rochtchina, Kifley, Wong, Hsu, Lee, Zhang, & Wang, 2010; Liew, Wang, Cheung, Zhang, Hsu, Lee, Mitchell, Tikellis, Taylor, & Wong, 2008a; Masters, 2004; Landini, Misson, & Murray, 1993). These studies gave inconsistent results of  $D_{\rm F}$ , possibly due to different methodologies used by different investigators. The  $D_{\rm F}$ measurement is also dimensionless (Liew et al., 2008a) which makes it hard to do a comparative analysis on the subject. D<sub>F</sub> measurement can take a value for instance of 1.4858. However, there is no direct physical meaning that can be interpreted by merely looking at one single  $D_F$  value. Therefore, the main goal of this study was to examine vessel density parameter which may potentially serve as a tool to study the morphology of human retinal vessel. The vessel density parameter is also dimensionless, however, the parameter is able to produce more explicit results when using a single datum as compared to the retinal vessel complexity measurement using D<sub>F</sub>.

Previous studies on  $D_F$  were more on the elderly population aged 49 years old and older (Azemin et al., 2012; Li et al., 2010; Liew et al., 2008a). A study conducted by Landini et al. (1993) included data from a younger age group, ranging from 14 to 73 years old, however the number of subjects were very limited, only 23 subjects' data was used for the large age spectrum leading to low variations among samples. Moreover, Landini et al. (1993) hand-traced the retinal vessel and the method of image acquisition was invasive (Avakian, Kalina, Helene Sage, Rambhia, Elliot, Chuang, Clark, Hwang, & Parsons-Wingerter, 2002; Landini et al., 1993; Remky, Arend, & Hendricks, 2000). In the study with invasive approach, fluorescein dye was used.