ANALYSIS OF IMAGES OF THE HUMAN RETINA

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ABSTRACT

The microvasculature is implicated in the development of cardiovascular disease, and the eye offers a unique window through which the retinal microvasculature can be imaged and evaluated. Retinal fundus photography allows computer aided quantitative measurements characterising vascular geometry that are believed to relate to vascular disorder and cardiovascular risk.

Measurement of vessel diameters from non-fluorescein images is challenging for numerous reasons. A novel measurement technique, the Sliding Linear Regression Filter (SLRF) is proposed to overcome deficiencies in earlier approaches, and confidence gained in its performance by comparison with manual measurements by a skilled clinician.

Non-dimensional parameters are preferred to characterise the vascular geometry to avoid dependence on refraction of the eye. Departure of diameter relationships from optimal conditions at arterial bifurcations is hypothesised to indicate endothelial dysfunction and to predict risk of atherogenesis and cardiovascular risk. Other parameters of interest include vascular length/diameter ratios and tortuosity.

A computer based semi-automatic grading tool using the SLRF method has been developed and applied to images from the Beaver Dam Eye Study in a prospective case-control study to explore associations between retinal vascular geometry and incident death from ischemic heart disease (IHD) and stroke over a 10 year period. Results were obtained from 126 IHD and 28 stroke cases, together with 528 age and gender matched controls.

Disordered arterial bifurcation optimality was associated with death from IHD, consistent with the underlying hypothesis. Furthermore, this association appears independent of other recognised risk factors, suggesting that it adds additional prognostic value.

Elevated conventional arterial L/D ratio was associated with death from stroke, but this association was not maintained after adjustment for systolic blood pressure.

An interesting but unexpected association between reduced simple tortuosity and death from IHD was found, although this should be treated with some caution, due to eccentricities in behaviour of this parameter.
DECLARATION OF ORIGINALITY

This thesis reports on my own work, and all other inputs are appropriately referenced.

PUBLICATIONS ARISING

The following publications have arisen from the work reported in this thesis:


I gratefully acknowledge the support and guidance from my supervisors Dr Anil Bharath and Prof Simon Thom, and similarly from the Principal Investigators of the exploratory study based on images from the Beaver Dam Eye Study, Prof Simon Thom, Prof Alun Hughes and Prof Nishi Chaturvedi. In addition I appreciate several helpful suggestions from Prof Kim Parker.

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Dr Paresh Mistry who performed measurements of retinal vascular diameters through the cardiac cycle (Chapter 9).
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Median and middle quartiles of simple tortuosity by bin of path length
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# GLOSSARY OF TERMS

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<tr>
<td>AI</td>
<td>Artificial Intelligence</td>
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<td>ALSPAC</td>
<td>Avon Longitudinal Study of Parents and Children</td>
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<td>AMD</td>
<td>Age Related Macular Degeneration</td>
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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ARIC</td>
<td>Atherosclerosis Risk in Communities Study</td>
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<td>AVR</td>
<td>Arteriovenous Ratio</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>BP</td>
<td>Blood Pressure</td>
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<td>CAIAR</td>
<td>Computer Aided Image Analysis of the Retina</td>
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<td>CCD</td>
<td>Charge Coupled Device</td>
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<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CMOS</td>
<td>Complementary Metal-Oxide Semiconductor</td>
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<td>CRA</td>
<td>Central Retinal Artery</td>
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<td>CRAE</td>
<td>Central Retinal Artery Equivalent Diameter</td>
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<tr>
<td>CRVE</td>
<td>Central Retinal Vein Equivalent Diameter</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>DBP</td>
<td>Diastolic Blood Pressure</td>
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<td>DM</td>
<td>Diabetes Mellitus</td>
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<td>DR</td>
<td>Diabetic Retinopathy</td>
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<td>ECG</td>
<td>Electrocardiogram</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>ETDRS</td>
<td>Early Treatment Diabetic Retinopathy Study</td>
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<td>FA</td>
<td>Fluorescein Angiography</td>
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<tr>
<td>GUI</td>
<td>Graphical User Interface</td>
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<td>HbA1c</td>
<td>Glycosylated Haemoglobin</td>
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<td>HDL</td>
<td>High Density Lipoproteins</td>
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<td>ICDA</td>
<td>International Classification of Diseases Adapted</td>
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<td>IHD</td>
<td>Ischemic Heart Disease</td>
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<td>L/D</td>
<td>Length to Diameter</td>
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<td>LDL</td>
<td>Low Density Lipoproteins</td>
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<td>LoG</td>
<td>Laplacian of Gaussian</td>
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<td>LSD</td>
<td>Least Significant Difference</td>
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<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<td>NHS</td>
<td>National Health Service</td>
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<td>OCTA</td>
<td>Optical Coherence Tomography Angiography</td>
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<td>PDR</td>
<td>Proliferative Diabetic Retinopathy</td>
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<td>RISA</td>
<td>Retinal Image multiscale Analysis</td>
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<td>RPE</td>
<td>Retinal Pigmented Epithelium</td>
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<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<td>SLRF</td>
<td>Sliding Linear Regression Filter</td>
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<td>WESDR</td>
<td>Wisconsin Epidemiologic Study of Diabetic Retinopathy</td>
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<td>WMH</td>
<td>White Matter Hyperintensities</td>
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CHAPTER 1
INTRODUCTION

The fundus of the eye allows direct observation of the branching microvascular system overlying the surface of the retina. The invention of the ophthalmoscope (or fundoscope), a hand held instrument used to view the ocular fundus, is widely attributed to Hermann von Helmholtz in 1851 [98], although a model of a device intended to serve the same purpose is reported to have been shown by Charles Babbage some seven years earlier [98]. Using this new instrument, it is reported that Heymann described the signs of hypertensive retinopathy in 1856 [98]. In 1879, the eminent London neurologist, William Gowers first published his “Manual and Atlas of Medical Ophthalmoscopy” (later editions [39] also included pioneering work by the ophthalmic surgeon Marcus Gunn) which served to popularise the use of the ophthalmoscope in clinical practice. Subsequently ophthalmoscopy has become widely used to detect the vascular involvement of several disease processes, most notably hypertension [17] and diabetes [30].

In the context of hypertension, Keith, Wagener and Barker in 1939 [54] showed that signs of hypertensive retinopathy, including generalised and focal arteriolar narrowing, arteriovenous nicking, retinal haemorrhages, cotton-wool exudates and swelling of the optic disc were predictive of increased mortality in hypertensive patients. They described the well-known system bearing their names that categorizes hypertensive retinopathy into four grades representing increasing severity. More recent population based studies have confirmed that retinopathy predicts cardiovascular events [134][138], although modifications of the original Keith, Wagener and Barker scheme have been proposed [141] in recognition that the four original grades were not well associated with the severity of systemic disease. Nevertheless, the grading of hypertensive retinopathy is essentially a subjective process, suffering from poor reproducibility between observers [25], and its value in the routine management of hypertensive patients has been called into question.
because retinopathy is uncommon in mild hypertension, and half of people without retinopathy still have hypertension that poses a risk to cardiovascular health [123].

Hypertension and diabetes are both recognised risk factors for the development of atherosclerotic disease of the cardiovascular system, which is a major cause of global vascular morbidity and mortality, giving rise to ischemic stroke, ischemic heart disease (IHD) and peripheral vascular disease [44]. Although mortality rates for stroke and IHD have declined over recent decades (more steeply in developed countries) [44] they still accounted for close to one quarter of all deaths in 2016 among upper and upper middle income countries [145]. Atherosclerosis is an inflammatory disease, principally affecting the large and medium sized elastic arteries, but there is a growing recognition of the role of endothelial dysfunction in its early stages [97][107], and the potential involvement of smaller vessels. Rarefaction and remodelling of resistance arteries and arterioles are associated with hypertension, which may lead to injury of vessel walls and dysfunction of the endothelium [10][63][114]. Furthermore, endothelial function is important in the autoregulation of microvascular networks [41], such as in the retinal circulation, to maintain optimum hemodynamic conditions. These considerations suggest that altered microvascular structure or function may be associated with the early development of atherosclerotic disease.

Hemodynamic principles provide insight into geometrical features of microvascular networks that optimise their function. Murray in 1929 [78] gave theoretical consideration to the optimum geometry of a bifurcating vessel to minimise the power loss associated with junction, yielding the well known Murray’s law, and others have built upon his initial treatment. Chapter 2 of this thesis provides a survey of the physiological background and theoretical treatment of vascular geometry, together with evidence that healthy vascular systems tend to conform to Murray’s law, but that significant departures exist in the presence of disease. In particular, a number of previous studies have shown associations between abnormalities of retinal vascular geometry and systemic disease.

Based on the foregoing, it is hypothesised that abnormalities in the retinal vascular geometry are associated with dysfunction that predisposes the vascular system to
atherogenesis, and hence that such abnormalities should predict subsequent cardiovascular events. Unlike the subjective assessment of retinopathy, measurements of vascular geometry are expected to be objective and reproducible, and lend themselves to a high level of automation based on computerised image processing.

Red-free or colour photographs of the retina, suitable for analysis of vascular geometry, may be captured readily and comparatively non-invasively by modern retinal fundus cameras such as the Zeiss FF450plus (illustrated in Figure 1-1), routinely used at Imperial College London.

Figure 1-1: The Zeiss FF450plus retinal fundus camera in a clinical setting
The procedure for capturing retinal photographs is described in greater depth in Chapter 3, including a discussion of different imaging techniques and capture media. Traditionally, photographic film has been used for the purpose of recording retinal images, and several interesting retinal image were captured originally in this format. More recently direct capture of digital images by retinal cameras has become universal, based for example on Charge Coupled Device (CCD) sensors, offering a number of benefits. Film based image sets of interest have been scanned into digital form to facilitate subsequent analysis.

A typical retinal image with a 30° field of view, centred on the optic disc, is illustrated in Figure 1-2, highlighting the principal anatomical features. Both colour and red-free images are captured without in vivo contrast agent (unlike fluorescein angiography) and are thus suitable for routine clinical screening. However, the image of the retinal vessels is formed in such circumstances by a complex interaction of optical scattering, absorption and reflection processes, giving rise to an intensity profile across the retinal vessel that lacks distinct edges, and may also suffer several confounding features such as the central light-reflex, also referred to as the “light streak” or “silver-wiring”, that is sometimes observed. For this reason, measurements of retinal vessel diameter are particularly challenging in such images, and several measurement methods have been proposed in the literature. Chapter 4 reviews a number of such methods, highlighting drawbacks that have been perceived with these approaches.
In an attempt to overcome some of the drawbacks in existing methods, the author has proposed an alternative technique, known as the Sliding Linear Regression Filter (SLRF), described in Chapter 5, to perform measurements of retinal vascular diameters from red-free or colour images. The method involves use of a sliding window to assess the rate of change of intensity with distance across the vessel, in order to minimise the impact of noise, and considers the edge of the vessel to correspond to the position of greatest rate of change.

The magnification of a retinal image relating the size of a feature on the image to the actual size in the retina cannot be regarded as a constant, since it is dependent on the optical characteristics of a particular subject’s eye. In order to avoid the confounding effects of such variations, the work reported here has concentrated on non-dimensional parameters characterising the retinal vascular geometry, typically formed from ratios of distances, that are expected to be more robust. The parameters that are of particular interest here are:
- Diameter relationships at vascular bifurcations
- Bifurcation angles
- Length to diameter relationships of vascular segments joining bifurcations
- Vascular tortuosity

Careful attention is required to the procedures for measurement of such parameters to ensure good reproducibility from retinal images captured in a clinical environment. For example, the junction exponent is a widely used measure of the relationship of diameters at a bifurcation, but has been found to behave poorly in the presence of measurement noise. Furthermore, measurements of length to diameter ratios are influenced by the ability to visualise small vessels terminating a vascular segment that may be imperceptible in an image of impaired quality. Chapter 6 addresses such issues, and proposes measurement procedures and in some cases surrogate parameters to overcome such difficulties.

Chapter 7 describes a computer assisted tool, developed by the author, to apply the SLRF method to measure vascular diameters of selected vessels, and then compute the non-dimensional parameters characterising the retinal vascular geometry mentioned above.

Retinal images from the Beaver Dam Eye Study [59] represent an ideal data set in which to test the hypothesis that alterations in retinal vascular geometry predict subsequent cardiovascular disease. This prospective population-based study included 4926 individuals who were examined at baseline, including retinal photography, and were followed-up for morbidity and mortality (with cause determined from death certificates) over a 10 year period. In Chapter 8, an exploratory study is described to test the foregoing hypothesis, in which retinal vascular geometry was assessed using the computer assisted tool based on the SLRF method of vascular diameter measurements. Results are reported in a sub-set of 682 Beaver Dam subjects under 75 years of age, including 126 deaths from ischemic heart disease and 28 deaths from stroke over a 10 year period, supporting the hypothesis.
Finally in Chapter 9 the findings are discussed, and in Chapter 10 conclusions drawn, on the value of retinal vascular geometry assessment in the understanding and management of cardiovascular disease, including suggestions for follow-on studies.
2.1. Atherosclerotic Disease of the Large and Medium Arteries

Atherosclerosis is a progressive disease of the arterial wall in the large and medium sized vessels, initially exhibiting thickening of the intimal layer, and eventually leading to arterial stenosis through development of lesions containing deposits of lipid, fibrous connective tissue, calcium and/or blood clots [8][96][97][115]. The progression of the disease is normally slow, and while early signs of it can often be detected even in childhood, it is common for symptoms to occur only decades later. It is also a highly complex disease in which a range of different interactions between constituents of the blood and arterial wall are implicated. The pathogenesis of atherosclerosis involves a sequence of many steps in the formation of an atherosclerotic plaque, commencing with the penetration of plasma low density lipoproteins (LDL) through the endothelium, leading to an inflammatory reaction, foam cell formation, necrosis, smooth muscle cell proliferation and calcification. Atherosclerosis may initially obstruct blood flow, but damage to the arterial wall may eventually become sufficiently severe to provoke thrombus formation, either from plaque rupture (of thin cap atheromas) or erosion, with subsequent embolism leading to distal vascular events [8].

The prevalence and complexity of atherosclerosis have given rise to a growing and interdisciplinary approach to study of the disease. The influence of hemodynamics in atherogenesis has received notable attention. In 1971, Caro et al [13] compared the geometrical distribution of atheroma with the expected shear stress at these locations. This and subsequent work [8][37] have contributed to a body of evidence pointing to a correlation between the sites at which atherogenesis occurs and the existence of low shear, oscillating shear, and disturbed flow associated with low shear. The carotid bifurcation and the coronary arteries are among the most commonly affected vessels, leading to risk of stroke and myocardial infarction respectively.
The mechanisms by which these factors may act are not fully understood [8], but evidence suggests that chronic exposure to low shear stress leads to downregulation of endothelial nitric oxide synthase, leading to greater vulnerability to vascular injury [124].

Substantial evidence exists associating the extent and severity of atherosclerotic lesions with hypertension [37]. Under elevated blood pressure, lesions can be found in locations which are usually spared of disease, in addition to those normally predisposed to plaque formation. However, early atherosclerosis may be seen in clinically normotensive individuals, suggesting that hypertension may accelerate atherogenesis, rather than being a necessary factor. In hypertension, increased transmural pressure gradients may tend to increase diffusion of atherogenic substances into the intima, and additionally, increases in wall thickness and cross-sectional area may retard transmural clearance of lipids.

In addition to hypertension, other recognised risk factors for atherosclerotic disease include increasing age, smoking, hyperlipidemia, diabetes mellitus, obesity, and in the community-based Framingham Heart Study these conditions were all found to be associated with impaired endothelial function, determined by measurement of flow mediated dilatation [7].

Endothelial dysfunction is also associated with hypertension [10][63]. In the Multi-Ethnic Study of Atheroclerosis [105] it was found that reduced flow-mediated dilatation did not predict incident hypertension independently, suggesting that impaired endothelial function is generally a sequela of hypertension, rather than playing a major role in its development. However, evidence exists that the reverse order of events may occur, and that in certain individuals, endothelial dysfunction may predispose them to development of incident hypertension [10][63].

The involvement of endothelial dysfunction in atherogenesis has received growing recognition [107][124]. McLenachan et al [72] reported in 1991 the loss of endothelial-dependent dilatation in Macaca fascicularis monkeys at an early stage in the development of atherosclerosis, induced by a cholesterol rich diet, prior to the appearance of occlusive disease. Subsequent work led to the conclusion that “a
dysfunctional endothelium will generate a prothrombotic environment favouring
development of atherosclerotic lesions and thrombotic complications” [19].
Furthermore, endothelial function may be impaired by programming effects as well as
recognised cardiovascular risk factors as early as the first decade of life, and that
“endothelial function … appears to be of prognostic value independent of traditional
cardiovascular risk factors” [106].

2.2. Involvement of the Microcirculation

Given that endothelial function also plays a crucial role in the regulation of
hemodynamics in the resistance vessels [41], it may be postulated that altered
microcirculatory structure or function is also associated with the early development of
atherosclerotic disease, which is gaining increasing recognition [10].

The microcirculation has been shown by pressure profile analysis to be a major site of
vascular resistance [114], and hence of great importance in understanding the
mechanisms giving rise to hypertension. Some insight to the relevant factors can be
gained from the Hagen-Poiseuille law

\[ R = \frac{8L\eta}{\pi r^4} \] \hspace{1cm} \{2-1\}

giving the resistance \( R \) of a straight vessel of length \( L \) and radius \( r \), carrying fluid of
viscosity \( \eta \), assuming the flow is laminar and the fluid incompressible. In the case of
microvascular beds, there are a number of coupled parallel vessels, and the combined
total resistance of \( n \) such vessels is given by

\[ R_{tot} = \frac{1}{\sum_{i=1}^{n} \frac{1}{R_i}} \] \hspace{1cm} \{2-2\}

where \( R_i \) represents the resistance of the individual vessels.

From these relationships, it may be deduced that microvascular structure has a major
effect on vascular resistance and the following structural characteristics have been
implicated in hypertension [95][114]. It is unclear whether raised blood pressure (BP)
precedes or follows alterations in microvascular structure; animal models suggest that such structural alterations might exist in a prehypertensive phase prior to significant changes in BP, but data in humans is scarce [95].

- **Arteriolar lumen diameters.** The diameter has a powerful effect on vascular resistance, by virtue of the inverse fourth power in the Hagen-Poiseuille law. While this appears to be the primary mechanism of short-term control of resistance, the picture in hypertension is more complex. In early primary hypertension, arteriolar diameters appear not to be significantly changed, whereas in later stages, decreased diameters have been observed in various microvascular beds [114]. However, in secondary hypertension, decreases in arteriolar diameter appear to be consistent early indicators of the hypertensive process [114].

- **Arteriolar media:lumen ratio.** Evidence exists that an increase in this ratio (without significant change in the volume of wall tissue) in small arteries and arterioles is present in hypertension as a result of rearrangement of cells in the vessel wall (known as eutrophic remodelling) [95]. It is postulated that this is an important mechanism giving rise to increased resistance, due to the heightened effect of any hypertensive stimulus (referred to as the vascular amplifier hypothesis) although this remains subject to debate [95].

- **Microvascular rarefaction.** The term ‘rarefaction’ refers to a decrease in the number of parallel vessels, leading to an increase in resistance by virtue of the effect of equation \(2-2\). There is a good deal of evidence that microvascular rarefaction is an important pathogenic mechanism in hypertension, appearing even in young borderline hypertensive patients [114].

Barker *et al* [5] have found that low birth weight is a strong predictor of occurrence of hypertension later in life, suggesting that circulatory characteristics likely to give rise to hypertension may occur as early as the fetal stage.

Further insight into the function of the microvascular network can be gained from other characteristics of its architecture and geometry [130]. Murray, in 1926 [78] considered the geometry of an arterial bifurcation, seeking to minimise the power
required to maintain the circulation through the bifurcation, including the effect of
viscous drag, as well as the metabolic energy to maintain the blood volume. His
initial work studied the effect of the junction exponent $x$, defined by the relationship

$$d_0^x = d_1^x + d_2^x \quad \{2-3\}$$

where $d_0$, $d_1$, and $d_2$ are the diameters of the parent and daughter vessels respectively
(Figure 2-1).

![Illustration of a typical vascular bifurcation](image)

**Figure 2-1: Illustration of a typical vascular bifurcation**

The power required to maintain flow in a vessel is given by

$$f \Delta p = f^2 R \quad \{2-4\}$$

where $f$ is the volumetric flow, $\Delta p$ is the pressure drop along the vessel, and $R$ is the
resistance from Pouseuille’s Law in equation \{2-1\}. Evidently, due to the impact of
the $r^4$ term, the power cost of maintaining flow in small vessels is increased substantially, but this is offset to some extent by the power required to maintain the blood volume. Assuming that the latter varies linearly with volume, the associated power loss can be represented by

$$mLr^2$$ \hspace{1cm} \{2-5\}

where $m$ is a metabolic constant. Summing the two contributions to power loss, and differentiating to find the minimum with respect to the radius $r$, it follows that under optimal conditions of minimum power loss, the flow $f$ is given by

$$kr^3 \text{ where the constant } k = \frac{m\pi}{\sqrt{16\eta}}.$$ \hspace{1cm} \{2-6\}

Since flow is conserved at a bifurcation, it may be stated that

$$f_0 = f_1 + f_2$$ \hspace{1cm} \{2-7\}

from which it follows that the optimum value of the junction exponent $x = 3$ minimises power loss in the junction, a result known as Murray’s Law.

The derivation of Murray’s law relies upon the Hagen-Poiseuille equation \{2-1\} which assumes laminar, non-accelerating flow of an incompressible fluid. Flow in large vessels is pulsatile and in certain circumstances may be turbulent, and hence better compliance with Murray’s law might be expected in smaller vessels where the underlying assumptions are more closely satisfied.

Sherman [104], and LaBarbera [62], based on data from a variety of other workers, have shown that with the exception of the very largest vessels, both arteries and veins appear to follow Murray’s law well.

Griffiths et al [41] suggested that endothelium-derived relaxation factor (EDRF), known to be nitric oxide, could be responsible for long-term regulation of arterial calibre in accordance with Murray’s Law. He subsequently demonstrated [42] that the junction exponent in the vasculature of a rabbit ear was maintained close to the
Murray optimum of 3, whereas when EDRF was inhibited, the junction exponent was altered. Furthermore, Hutchins et al [50] in examination of human small coronary arteries found junction exponents to be consistent with Murray’s Law in normal subjects, but observed a reduction in junction exponent in the circumstance of heart disease.

These findings raise the prospect that departures from the optimal configuration predicted by Murray might be indicative of endothelial dysfunction, and hence reflect increased risk of subsequent atherosclerotic disease.

Subsequent work by Murray [79] considered the impact of the conservation of flow (equation 2-7) on the optimum branching angle at an arterial bifurcation, based on the principle of virtual work, and concluded that the minimum angle should be 75°, although noting that smaller angles are often found. Later work by Zamir et al [150][151] went on to address the effect of asymmetry in bifurcating vessels, defining a non-dimensional asymmetry ratio

$$\alpha = \frac{d_2^2}{d_1^2} \quad \{2-8\}$$

and deriving expressions for the angles $\theta_1$ and $\theta_2$ (Figure 2-1) that the larger and smaller daughter vessels make respectively with the direction of the parent vessel, as a function of the asymmetry ratio $\alpha$. The angle relationships were derived under four different optimisation criteria, these being

- minimisation of lumen surface area
- minimisation of lumen volume
- minimisation of power required
- minimisation of total drag force acting on the lumen walls

yielding a different relationship between $\theta_1$, $\theta_2$ and $\alpha$ in each case.

From the derived relationships, two general qualitative principles were established by Zamir [150], regardless of the applicable optimisation principle. Firstly, “when a
blood artery gives rise to a relatively small branch, the angle of that branch will be close to a right angle and the parent vessel will continue almost unchanged in both size and direction”. Secondly, “when a blood artery undergoes a bifurcation, the larger branch will make a smaller angle with the direction of the parent artery than will the smaller branch”.

Measurements of arterial bifurcation angles from retinal images have been presented [152], exhibiting considerable scatter around the theoretically derived curves, suggesting that more than one optimality principle may be involved, although with some bias towards the principles of minimising lumen volume and pumping power, which is consistent with the optimisation criteria adopted in Murray’s Law.

In the most general case, considered by Woldenberg and Horsfield [132], the theoretically derived optimum total bifurcation angle

\[ \psi = \theta_1 + \theta_2 \]  

will depend, for a specified optimality principle, on the junction exponent \( x \) as well as the asymmetry ratio \( \alpha \). For a moderately symmetrical bifurcation (say \( \alpha >0.4 \)) the expected angle \( \psi \) is comparatively insensitive to \( \alpha \), and for a bifurcation compliant with Murray’s Law and subject to the principle of minimum power loss, the expected angle \( \psi \) may be regarded as a constant 75 degrees. However, deviations from this value are predicted as the junction exponent \( x \) varies from the Murray optimum of 3.

One of the limitations of Murray’s approach is that it considers only a single bifurcation in isolation, without taking into account the resistance of the distal components of the remaining vascular tree. Zhou and Kassab [154] [53] sought to address this shortcoming by adopting a vascular model consisting of a ‘stem’ vessel joining successive bifurcations, and a ‘crown’ which is considered to be the entire vascular tree distal to the stem (as far as the first capillary bifurcation). Based on the self similar, fractal nature of the tree, and adopting an optimisation criterion to minimise the total power loss (including resistance and metabolic components as assumed by Murray), they derived a series of ratios linking volume, flow, cumulative...
arterial length and vascular diameters at junctions between the stem and the crown. Of particular interest is the relationship

\[
\frac{Q}{Q_{\text{max}}} = \left( \frac{D}{D_{\text{max}}} \right)^{\delta} \quad \{2-10\}
\]

relating flow \(Q\) and diameter \(D\) of a vessel to those values \(Q_{\text{max}}\) and \(D_{\text{max}}\) in the most proximal stem, where

\[
\delta = \frac{4(e' + 1)}{3e' - 2} \quad \{2-11\}
\]

corresponds to the optimal junction exponent, and the parameter \(e'\) represents the ratio of metabolic to viscous power loss.

Furthermore

\[
\frac{D}{D_{\text{max}}} = \left( \frac{L}{L_{\text{max}}} \right)^{\chi} \quad \{2-12\}
\]

relates the diameters as defined previously to the cumulative length of the crown \(L\) and the value for the entire tree \(L_{\text{max}}\), where

\[
\chi = \frac{1}{\delta} \quad \{2-13\}
\]

providing insight into the progression of the geometry of the tree into its distal regions.

A consequence of this approach is that the optimum junction exponent resulting from this model is not a constant as predicted by Murray, but is dependent on the physiological parameter \(e'\). Kassab [53] presented results derived from anatomical data of 18 different arterial trees in a variety of species, indicating junction exponents ranging from 2.06 to 4.18 in different trees. In later work Huo and Kassab [49] found agreement with Murray’s law in arterioles, but with reduced junction exponents in larger vessels (of higher Strahler order [68]).
2.3. Early Findings in Retinal Vascular Geometry

The retinal circulation is an end-arterial system that does not anastamose [90]. On entering the eye at the optic disc, the central retinal artery bifurcates into the superior and inferior papillary arteries, that in turn bifurcate into temporal and nasal branches. The diameter of the central retinal artery has been estimated to be in the region of 200 μm [137], and the temporal and nasal branches distal to the second division of the central retinal artery are considered to have the structure of arterioles, rather than arteries [113]. Retinal arterioles exhibit autoregulation through endothelial action, but are not innervated by the autonomic nervous system (unlike the choroidal vessels) [90][29], and thus measurement of geometry in such vessels is not confounded by autonomic activity.

One of the earliest geometrical parameters to receive attention in the retinal vasculature has been the ratio of arterial to venous diameters, generally known as the arteriovenous ratio (AVR), as described by Stokoe and Turner in 1966 [113] although citing references in the literature as early as 1879. Interest in AVR was focussed on quantification of generalised arterial narrowing associated with hypertension and ageing, whereas normalisation by the venous diameter provides a non-dimensional measure that is more robust to variations in the refractive characteristics of the eye.

Stoke and Turner highlighted the necessity to ensure that the arterial and venous diameter measurements contributing to the ratio are obtained from a comparable order of division in their respective trees, and noted that “this is difficult, tedious, and often impossible by casual ophthalmoscopy, or even by retinal photography”, since the arterial and venous trees tend to be dissociated, especially in the peripheral regions. More recently, this issue has been overcome, based on work by Parr and Spears [83][84] and subsequently Hubbard [46] by relating arterial and venous diameter measurements from within the vascular trees to equivalent diameters of the central retinal artery and vein respectively, which can then be used to calculate AVR.

Parr and Spears [84] performed an analysis of diameter measurements at arterial bifurcations from a series of red-free retinal images from normotensive adults, and then went on to derive an empirical formula giving parent arterial diameter as
\[ d_{a_0} = \sqrt{0.87d_{a_1}^2 + 1.01d_{a_1}^2 - 0.22d_{a_1}d_{a_2} - 10.76} \] \{2-9\}

where \( d_{a_1} \) and \( d_{a_2} \) are the diameters of the larger and smaller daughter vessels respectively, all diameters being measured in \( \mu \text{m} \). Hubbard [46] went on to derive a similar formula for venous trees, giving the parent venous diameter as

\[ d_{v_0} = \sqrt{0.72d_{v_1}^2 + 0.91d_{v_1}^2 + 450.05} \] \{2-10\}

where the daughter venous diameters are represented by the same nomenclature, again with all diameters measured in \( \mu \text{m} \).

In order to apply their technique to calculate the equivalent diameter of the central retinal artery, Parr and Spears [83] proposed reconstruction of the entire arterial tree, a complex and time-consuming process. However, Hubbard [46] proposed a simplified algorithm in which the pairing of daughter vessels in actual bifurcations are disregarded, and instead, the largest measurement is paired with the smallest, the next largest with the next smallest, and so on, and the parent vessel diameter is calculated from the assigned pairs. The resulting parent diameters (together with any odd vessel) are carried to the next order of division, where the process is repeated until a single parent diameter is obtained, representing the equivalent diameter of the central retinal vessel diameter. This process is illustrated in Figure 2-2 which represents a series of diameter measurements, which are typically made within an annular ring superimposed on a disc centred retinal image, and then paired so that their parent diameters can be calculated, eventually yielding the equivalent central arterial (CRA) diameter. A similar process is performed for the venous tree, allowing the AVR to be calculated from the ratio of the equivalent diameters of the central retinal artery and vein.
Evidence of the potential of AVR [130], measured using the Parr, Spears and Hubbard method, to predict cardiovascular disease has come from prospective population based studies, such as the Atherosclerosis Risk in Communities (ARIC) [2] as well as the Beaver Dam Eye Study introduced earlier. Reduced AVR has been shown to predict incident hypertension over a 3 year period in ARIC [139] and over a 10 year period in Beaver Dam [142]. Furthermore, in ARIC, reduced AVR was shown to predict stroke independently of other risk factors [134], coronary heart disease in women (although not men) [135], and type II diabetes mellitus [136]. In Beaver Dam, reduced AVR was found to predict cardiovascular death in a small cohort of 879 younger persons (aged 43-74 years), although not in older subjects [138].
However, in the full Beaver Dam cohort of 4926 subjects, an interesting U shaped association was found, in which unusually small or large values of AVR were associated with increased mortality over 10 years, either from all causes, or specifically from vascular disease [140].

It has been pointed out [130] that the empirical Parr and Spears formula was derived from observations in healthy normotensive individuals, in which good adherence to Murray’s law might be expected. Comparison of the diameter relationships at junctions implied by Parr and Spears with those predicted by Murray confirms a strong correlation over a wide range of bifurcations, normally within a tolerance of better than ± 0.05% of parent diameter, the only exceptions being in highly asymmetrical junctions. Against this background, it seems credible to suggest that AVR measured by the Parr, Spears and Hubbard approach may be sensitive not only to general arterial narrowing, but also to deviations from the optimum bifurcation geometry predicted by Murray, which may provide some insight into the aforementioned U shaped association between AVR and cardiovascular disease.

Other characteristics of microvascular geometry have also received attention in the retina. Stanton et al [110] found in a study of 100 subjects (74 untreated hypertensives and 26 normotensive volunteers) that the ratio of arterial to venous vascularity, defined as the sum of arterial and venous diameters respectively, decreased significantly with increasing blood pressure. This finding is consistent with the expectation that arterial rarefaction and generalised narrowing gives rise to increased peripheral resistance.

In a further study [111], based on manual measurements from fluorescein retinal angiograms taken from a sample of 25 subjects, divided into normotensive and hypertensive groups, a significant decrease was shown in the junction exponent $x$ with age, which concurs with other reports of endothelial dysfunction with ageing [28]. Additionally, in the same study, a reduction of bifurcation angle $\psi$ in hypertensives was found in comparison with normotensives, and a reduction of $\psi$ in both groups with age.
King et al [56] introduced another geometrical parameter, the arterial length to diameter (L/D) ratio, measured over a vessel segment connecting consecutive bifurcations. In a small study of 12 individuals, half untreated hypertensives, and the remainder normotensive, they found significantly increased L/D ratios in the hypertensives, and also significantly narrower bifurcation angles. The finding in L/D ratios is again consistent with the expectation of arterial rarefaction and generalised narrowing in hypertension, although a potential confounding factor in this study was a substantial difference in age (approx. 20 years) between the two groups. The findings of reduced bifurcation angle $\psi$ in hypertension were also consistent with the earlier study mentioned above [111].

Interesting associations have been reported between low birth weight and increased cardiovascular mortality [4] as well as increased prevalence of hypertension [64] [21]. These findings have been reinforced by Poulter et al [89] who have found similar associations in same sex twins, suggesting that the effect is independent of maternal and environmental characteristics. Chapman et al [14] found a significant reduction in retinal bifurcation angles in men of low birth weight, independently of blood pressure, indicating an association between low birth weight and altered microvascular architecture. Narrowed bifurcation angles are predicted to give rise to reduced circulatory energy efficiency, and may also be associated with reduced microvascular density [55]. Hence these results may point to a mechanistic link between low birth weight and subsequently increased cardiovascular risk.

These findings lend support to the view that altered microvascular architecture may be evident in the early stages of conditions giving rise to increased cardiovascular risk, when they may be largely asymptomatic.
CHAPTER 3
ACQUISITION OF RETINAL IMAGES

This chapter provides a review of the process of retinal image acquisition, typically employed in a clinical setting. It commences with a brief description of the anatomy and optics of the eye, followed by a description of a mydriatic fundus camera, using as an example the Zeiss FF450plus. It goes on to consider the different techniques for retinal imaging, with particular attention to the capture of red-free images. Alternative media, onto which retinal images may be captured, are also discussed. Photographic film has traditionally been used, but direct capture by digital sensors offers several benefits and is increasingly common.

3.1 The Eye

The anatomical structure of the eye was described by Gray in 1918 [40], and his illustration of a horizontal section through the eyeball is reproduced in Figure 3-1 below.

The optical path through the eye which focuses the desired image into the retina includes

- the cornea, a dense, circular, transparent, non-vascular structure that projects in front of the sclera

- the anterior chamber, containing the aqueous humour, consisting predominantly of water with small amounts of sodium chloride

- the iris, which forms a variable size circular aperture (the pupil), regulating the amount of light entering the eye

- the lens, the curvature of which is controlled by the ciliary muscle, to adapt the optical path to focus near or far field objects
the vitreous humour, a transparent viscous substance consisting of water, albumin and some salts that is contained within the hyaloid membrane; a prolongation of the latter forms the hyaloid canal, containing lymph fluid, running from the centre of the optic nerve to the lens.

In order to model the optical behaviour of the eye, a number of schematic eyes have been proposed [108], of which one of the most widely used is the Gullstrand eye no 1. This is an example of a paraxial model and incorporates many simplifications, including the specification of spherical refracting surfaces which are rotationally symmetric, the coincidence of visual and optical axes, and constant refractive index of the lens. A real eye does not conform to these; surfaces are aspheric, the optical and
visual axes differ by around 5°, and the refractive index varies throughout the lens. Accordingly, such models do not predict well aberrations at large pupil diameters or over wide angles.

The Gullstrand No 1 model is specified in terms of a number of refracting surfaces at positions (for a relaxed eye) as summarised in Table 3-1 below.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Surface</th>
<th>Radius (mm)</th>
<th>Refractive Index</th>
<th>Thickness (mm)</th>
<th>Position (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>Anterior</td>
<td>7.700</td>
<td>1.376</td>
<td>0.500</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>6.800</td>
<td>1.376</td>
<td>0.500</td>
<td>0.500</td>
</tr>
<tr>
<td>Aqueous</td>
<td></td>
<td></td>
<td>1.336</td>
<td>3.100</td>
<td></td>
</tr>
<tr>
<td>Anterior lens cortex</td>
<td>Anterior</td>
<td>10.000</td>
<td>1.386</td>
<td>0.546</td>
<td>3.600</td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>7.911</td>
<td>1.386</td>
<td>4.146</td>
<td></td>
</tr>
<tr>
<td>Lens core</td>
<td></td>
<td></td>
<td>1.406</td>
<td>2.419</td>
<td></td>
</tr>
<tr>
<td>Posterior lens cortex</td>
<td>Anterior</td>
<td>-5.760</td>
<td>1.386</td>
<td>6.565</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Posterior</td>
<td>-6.000</td>
<td>1.386</td>
<td>7.200</td>
<td></td>
</tr>
<tr>
<td>Vitreous</td>
<td></td>
<td></td>
<td>1.336</td>
<td>17.185</td>
<td>24.385</td>
</tr>
<tr>
<td>Retina</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3-1: Characteristics of the Gullstrand schematic eye no 1 [108]

The retina is the structure of light sensitive neural cells that forms the image and conveys it to the brain via the optic nerve. A particularly high concentration of such cells is found in the macular region of the retina, and particularly at the fovea, where the most detailed vision is available. At the posterior surface of the retina is the retinal pigmented epithelium (RPE), a layer of pigmented cells containing melanin, that absorbs shorter wavelength light [24]. A highly reflecting layer also lies at or close to the RPE [24]. Posterior to the RPE is the choroid, a heavily vascularised layer of tissue also containing melanin.
The retinal circulation is connected to the central retinal artery and vein, which are embedded within the optic nerve and enter the eye via the optic disc, before bifurcating into superior and inferior branches, and again into the nasal and temporal arcades. The retinal vessels are embedded in the neural tissue, very close to the anterior retinal surface, and are hence readily visualised by the ophthalmoscope and photographed by the retinal fundus camera.

### 3.2 The Fundus Camera

The optics of the retinal fundus camera are designed to capture plane images of the retina, taking into account that the retinal surface is essentially hemi-spherical, and also that the eye itself introduces additional refraction into the optical path. A schematic diagram of the optical path through a typical mydriatic fundus camera, as shown by Suzuki [116], is reproduced below in Figure 3-2.

![Schematic illustration of optical path of retinal fundus camera](image)

**Figure 3-2: Schematic illustration of optical path of retinal fundus camera [116]**

A classical mydriatic camera requires dilation of a subject’s pupil (mydriasis) prior to retinal photography. Conventionally, the pupil of the eye to be imaged is dilated 15 mins minutes prior to photography by administration of a topical mydriatic agent (e.g.
tropicamide). An example of a mydriatic retinal camera is the Zeiss FF450plus, illustrated in Chapter 1; for this camera, a pupil diameter of at least 4mm is necessary, and ideally 6mm is desirable.

The camera must illuminate the ocular fundus in order to form an image from a combination of reflection, scattering and absorption processes. Two sources of illumination are normally provided; continuous low level illumination is used for setting up and focussing the image by the operator via an eyepiece, and once this has been achieved, a short duration higher intensity Xenon flash is used (via a similar optical path as the low level illumination) to capture the image onto the media or sensor. Provision is made to introduce a variety of colour filters in front of the light/flash source, for different imaging techniques, as discussed later.

Specific details of the Zeiss design are provided by Bengtsson and Krakau [6]. The illumination from the fundus camera is focussed into an annular ring that is optimally positioned at the principal plane of the subject’s eye. Within this ring, an aperture exists through which light emerging from the ocular fundus reaches the camera, as illustrated in Figure 3-3. Provided the plane of focussed illumination is correctly positioned by the operator, this arrangement ensures that any illuminating light dispersed by the tear film at the corneal surface does not interfere with the capture of the image. Incorrect positioning of the camera will be readily apparent to the operator, since flare will be visible at the edge of the image, and overall contrast will be impaired.

The illuminating ring has an outer diameter of ~6.5mm, and an inner diameter of ~4mm (measured on the Zeiss FF450plus camera). The capture aperture is given by Bengtsson [6] to be “about 2mm in diameter”.

The operator of the camera must first adjust the eyepiece to correct for any ametropia of their own eye, so that the cross-hairs in the eye-piece are clear and in focus. The subject is positioned in front of the camera, with chin and forehead placed on supports at the front of the camera to provide a stable position, after which the camera is positioned such that the retina can be perceived through the eyepiece. External fixation devices are provided on which the subject can focus with the eye not being photographed, to promote a stable eye position. The position of the field of view on the retina is selected by the photographer through movement of the fixation device. For patients with poor vision in the other eye, an internal fixation option is also available which appears within the field of view of the eye under examination. However, the latter device results in a silhouette in the resulting image, which may hinder subsequent analysis.

The position of the camera in relation to the subject’s eye can be adjusted finely to optimise the image by means of a mechanical positioning arrangement, driven by a small joystick at the rear of the camera. The focus is adjusted by the operator so that the retina and vessels appear clear, and the image is captured by depression of a button on the top of the joystick.
The particular characteristics of the Zeiss FF450+ [160] can be summarised as follows:

- Three field of view angles are available, user selectable at 20°, 30° or 50°.

- Retinal image size on 35mm film is 26mm diameter, vertically limited to 24mm.

- Design distance from front objective lens to subject’s eye is 42mm.

- The eyepiece can be adjusted for ametropia of the operator’s eye in the range ±8D.

- The focus range of the wide angle objective lens is able to compensate for ametropia of the subject’s eye in the range ±30D.

- Several camera ports are available, to which can be attached a variety of cameras or image sensors, although normally only a single port can be active at a time. The active port is selected on the operator’s control panel and effected through adjustment of the internal optical path by motorised actuators. The first (bottom) camera port is suitable for a 35mm film Single Lens Reflex camera, whereas the remaining (top) ports are normally available for electronic image sensors.

- The exposure of the photographic image is controlled by the duration of the flash. It is adjusted through the operator’s control panel in steps ±0.25 EV.

An alternative type of retinal camera, termed non-mydriatic, allows retinal photographs to be acquired without administration of a mydriatic agent. Typically a subject will be kept in a darkened room for a few minutes to allow the pupils to dilate naturally. A non-mydriatic camera uses infra-red illumination in conjunction with electronic sensors to compose and focus the image, so as to avoid constriction of the pupil. The image is then acquired using a white light flash as in a conventional camera, so it is not possible to take a sequence of photographs of the same eye, since the flash will provoke pupil constriction. Non-mydriatic cameras typically capture retinal images with a 45° field of view.
A further characteristic of the retinal photographic process which is of particular interest is the magnification relating the actual dimension of a feature on the retina (e.g. a vessel) with its dimension on the captured image. This has been addressed by Littman [66] and later by Rudnicka et al [99], who adopted the following nomenclature and equation for small features that allow paraxial approximations to be made:

\[ t = pqs \]

where

- \( t \) is the true size of a feature on the retina (mm)
- \( p \) is a camera correction factor (°/mm) relating the angular dimension of a retinal feature exterior to the eye with the size of the feature in the plane of image capture
- \( q \) is a characteristic of the subject’s eye (mm/°) relating the true dimension of a retinal feature to the angular dimension exterior to the eye
- \( s \) is the size of the feature on the fundus image (mm).

The Zeiss camera is designed according to the principle of telecentric optics, meaning that a specified distance on the image plane (e.g. captured on film) corresponds to a constant angular dimension throughout a wide ametropic range in the subject eye [66]. Under such conditions, the parameter \( p \) above is purely a characteristic of the fundus camera, and can be regarded as a constant for a given field of view, provided the camera is aligned and focussed correctly. In addition to the Zeiss, a number of other retinal fundus cameras also employ a telecentric design, and although the exact value of \( p \) will vary between them, its value for a 30° field of view is typically in the region of 1.3 °/mm for an image captured on 35mm film [99]. However not all retinal cameras are telecentric, and for those that are not, the value of \( p \) is not constant, but normally varies linearly with the refractive properties of the subject eye [99].
The value of $q$ is in all cases a characteristic of the subject’s eye, independent of the design of the retinal camera. Littman [66] discusses the parameters of the eye influencing the value of $q$, and concludes that it is most strongly dependent on the anterior corneal radius ($r_1$) and the axial length, whereas the thickness of the cornea, the depth of the anterior chamber, and the thickness of the lens have such a small effect that they can be disregarded. The posterior corneal radius is also important, but can be accounted for by assuming that it is linked to the radius of the anterior surface by a constant factor. The value of $r_1$ can be readily measured in the clinic by use of a keratometer. It is also possible to measure the axial radius by ultrasound which may give the best accuracy, but this is an unwelcome procedure, and can be avoided by instead measuring the overall ametropia ($A$) as a surrogate, and using a set of published curves [66] to derive the value $q$ from measurements of $r_1$ and $A$. For the Gullstrand schematic eye no 1 (Table 3-1), the value of $q$ is equal to 29.8 mm/°.

From the foregoing, it is evident that deriving absolute measurements of features on the retina adds complication due to the dependence on the characteristics of the eye and the consequent need for additional measurements of its optical characteristics. Accordingly, emphasis is placed in this work on the measurement of dimensionless characteristics of the retinal vasculature, as discussed in Chapter 6, that avoid the explicit need to consider the magnification.

### 3.3 Retinal Fields

The existing procedures for clinical photography of the retina have been developed to support grading of retinal pathology such as diabetic retinopathy or age related macular degeneration (AMD) rather than routine surveillance of vascular geometry. From this background, seven standard retinal fields have been specified, each corresponding to a single image captured by a 30° field of view, and defined by a specific location in the retina by reference to the position of the optic disc [101]. These fields are illustrated in Figure 3-4 in the right eye; in the left eye, the same fields are defined in mirror image. The optic disc is represented by the orange circle, with the principal vascular arcades being shown in each quadrant, and the macula is represented by the dotted circle on the temporal side of the disc.
Field 1 is conventionally centred on the optic disc, although in a modified variant, the field may be centred instead on the temporal side of the disc, so as to include more of the macula within the field. Of the standard seven fields, field 1 is the most suitable for analysis of vascular geometry; it is the only field in which the optic disc is completely visible, ensuring that the arterial and venous trees can be tracked from the central vessels emerging in the disc, and furthermore gives visibility of the vascular arcades in all four quadrants.

Field 2 is centred on the macula, and is of particular importance for any disorders affecting the macula, such as retinopathy or AMD. Fields 3, 4 and 5 extend to the
peripheral regions of the temporal perspective, and likewise fields 6 and 7 cover the nasal side.

Retinal photography across all seven standard fields represents the gold standard for ocular surveillance in clinical trials [101], especially when combined with stereoscopic techniques, where two images are captured of each field, with a small horizontal displacement of the retinal camera introduced between capture of each image. However, for analysis of vascular geometry, neither 7 field photography, nor stereoscopy are optimum, and other approaches may be more suitable, such as the superior and inferior temporal views, possibly in conjunction with the similar fields on the nasal side. These are illustrated in Figure 3-5, again for the right eye; the optic disc is positioned at the edge of the appropriate quadrant of the camera’s field of view, so that the vascular arcades approximately follow the diagonal of the retinal field. This approach allows measurements to be made in more distal orders of the vascular arcades than can be achieved from field 1 of the seven field scheme. However, these fields are much less convenient for subjective grading of retinal pathology, and so would not be suitable for studies where this was a requirement.
3.4 Retinal Imaging Techniques

In common with many similar mydriatic retinal fundus cameras, the Zeiss supports three imaging techniques through the application of alternative filters into the optical path(s), as discussed in the subsequent sections:

- fluorescein angiography involving blue/yellow excitation/barrier filters
- red-free imaging involving green filtration of the illumination
- colour imaging involving no filtration.
3.4.1 Fluorescein Angiography

This procedure offers the most detailed images of the retinal vasculature and is described in depth by Morris [77]. A bolus of fluorescein is administered intra-venously, typically via a superficial vein in the arm or hand, and reaches the retinal circulation up to 18 seconds later. Imaging takes place by exciting the fluorescein with illumination from the retinal camera via the blue excitation filter with a peak transmission in wavelengths of the range 485-500 nm. Fluorescent emissions then arise with peak intensity in the range 520-530 nm, and the yellow barrier filter in the imaging path permits only this fluorescent light to reach the film or sensor.

This procedure is considerably more invasive that other imaging techniques, and the need for intra-venous administration increases the time and expertise required to perform photography. Furthermore, side effects from the fluorescein are common, including staining of the skin, sclera, or mucous membranes, as well as some secretions. Mild to moderate adverse reactions may also occur, including nausea, vomiting, dizziness/lightheadedness due to a vasovagal response, urticaria, syncope, phlebitis and localized nerve palsy. Severe adverse reactions have also been noted rarely, including seizures, breathing difficulties, circulatory shock, or cardiac arrest. The Fluorescein Angiography Complication Survey [149] indicates that death occurred in 1 out of 220,000 angiograms.

For the reasons described above, fluorescein angiography is not considered appropriate for routine screening, nor for epidemiological studies, and so its application to analysis of clinical retinal images is extremely limited. Accordingly, the emphasis of the current work has been on analysis of images from less invasive procedures such as red-free imaging discussed below.

3.4.2 Red-Free Imaging

Acquisition of red-free photographs of the retinal vasculature requires no administration of contrast agent to the subject. Instead, a green filter is introduced into the illumination path of the retinal camera to enhance the contrast between blood filled vessels and the retinal background.
The formation of red-free images is a complex process, but in summary, green filtered light is predominantly scattered from anterior retinal layers at or close to the retinal pigmented epithelium (RPE) [24][101], but blood within the lumen of retinal vessels strongly absorbs green light, thus giving rise to increased contrast between the background, and the vessels. This principle can be appreciated by considering the spectral transmittance of blood. Friebel et al [33] report the absorption coefficient of flowing oxygenated blood in physiological concentration, based on measurements using an integrating sphere spectrometer, in combination with inverse Monte-Carlo simulation. Using the absorption law [86]

\[ I = I_0 e^{-\mu_\alpha x} \]

where

- \( I \) and \( I_0 \) are the transmitted and incident intensity respectively
- \( \mu_\alpha \) is the absorption coefficient, and
- \( x \) is the linear distance through the material,

the absorption coefficient was used to calculate the spectral transmittance of a 150\( \mu \)m layer of blood, and also for a 50\( \mu \)m layer, roughly corresponding to the range of path lengths through vessels in the retinal microvasculature. This is illustrated in Figure 3-6, which also shows the transmission spectrum of a typical green glass filter (Schott VG6) [159] of 2mm thickness.

As can be seen the green filter has the effect of illuminating the vessels in the spectral range where blood is moderately to strongly absorbing, so that through the retinal camera’s eyepiece, the vessels appear dark, whereas the background appears bright due to scattering. The resulting image may be captured on a monochrome sensor or film emulsion; in the latter case, negative film would be most commonly used, in which case the vessels would appear light against a dark background.
It may also be appreciated that, particularly for smaller retinal vessels, the green glass filter may not give optimal contrast between vessels and background, due to the minor peak in transmittance of blood at around 500nm. A passband filter centred at 560nm, with a bandwidth of less than 100nm would be expected to give better results.

![Spectral transmittance of blood comparison with a green glass filter](image)

**Figure 3-6: Spectral transmittance of blood in comparison with a green glass filter**

Since the red-free image is formed by a complex interaction of scattering from retinal layers, together with absorption of incident and/or scattered light by intra-vascular blood, the vessels in red-free images exhibit much less distinct edges than those from fluorescein angiography, where the light is emitted directly from fluorescence within the vessel lumen. This is illustrated in Figure 3-7, which compares a fluorescein angiogram with a red-free retinal image of the same subject.

In addition, other confounding factors may also impact on vascular images captured by the red-free technique; retinal nerve fibres are sometimes visible, and may be confused for small vessels, and choroidal vessels may also introduce artefacts into the image. Such factors increase the challenges of accurately segmenting and measuring the retinal vascular architecture from red-free images.
The central light reflex or “light streak” that is sometimes observed along the centre line of vessels in red-free images has been postulated by Brinchmann-Hansen and Heier [12] to be due to scattering of light from the surface of a rough column of blood.

3.4.3 Colour Imaging

The final imaging technique to be considered here is colour retinal photography, for which no optical filtration is applied during photography. Where subjective grading of images for retinal pathology is required, particular affecting the macula, colour is regarded as essential, and accordingly, retinal image sets captured in a modern clinical environment are likely to be colour images.

Accordingly it is of interest to consider how well the retinal vasculature may be visualised and measured from a colour image. In principle, the desired contrast between the vessels and retinal background may be obtained by taking the green component of a colour image, so as to reproduce the principle of red-free photography. The spectral characteristics of colour film or CCD sensors may be considered to give a broadly similar effect to the green glass filter considered in the context of red-free imaging. However in some cases, the green sensitivity has been found to extend beyond the limits of the glass filter, giving rise to a reduction in contrast between vessels and background compared to genuine red-free imaging.
employing an optical filter. Accordingly, where a highly optimised red-free filter is available, capture of colour images with the red-free filter simultaneously in place may improve the contrast between vessels and background for the purpose of vascular measurements, but of course would require capture of a separate image for subjective grading of pathology.

3.5 Imaging Technology

Traditionally, photographic film (most commonly in 35mm format) has been used to capture images using a retinal fundus camera; monochrome negative film in the case of red-free images, and reversal film to give positive colour slides. However, the use of film has several practical drawbacks in a clinical setting, not least that the image is not available for inspection at the time of photography, so that the opportunity to take repeat images in case of problems may be lost. There is additional cost associated with the film itself as well as processing, and if a digital image is required for subsequent archiving, or automated analysis, further effort is required to scan the film. For these reasons, direct digital capture by CCD and/or CMOS devices mounted on the fundus camera has become widespread as the cost of such devices has fallen dramatically in recent years. Nevertheless many epidemiologically interesting retinal image sets (including the Beaver Dam Eye Study) were originally captured on photographic film, and so the ability to handle such images is important.

Film images can be readily scanned at a high spatial resolution of 2700 dpi or better using commercial film scanners, to yield a digital image consisting of a two dimensional array of pixels, each representing the intensity value at the corresponding point in the image. At this resolution, pixels forming a rectilinear grid would each represent approximately a 9\(\mu\)m \(\times\) 9\(\mu\)m square, and a retinal field from the Zeiss camera, for example, would occupy around 2760 \(\times\) 2550 pixels.

A typical film scanner is the Nikon LS-1000, which has been used at the International Centre for Circulatory Health for scanning of retinal photographs. This, and other similar devices, uses a linear tri-colour CCD array that is mechanically scanned across the film, capturing a series of lines to make up the two dimensional image. Red, green and blue intensity values are captured at each pixel position, each value of 8 bits,
allowing up to 256 discrete values to be represented in each colour plane. For retinal images to be subject to vascular measurements, the practice has been adopted at Imperial College London to capture and store such images in an uncompressed format (or using lossless compression), to avoid the risk of distortion of the intensity profile of the vessels by lossy compression techniques such as jpeg.

Film emulsions exhibit a generally problematic phenomenon known as ‘graininess’ that appears to a human observer as an irregular texture superimposed on the underlying image. Individual particles of silver halide in the emulsion are converted to silver following exposure and development. Such particles are typically less than 2μm in diameter [51] which is too small to directly represent the observed texture, but the tendency of individual particles to clump together gives rise to the perceived pattern which typically appears to have dimensions many times larger. In colour films, the same phenomenon is apparent, but is induced by clumps of dye clouds rather than silver particles. The apparent size of clumps of grain are affected by several factors; high speed (more sensitive) emulsions tend to exhibit larger grain, and push processing (longer or hotter development) tends to have the same effect.

In digitally scanned film images, the phenomenon of graininess may give rise to apparent noise, either in individual pixels, or in small groups of pixels. Industry specifications seek to quantify the effect by a parameter known as the diffuse rms granularity [157], which is defined to be the root mean square (rms) variation in density measured using an aperture of 48 μm diameter over a film of uniform optical density of 1.0 D (transmitting 10% of incident light). Insight may be gained into the variation that may be expected in other scales, through appeal to Selwyns’ Law [156] which states that

$$\sigma \sqrt{2a} = \text{constant}$$

where \(a\) is the aperture area, and \(\sigma\) is rms variation in density. For a fine grain film emulsion, the industry standard rms granularity is typically 0.01, and application of Selwyn’s law suggests that with pixel sizes used in a 2700 dpi scanner, the expected rms variation in density would increase to 0.047. The optical density is defined [156] to be \(\log_{10}(1/T)\) where \(T\) is transmittance, suggesting that intensity noise (1 sd) in the
digitised image in excess of 10% of the background transmittance can be expected. However, this quantitative approach gives only a sparse description of the overall characteristics of grain noise, since it does not reveal anything about the scale (i.e. spatial frequency) of the noise, nor about its characteristics at different background densities.

It is instructive to compare the characteristics of film in this regard with the alternative of direct digital capture by an electronic sensor such as a two dimensional CCD or CMOS array. Each element in the array represents a single pixel, and the sensor produces an analogue output at each pixel representing the total exposure to light within a set period. The sensor is normally incorporated into a device which digitises the analogue outputs to yield an entirely digital image.

Only a few years ago, the cost of high resolution electronic image sensors was prohibitively high, and so only medium resolution sensors were normally available in retinal fundus cameras. The Basler A101 is an example of a medium resolution monochrome CCD back that has been used at Imperial College London with the Zeiss retinal camera, giving a digital image of the retinal field of 1300 x 1030 pixels. This represents a noticeably smaller number of pixels than typically available from the same field scanned from 35 mm film (2760 x 2550), but the impact of grain noise in the film image reduces useful information content of the additional pixels. This can be appreciated from viewing Figure 3-8 below, which illustrates a vessel from a red-free retinal image captured with a Basler A101 digital sensor (left) compared to the same vessel captured on film and scanned at 2700 dpi. Although the film image contains a considerably greater number of pixels, the impact of graininess can be readily seen, and will give rise to additional noise in the intensity profile across the vessel. While electronic sensors suffer from pixel noise arising from statistical sampling, and electrical noise, these effects appear to be modest in comparison with the impact of film grain. For the sake of the comparison, the CCD image has been reversed, so that the dark vessel appears light, to match the presentation from the negative film.
Cost effective high resolution colour CCD and CMOS sensors are now commonplace. Most such devices employ a Bayer pattern to capture colour, whereby each pixel is sensitive to only a single colour, through the placement of primary red, green and blue filters over the array of pixels in the pattern illustrated in Figure 3-9. Half of the pixels are sensitive to green, with the remainder equally divided between red and blue.

However, care is needed in interpreting the total number of pixels offered by Bayer pattern CCD or CMOS sensors in the particular context of vascular measurements. The vascular boundaries are defined by the intra-vascular blood column, which, as discussed earlier, is predominantly visible only in the green part of the spectrum. Hence the red and blue sensitive pixels contribute negligible relevant information, and the effective resolution relating to vascular features is notably lower than might be suggested by the total number of pixels available.
A typical Bayer pattern CCD camera back employed in retinal imaging is the AVT Oscar F-510C, which provides a total of 2588 x 1958 pixels within a retinal field. However, since only half of all the pixels are green sensitive, it may be crudely considered that the effective resolution for vascular imaging is reduced by a factor of $1/\sqrt{2}$ i.e. to 1294 x 979. This is very similar to that of the medium resolution monochrome Basler CCD device, although the camera electronics will typically interpolate to provide a green value at each pixel position, even where green sensitive information is not available.

A further comparison of interest between film and electronic sensors concerns their response to illumination. In general CCD sensors are more sensitive than film, which is valuable in retinal imaging since a lower flash intensity can be used and is more comfortable for the subject. Furthermore, the response of CCDs (recorded brightness) to exposure is inherently linear. Their dynamic range is limited at the low exposure end by the dark noise, and at the high end by saturation of the sensor and/or the digitising electronics. For a medium quality CCD device yielding an 8 bit brightness value at each pixel, the dark noise may be considered to be of order one bit [155], and the maximum brightness is 255. The sensor can reasonably be expected to be linear between these extremes, meaning that the dynamic range is 1:255. Higher dynamic range can be obtained from specialist scientific grade CCD sensors, albeit at higher cost.

On the other hand, the response of film to illumination is intrinsically non-linear, and is typically plotted on a log-log scale relating log exposure to optical density ($D$), defined to be $\log_{10}(1/T)$, where $T$ is the transmittance in the range 0 to 1. The characteristic curve for Kodak Ektachrome 64 [158], a colour reversal film often used for retinal imaging, is illustrated in Figure 3-10.
From this scale, it can be appreciated that the dynamic range of exposure over which the film is sensitive is of the order 1:700; somewhat greater than for the CCD sensor considered earlier. However, bearing in mind the impact of the log-log presentation of the characteristic curve, the range of exposure in which the film exhibits approximately linear behaviour is substantially smaller than this. From inspection of the characteristic curve plotted on linear axes, the response of the film may be reasonably considered as linear over an exposure range of perhaps only 1:7.

The lack of linearity of film is not necessarily a major impediment for vascular measurements; the exposure range across a retinal vessel is generally quite small, and may reasonably be considered to lie within the linear range of the film. In principle, non-linearities in the film may also be accommodated during the scanning process. Furthermore, the non-linear response of the film may in fact offer some benefits in retinal imaging generally. When a retinal photograph is optimally exposed to give detail of the vascular or macular features, other highly reflective parts of the retina such as the optic disc appear extremely bright, but nevertheless are of interest in subjective grading of pathology. The graceful reduction in sensitivity of the film in such regions of high exposure allows some detail to be retained in film images, that might saturate a CCD sensor and hence be lost. The latter characteristic is also
relevant in the red channel of a colour image, where film is inherently less susceptible to saturate in an over-exposed image compared to a CCD sensor [47].

The final aspect of retinal image capture that merits discussion is the issue of colour balance of the medium. In a correctly exposed film image, the colour balance of the emulsion is outside the direct control of the photographer, other than through the choice of film or insertion of filters in the optical path. On the other hand, in digital devices, extensive control of the colour balance is available, through direct or indirect control of the electrical gain of the red, green and blue channels. Hubbard et al [47] has drawn attention to the wide variation in characteristics of retinal images produced from digital devices in comparison with film. They observed that “the quality of the best digital images matched that of the best film”, but that the overall sample of digitally captured images “suffered from wider variability in its tonal resolution” and that “the most critical factor is colour balance”. It has been observed in digital image sets examined at Imperial College London that the default settings of commercial CDD cameras often give a particularly warm image due to elevated red gain that may give a more pleasing image in everyday use, but is inappropriate for retinal photography. Red bias in a retinal image may give rise to widespread saturation of the red channel, destroying detail that may be clinically useful. Hubbard et al [47] have recommended that in order to offer the best potential for subjective grading of pathology, colour balance should be set so as to achieve optimal ratios of the red, green and blue intensities in a retinal image, averaged over a macular centred region of interest, as follows:

- Green/Red ratio = 0.50
- Blue/Red ratio = 0.17

However, it is not clear to a photographer how a digital capture device attached to a retinal camera should be set in order to achieve such ratios, other than through a process of trial and error. There would appear to be value in defining a standard calibration procedure to establish settings for such devices, as a topic for future investigation.
CHAPTER 4
PROCESSING OF RETINAL IMAGES – EARLY APPROACHES

Quantitative geometrical features of interest in the retinal vasculature include the relationship of diameters at vascular bifurcations, as well as length to diameter ratios of vascular segments joining bifurcations. Measurement of vascular diameters is fundamental to the derivation of these features, and hence is a crucial step in quantifying the retinal vascular geometry. Since the optimality of bifurcations involves a combination of three distinct diameter measurements, it is particularly important to reduce the noise in such measurement as far as possible, in order to minimise the scatter in the overall measure of optimality, and hence maintain its statistical power.

However, from the discussion of the previous chapter, it will be evident that the measurement of vascular diameters from red-free or colour retinal images is particularly challenging for a number of reasons:

- the indistinct vessel edges arising from the scattering and absorption processes inherent to the image
- the presence of the central light reflex, possibly arising from light scattering from the surface of the blood column
- the confounding effects of choroidal vessels and/or retinal nerve fibres on the retinal image
- intensity noise introduced during image capture either from photographic grain and/or pixel noise from CCD sensors.

An illustration of some of these factors can be seen in Figure 4-1, which shows the intensity plotted against the distance over a cross-section of the vessel (referred to hereafter as the intensity profile) from a typical retinal vessel in a red-free image.
captured on photographic film. Noise is clearly evident, possibly arising from grain in the film, as well as irregularities in the background, perhaps due to physiological features such as retinal nerve fibres. The central light reflex is also clearly visible in the centre of the vessel.

![Image of retinal image with intensity profile](image)

**Figure 4-1: Intensity profile from a vascular cross-section in a red-free retinal image**

A generalised approach to processing of digitised images may be considered to consist of the following distinct steps:

a) image enhancement (*e.g.* filtration of unwanted noise)

b) segmentation, in which individual components of the image are identified and segregated (*e.g.* vessels separated from background, and arterioles distinguished from venules), and

c) quantification of features in the segmented process (*e.g.* vessel diameters, angles and other parameters are measured).

In the case of red-free retinal image analysis, for the reasons highlighted earlier, traditional segmentation techniques, such as edge detection (*e.g.* by Sobel operators), or grey-level morphological analysis perform poorly [16]. Hence the distinction between these traditional steps may be less appropriate in retinal image analysis compared to other applications, and it is probable that a combination of several
techniques might be appropriate. For example, a segmentation technique may prove to be effective in identifying the presence of a vessel-like structure, but an entirely different measurement technique may be more appropriate to yield a reliable estimate of the vascular diameter, operating on the original image rather than the results of segmentation.

The work described in this thesis will focus particularly on the measurement of vascular diameters and the derivation of geometrical characteristics of the vasculature from such measurements. This chapter offers a review of a range of previous techniques aimed at measurement of vessel diameters in red-free and colour retinal images. Some techniques address this aspect exclusively, requiring identification of vessels as a prior process, whereas others set out to perform segmentation followed by measurement as a subsequent step.

4.1 The Half Height Method

Brinchmann-Hansen and Engvold [11] proposed in 1986 a method of measuring the width of a retinal vessel from a single intensity cross-section of a fundus photograph, based upon measurement of distance between the points of half intensity. This technique makes no attempt to segment the vessel from the background, and relies on prior identification of the vessel subjected to measurement, e.g. by manual inspection. In the original report on this method, the intensity cross-section of the vessel was obtained from a linear scan of a microphotometer, taking into account the characteristic curve of the film.

Prior to making a measurement of the vessel diameter, the intensity cross-section is filtered to reduce grain noise from the film, by use of an unspecified Fourier domain technique.

This principle of the measurement technique is illustrated in Figure 4-2, showing an idealised intensity profile across a bright vessel as would be observed on monochromatic negative film. The following steps lead to measurement of the vessel diameter

- The minimum background intensity levels $I_{bk}(l)$ and $I_{bk}(r)$ are established on the left and right sides of the vessel respectively, within one diameter away from the
vessel centre, and the mean background intensity $<I_{bk}>$ is obtained from these two measurements.

- Similarly, the peak intensity values $I_{pk}(l)$ and $I_{pk}(r)$ are taken from the left and right sides respectively of the central light reflex, and the mean value $<I_{pk}>$ calculated.

- The diameter of the vessel $W_0$ is determined from the width of the intensity profile at the intensity level half way between $<I_{bk}>$ and $<I_{pk}>$.

- If desired, the width of the central light reflex $W_r$ may be obtained at the intensity level half way between $<I_{pk}>$ and the trough of the reflex $I_{rf}$.

![Diagram](image)

**Figure 4-2: Estimation of vascular diameter by the half height method**

The half height approach has been utilised in other retinal vascular studies [81]. However, several drawbacks are evident. The determination of the vessel width at the mid point of the intensity profile is essentially arbitrary, without specific justification from optical or physiological considerations. Furthermore, Chapman et al [15] noted that “this method makes measurements based on only a limited amount of information in the intensity cross-section, and therefore would be expected to be susceptible to
image intensity noise and imperfections in the image acquisition process, such as the
effect of film grain”. In addition, anatomical features such as nerve fibres may also
introduce unwanted artefacts into the background region of the intensity profile, thus
distorting the measurement of the vessel diameter.

4.2 Single Gaussian Fitting

In recognition of the inadequacies of conventional edge detection methods in the
detection of retinal vessels, Chaudhuri et al [16] in 1989 proposed the use of matched
filters, based on a single Gaussian shaped profile, to identify and segment retinal
vessels. They applied a set of 12 kernels representing the intensity profile of a retinal
vessel at a constant width of 4 pixels over a fixed vessel segment of length of 9 pixels,
each kernel corresponding to a different vessel direction. The technique was aimed
principally at segmentation rather than measurement, and in this role, the fixed width
matched filter was not considered to represent a significant drawback, although this
would be expected to reduce the selectivity of extremely small or large vessels.

Zhou et al [153] in 1994 employed a single Gaussian model of the form

\[ g(x) = Ae^{-\frac{(x-c)^2}{2\sigma^2}} \]  \{4-1\}

to measure retinal vascular diameters in fluorescein angiograms, where \( x \) is the
distance across the vessel profile. The height \( A \), width \( \sigma \) and central position \( c \) of
the Gaussian curve were derived by fitting the model curve to three sample points
from the profile of the vessel under measurement. The edges of the vessel were then
taken arbitrarily as 1.96 \( \sigma \) either side of the mean. It should be noted that fluorescein
angiograms do not exhibit the central light reflex commonly found in red-free images,
and so the confounding effect of this feature would not cause any difficulty in such
angiograms.

Gang et al in 2002 [34] considered application of Gaussian Filters both to the
detection and measurement of vessel diameters in colour (i.e. non-fluorescein retinal
images). They proposed a modified form of a second-order differential Gaussian

\[ f(x) = \frac{1}{\sqrt{2\pi}\sigma^3}(x^3 - \sigma^2_x)\frac{1}{2\sigma^2} \]  \{4-2\}
where $x$ and $\sigma$ are as defined earlier. This gave rise to a more specific peak when convolved with a typical retinal vessel of the same size as the filter, thus improving the suitability for measurement of vessel diameter.

Nevertheless, in red-free or colour retinal images, the inability of a single Gaussian model to accommodate the central light reflex represents a significant drawback, potentially leading to erroneous diameter measurements when such a light reflex is prominent.

### 4.3 The Kick-Point Method

Rassam *et al* in 1994 [93] proposed a simplistic model of the optical path through a retinal vessel, taking into account the path length and absorption coefficient of the blood column and vessel wall. Under the assumption that light travelling through the vessel is collimated and passes through the vessel once, they deduced a model of the intensity profile that exhibits ‘kick-points’ where the intensity gradient (wrt distance) is discontinuous, corresponding to the edge of the lumen. In common with the earlier half height method, this technique was aimed purely at measurement of the vessel from its intensity profile, obtained by a prior process. Their theoretical intensity profile (as would be observed in a negative film image) across an artery of lumen 100 $\mu$m and a 15 $\mu$m wall is reproduced in Figure 4-3, from which the kick-points can be perceived readily.

The technique of measuring vessel diameter between kick points was exercised both *in vitro*, using plastic tubing of different calibre, and *in vivo*, using red-free retinal photographs from healthy volunteers. From this treatment they concluded that the arbitrary half-height method under-estimated the blood column width by around 16% in arteries, and by 17% in veins.

However this approach is open to much criticism. As pointed out earlier, a red-free (or colour) intensity profile of a retinal vessel is formed by a complex interaction of light scattering and absorption. The illumination of the vessel in a normal fundus camera is not collimated, and light from a given point in the vessel will reach the image plane via many different paths, which will generally have an overall blurring effect on the intensity profile. Accordingly, the underlying optical model appears unrealistic. In
the present author’s experience, typical clinical images rarely exhibit features characteristic of the
kick points described by Rassam et al, and image noise together with physiological artefacts would also render such points very difficult to detect reliably.

![Figure 4-3: Kick points in theoretical intensity profile [93]](image)

4.4 Double Gaussian Fitting

A more general model based approach to measurement of vessels was proposed by Gao et al [35] [36] in 1997, based on a double Gaussian model of the intensity profile across a retinal vessel. In common with the earlier half height method, this technique was aimed purely at measurement of the vessel from its intensity profile, obtained by a prior process.
Under the assumption that the intensity profile of the vessel arises from the attenuation of red-free light as it passes through the blood column, and that the attenuation coefficient is linear in radius across the vessel, it may be shown that a reasonable approximation of the intensity profile across the vessel is a Gaussian function. The central light reflex may be modelled [12] as an optical scattering from a rough column of blood, which under the assumption of elliptical scattering functions, gives rise to a narrow inverted Gaussian curve, which is summed with a main Gaussian curve, representing the image of the vessel itself.

The double Gaussian profile may be characterised by up to seven parameters, as illustrated in Figure 4-4, and modelled [36] by a function of the form

\[
Z(x; \hat{a}) = g_1(x; \hat{a}) - g_2(x; \hat{a}) \quad \{4-3\}
\]

where

\[
g_1(x; \hat{a}) = a_1 e^{\left(\frac{x-a_2}{a_3}\right)^2} + a_4 \quad \{4-4\}
\]

\[
g_2(x; \hat{a}) = a_2 e^{\left(\frac{x-a_6}{a_7}\right)^2} \quad \{4-5\}
\]

In this model, the function \(g_1\) represents the overall width of the vessel, giving the modelled intensity value as a function of distance \(x\) along the cross-section. Hence parameters \(a_2\) and \(a_3\) indicate the width and centre position of the vessel respectively and parameter \(a_4\) gives the background intensity. The function \(g_2\) represents the central light reflex which is commonly observed in red-free images, with parameters \(a_6\) and \(a_7\) indicating the width and central position of the reflex.
In order to determine the characteristics of a vessel by use of the double Gaussian model, values of the parameters $a_1$ to $a_7$ are found which produce the best-fit to the observed intensity profile. This may be achieved by a non-linear optimisation technique such as the Levenberg-Marquardt method [91], which aims to minimise the cost function

$$\chi^2 (\hat{a}) = \sum_{i=1}^{N} \left[ \frac{z_i - Z(x_i; \hat{a})}{s_i} \right]^2 \quad \{4-6\}$$

where $z_i$ is the observed intensity and $s_i$ is a weighting function at the $i$th point of a cross-section. The technique proceeds by a series of iterations, which progressively migrate from a steepest descent to an inverse Hessian procedure, as the minimum is approached. Given an estimate of the parameter set $\hat{a}$, subsequent iterations are determined from the equation
\[ \sum_{i} \alpha'_{ii} \delta a_i = \beta_k \] \{4-7\}

where

\[ \alpha'_{ij} \equiv \alpha_{ij} \left( 1 + \lambda \right) \quad \text{and} \]
\[ \alpha'_{jk} \equiv \alpha_{jk} \quad \text{for} \quad j \neq k. \] \{4-8\}

\[ \alpha \quad \text{and} \quad \beta \] are matrices determined analytically from the second partial derivatives of the cost function of the double Gaussian model:

\[ \alpha_{kl} = \sum_{i=1}^{N} \frac{1}{s_i^2} \left[ \frac{\partial Z(x_i; \hat{a})}{\partial a_k} \frac{\partial Z(x_i; \hat{a})}{\partial a_l} \right] \] \{4-10\}

\[ \beta_k = \sum_{i=1}^{N} \left[ z_i - Z(x_i; \hat{a}) \right] \frac{\partial Z(x_i; \hat{a})}{\partial a_k}. \] \{4-11\}

The non-dimensional constant \( \lambda \) controls the movement from steepest descent to inverse Hessian procedures. When the iteration proceeds in a favourable direction (\( i.e. \) the cost function decreases), the value of \( \lambda \) is decreased (conventionally by a factor of 10), and when an unsuccessful iteration is performed, it is increased (also conventionally by a factor of 10). The iteration proceeds until the value of the cost function decreases by a negligible amount for a number of successive iterations.

This method is in principle capable of determining the width and other characteristics of a vessel from a red-free image. However, in practical evaluation of this approach by the present author, a number of difficulties have become evident, which necessitate some adaptation of the overall approach. Foremost among these is the shape of the \( \chi^2 \) cost function arising from the double Gaussian model, which under certain conditions can exhibit a significant elongated valley, leading to slow and inconsistent convergence, particularly affecting the \( a_3 \) parameter (width of the vessel) which is one of the principal parameters of interest. This is illustrated in Figure 4-5 which shows
contours of constant cost for varying $a_3$ and $a_7$ of a fitted curve, with respect to a reference curve for which $a_3 = 10$ and $a_7 = 7$, all other parameters being constant ($a_1 = 128$, $a_2 = a_6 = 30$, $a_4 = 5$, $a_5 = 64$). This characteristic is further exacerbated by the tendency of the inverse Hessian iterative procedure to produce unhelpful steps unless in close proximity to a well defined minimum.

These difficulties can be mitigated by seeding the iteration such that it commences in a favourable position with both $a_3$ and $a_7$ set to less than their true values, so that the iteration is kept away from the valley in the cost function. Under these conditions, the steepest descent procedure can be expected to deliver a solution close to the true minimum, thus offering the inverse Hessian procedure the best chance to reach the true minimum.

In the presence of noise, which is characteristic of red-free images, problems may be expected in successful fitting of the negative Gaussian representing the light reflex. In the general formulation of the double Gaussian model presented above, the negative going Gaussian is unconstrained, which may result in it being fitted to a significant noise spike, yielding no information of physical significance. Hence, it has been
found to be advantageous to fix the position of the negative Gaussian to coincide with the centre of the main curve. This is effected by setting \( a_6 \) to be equivalent to \( a_2 \).

A further benefit has been obtained by adjustment of the strategy for setting the \( \lambda \) parameter. If \( \lambda \) is set too low, the inverse Hessian procedure may become dominant before the iteration has become sufficiently close to the true minimum, and larger than desirable steps may be achieved, placing the subsequent estimate in the valley in the cost function. By slowing down the reduction of \( \lambda \) following a successful step, the onset of the inverse Hessian procedure is delayed until the iteration is closer to the minimum point, which improves the consistency of convergence. A reduction in \( \lambda \) by a factor of 1.5 (compared to 10 normally recommended) following a successful step has been found to give good results. An increase in \( \lambda \) by a factor of 10 appears appropriate in the event of an unfavourable step.

The principal parameter of interest in the current study is the width of the vessel, indicated by the parameter \( a_3 \). To achieve reliable estimation of vessel width, a weighting function may be applied to give greater contribution to the cost function from points in the region of the vessel edges than elsewhere. This weighting is defined by

\[
s_n = \begin{cases} 
0.3 & \text{for } a_3^* < (x_n - a_2^*) < 3.5a_3^* \\
1.0 & \text{otherwise}
\end{cases} \tag{4-12}
\]

as indicated in equations \{4-6\}, \{4-10\} and \{4-11\}.

Nevertheless, an iterative step may be encountered which results in a reduction in the value of the cost function, but nevertheless produces a result which is not physically meaningful in the context of the fitting of a retinal vessel. The most common example is a width of the negative (reflex) Gaussian which exceeds the width of the main curve. Such occurrences normally arise from the inverse Hessian procedure reacting disadvantageously to the particular noise characteristics of the intensity data. An
effective measure for dealing with such cases has been found to be the rejection of the step and an increase of the parameter \( \lambda \) as if for an unfavourable step.

The double Gaussian fitting yields the parameter \( a_3 \) (corresponding to \( \sqrt{2} \) * standard deviation of the main Gaussian curve), measured in pixels, which is multiplied by a non-dimensional constant scaling factor (\( \gamma \)) to yield an estimate of vessel width which may be compared with that measured from fluorescein images. The value of \( \gamma \) has been arbitrarily set to a value of 2.33 to preserve compatibility with other studies employing this method.

Despite the adaptations discussed above to improve its practical performance, the fitting of the double Gaussian model has been found to be particularly intolerant to noise in the intensity profile of a red-free vessel. An evaluation (reported in Chapter 5) based on synthesised vessel profiles with \( a_3 = 5, 10 \) and 20 pixels, found that additive intensity noise gave rise to an rms error in estimating the vessel width of several pixels which appears considerably higher than desirable.

### 4.5 Scale Space Approaches

Most recently, methods based on scale space principles have received attention for analysis of images of the retinal vasculature. Martínez-Perez et al in 1999 [73] described such an algorithm known as RISA (Retinal Image multiScale Analysis) that involves a number of distinct steps:

First the original image is convolved with a series of Gaussian kernels \( G(x, y; s) \) of variance \( s^2 \), each kernel representing a different scale factor \( s \). Each image in the resulting sequence exhibits progressively increased blurring, corresponding to increased \( s \), which effectively suppresses features of characteristic dimension less than \( s \). The response of the subsequent feature extraction process is expected to be maximised in the image where the scale factor \( s \) corresponds to the dimension of the feature in question.

Feature extraction employs first and second order directional derivatives to identify ridge-like structures corresponding to vessels. The first order directional derivative
\[
\n\|\nabla I_s(s)\| = \sqrt{(\delta_x I_s)^2 + (\delta_y I_s)^2}
\]

\{4-14\}

describes the slope of the image intensity at a point in the image \(I_s(x,y)\) corresponding to scale factor \(s\). The second order derivatives information is derived from the symmetrical Hessian matrix

\[
H = \begin{pmatrix}
\delta_{xx} I_s & \delta_{xy} I_s \\
\delta_{yx} I_s & \delta_{yy} I_s
\end{pmatrix}
\]

\{4-15\}

having real eigenvalues and orthogonal eigenvectors. The eigenvalues \(\lambda_+\) and \(\lambda_-\) (such that \(\lambda_+ \geq \lambda_-\)) measure convexity and concavity in their respective eigendirections. For a bright vessel arising from a negative red-free image, pixels within the vessel will exhibit \(\lambda_+ \approx 0\) and \(\lambda_- \ll 0\), and at the vessel edges \(\nabla I_s \approx 0\). A further adjustment is necessary to account for the fact that larger vessels inherently yield greater contrast due to the larger column of blood. Accordingly the features are equalised through division by the diameter of a candidate vessel \(d = 2s\), giving rise to definition of the following features. The maximum of both of these throughout the range of scale factors \(s\) is sought.

\[
\gamma = \max_s \left[ \frac{\|\nabla I_s(s)\|}{d} \right]
\]

\{4-16\}

\[
\kappa = \max_s \left[ \frac{\max(\lvert \lambda_+ \rvert, \lvert \lambda_- \rvert)}{d} \right]
\]

\{4-17\}

A multi-stage iterative region growing algorithm is used to classify pixels as being either background or vessel. Seeds for each class are planted, based on histograms of the features, so that each pixel may be classified as background, region or unknown. The regions are then grown based on the 8 neighbouring pixels in two stages in which the classification constraints are progressively relaxed.
Finally, a labelling stage is performed in which branching and crossing points are identified, and the tree tracked. This allows the length and area of each vascular segment to be determined, and hence the diameter calculated.

This algorithm has been applied to the segmentation of retinal images in several local and public databases of retinal images with encouraging results [75] and results presented from the quantitative analysis of retinal geometry [74]. However, the diameter measurements produced by the algorithm arise from a complex interaction of blurring, feature extraction and region growing, and hence careful validation would be prudent to gain confidence in such measures from comparison with diameter measurements from alternative methods derived more directly from the intensity profile of vessels. Furthermore, initial versions of the programme were computationally demanding, requiring images for analysis to be downsampled, thus reducing the potential resolution of diameter measurements, and preventing direct comparison of measurements from high resolution images (although the most recent versions are more computationally efficient).

Another technique based on scale-space principles is a method known as CAIAR (Computer Assisted Image Analysis of the Retina) [127] [82], the development of which was partially motivated by a desire to reduce the computational load of the earlier RISA approach. The algorithm is based on a maximum likelihood fitting of a single Gaussian profile over a scale space framework at just four different scales; fewer than typically employed in early versions of RISA.

CAIAR has been shown to be capable of reliably identifying retinal vessels, and also produces measurements of tortuosity and vessel diameter. Tortuosity is measured by grouping vessel centreline locations into contiguous structures by a connected component algorithm, followed by application of an algorithm that successively divides sections of vessels into two parts until a specified length threshold is reached. The chord and arc lengths of the segments are combined in a number of ways to give a total of 14 different measures of tortuosity. Measures of tortuosity in infants from CAIAR correlated moderately well with grading by expert ophthalmologists [127] and in ten year old children showed strong associations [82].
Measurements of vessel width in CAIAR are obtained either from the width ($\sigma$) of the fitted single Gaussian curve, or else from the isotropic contrast computed at the vessel centreline from a Laplacian of Gaussian (LoG) filter. However, the use of a single Gaussian model to fit the vessel profile represents a limitation as highlighted in Section 4.2 above, since it does not allow the central light reflex to be specifically taken into account. Furthermore, in infants, vessel width measures from CAIAR correlated less well with experts grading than for tortuosity [127].
CHAPTER 5
THE SLIDING LINEAR REGRESSION FILTER (SLRF) METHOD

The early approaches outlined in the foregoing chapter all exhibit some drawbacks in the measurement of retinal vascular diameters. The Sliding Linear Regression filter technique has been developed by the present author to explore whether this approach has potential to overcome or reduce the impact of such drawbacks. In common with the half height method and some other techniques described previously, the SLRF method is intended to be applied to a single intensity profile across a vessel in a red-free or colour retinal image. In other words, it is aimed purely at measurement of diameter of a vessel that has been identified by a prior process.

The principle of using intensity gradient to identify the edges of a retinal vessel was promoted by Suzuki in 1995 [116], in the context of direct measurement of vascular diameter by a linear CCD sensor embedded in a fundus camera. The SLRF method described here builds upon that principle, and is generalised to apply to measurement of vessels from two dimensional retinal images. Furthermore, the SLRF method introduces a variable size window derived from an initial estimate of vessel diameter, aimed at optimising the response specifically for the dimension of vessel under measurement.

5.1 Cross-Section Extraction

The SLRF method is applied to one or more intensity profiles derived from cross-sections of a vessel, normal to the direction of flow, extracted from a digitised image. Each cross-section gives the observed image intensity values \( z \) corresponding to a series of discrete values of linear distances \( x \) along the cross-section. An individual cross-section is characterised by its position and direction, both of which are continuously variable (\( i.e. \) not constrained to coincide with individual pixels). The position is specified by the co-ordinate pair \((r, c)\) representing the row and column respectively of the centre point of the cross-section, at which \( x = 0 \) by definition. The direction is given by \((\Delta r, \Delta c)\) representing the normalised vector perpendicular to the
cross-section \(i.e.\) parallel to the vessel direction). These principles are illustrated in Figure 5-1.

![Figure 5-1: Extraction of an intensity cross-section](image)

Before the extraction process can proceed, it is necessary to determine whether the cross-section is orientated more closely to the row or column direction of the image. If \(|\Delta r| \geq |\Delta c|\) then the cross-section is considered to be row orientated; otherwise it is column orientated.

The extraction proceeds to determine the set of intensity values \([z_1..z_N]\) corresponding to the set of distance values \([x_1..x_N]\). For a row orientated cross-section, the incremental distance between each discrete point along the cross-section is calculated as

\[
\Delta x = \frac{1}{|\Delta r|} \quad \{5-1\}
\]

so that at a general point \(m\) along the cross-section the distance from the centre point \((r, c)\) is given by
\[ x_m = \left( m - \text{ceil} \left( \frac{N}{2} \right) + \text{sign}(\Delta r)(c - \text{floor}(c)) \right) \Delta x \] \hspace{1cm} \{5-2\}

and the intensity is given by

\[ z_m = (1 - k_m) z\left( \text{floor}(r_m), c_m \right) + k_m z\left( \text{floor}(r_m) + 1, c_m \right) \] \hspace{1cm} \{5-3\}

where

\[ r_m = r + \left( m - \text{ceil} \left( \frac{N}{2} \right) + \text{sign}(\Delta r)(c - \text{floor}(c)) \right) \frac{\Delta c}{|\Delta r|} \] \hspace{1cm} \{5-4\}

\[ c_m = \text{round}\left( \text{floor}(c) - \left( m - \text{ceil} \left( \frac{N}{2} \right) \right) \text{sign}(\Delta r) \right) \] \hspace{1cm} \{5-5\}

\[ k_m = r_m - \text{floor}(r_m). \] \hspace{1cm} \{5-6\}

An analogous procedure is employed when the cross-section is column orientated.

### 5.2 SLRF Measurement

The SLRF method is based upon the fitting of a line by least squares linear regression, relating image intensity against distance along the cross-section, within a window of \( W \) points centred on the \( n \)th point. The window is progressively moved by a single point at a time across the entire cross-section of interest, and the gradient of the best least-squares line recorded against the \( n \)th point as \( m_n \). Hence

\[ m_n = \frac{W \sum_i x_i z_i - \sum_i x_i \sum_i z_i}{W \sum_i x_i^2 - \left( \sum_i x_i \right)^2} \] \hspace{1cm} \{5-7\}

for all \( i \) such that \(-W/2 \leq (i-n) \leq W/2\)

This principle is illustrated in Figure 5-2, which illustrates the movement of the sliding window across an idealised intensity profile.
To determine the positions of the maximum positive going and negative going slope, the resulting values of \( m \) are subject to a threshold test, and those points falling beyond a specified range are used to compute the actual position of the edge using the first moment of intensity gradient over a number of points. Hence

\[
x_{\text{edge}} = \frac{\sum_{i} x_{i} m_{i}}{\sum_{i} m_{i}} \quad \{5-8\}
\]

for all \( i \) such that \( m_{i} > k_{i} m_{\text{max}} \) and \( -m_{i} > k_{i} m_{\text{max}} \)

where \( m_{\text{max}} \) is the maximum magnitude of slope, and \( k_{i} \) is an empirically determined constant, for which a value of 0.5 has been found to yield acceptable results. The vessel width is given by the difference between \( x_{\text{edge}} \) values.

Figure 5-3 illustrates the intensity gradient produced by the SLRF method from the example noise free profile shown in Figure 5-2, together with the gradient thresholds.
and the derivation of vessel edge positions. Ordinarily, the strongest +ve and –ve going peaks correspond to the vessel edges, with the smaller inner peaks arising from the central light reflex (if present).

Figure 5-3: Derivation of vessel edge positions from intensity gradient

The use of least squares linear regression to determine intensity gradient within the window was originally proposed in the interests of simplicity. However consideration has also been given to whether any benefit may be derived from use of alternative techniques, such as robust linear regression aimed at minimising the absolute deviation [91], or else fitting of higher order curves by Savitsky-Golay filters [91], taking the analytical derivative to determine the intensity gradient. However, in both synthesised noisy images and clinical images, neither of these approaches demonstrated any advantage over use of least squares linear regression, which was accordingly retained.

5.3 Choice of Window Size

An important consideration in the SLRF method is the choice of window size $W$. Generally, a smaller window size will give better resolution of edges of small vessels, whereas a larger window size will give better noise rejection. It may be concluded
that the window size should be adapted to reflect the size of the vessel under examination, and such a strategy would be expected to improve the linearity of the diameter determination. A suitable estimate of the vessel width $w_{est}$ prior to application of SLRF may be obtained by a variety of different methods from which an appropriate value of $W$ may be derived:

$$W = k_w w_{est} \quad \{5-9\}$$

where $k_w$ is an empirically determined constant, and $W$ is then adjusted to the next highest odd number.

A method to obtain $w_{est}$ based upon the half height approach discussed in the previous chapter has been found to give acceptable results, in which an intensity threshold is set initially at the mid point between minimum and maximum intensity values, and subsequently adjusted if necessary to seek just two points where the threshold is crossed, these points then being taken as the vessel edges.

Some insight into the appropriate value of the constant $k_w$ can be gained from consideration of the sensitivity of the vessel diameter output by the SLRF method to the window size $W$. To illustrate this, a series of noise-free single Gaussian intensity profiles were prepared as inputs to the SLRF method, and the resulting vessel diameters recorded for different values of $W$. The results are shown in Figure 5-4. It should be emphasised that this treatment is not intended to serve as a validation of the SLRF method, since the single Gaussian profile is not regarded to represent a gold standard for the intensity profile of a retinal vessel. It merely represents a convenient analytical profile to explore the impact of variation in the window size on SLRF diameter determination.

As may be observed, the diameter determined by the SLRF method appears comparatively insensitive to the window size $W$ for a range of values, until it is increased to a size, dependent on the vessel under measurement, where the measured diameter starts to increase markedly. It is desirable to select a value of $k_w$ so as to give
the largest practicable window size $W$ (to improve noise rejection), while remaining well within the region where diameter estimation remains insensitive to $W$.

The technique used to make an initial estimate of the vascular diameter, based on the half height approach has a tendency to underestimate the diameter in comparison with the SLRF method. Taking this into account, a value of $k_w = 0.43$ has been found to be appropriate, and gives rise to window sizes for the corresponding noise-free Gaussian profiles which are indicated by the circled points in Figure 5-4.

![Figure 5-4: Illustration of the variation in diameter returned by the SLRF method in noise-free single Gaussian intensity profiles for different values of window size](image)

It may also be seen from the figure that for the smallest profile considered, the diameter returned by the SLRF method exhibits more substantial dependence on varying $W$. Accordingly, the diameter measurements from the SLRF method for vessels less than 10 pixels in diameter should not be considered reliable.

### 5.4 Robustness

Ordinarily, the selection of the most prominent positive and negative going peaks in the intensity gradient (as illustrated in Figure 5-3) is uncomplicated and leads directly
to the identification of the vessel edges, without undue interference from the effects of the central light reflex. However, it is possible to encounter occasionally a vessel where a particularly prominent light reflex or noise peak generates an intensity gradient comparable to those of the vessel edges, leading to ambiguity as to the correct location of the vessel. Furthermore, where vessels cross, there may be no credible peaks in intensity gradient from which a measurement can be made. Accordingly, additional rules have been built into the SLRF method to detect and resolve such issues.

First, the peaks in intensity gradient are identified as positive or negative (defined to be where intensity gradient is >0 or <0 respectively), and sorted in order of increasing position $x$.

The SLRF method is designed to be applied to a bright vessel, resulting for example from a photographic negative of a red-free image, and so a vessel edge by definition must commence with a positive peak in intensity gradient in the direction of increasing $x$. Accordingly, any initial negative going peak is rejected as spurious.

Following this, all possible candidate vessels are identified from either

- consecutive positive and negative peaks, or else

- a sequence of a positive peak, representing the candidate edge, followed by an intervening pair of negative and positive peaks representing the light reflex, followed by a negative peak representing the opposite candidate edge.

The candidate vessels are each assessed for credibility by considering the following criterion. Firstly, a certain level of symmetry in the intensity profile is required for the candidate vessel to be retained:

$$\left|\frac{m_- - m_+}{m_- + m_+}\right| < \frac{k_m}{2} \quad \{5-10\}$$

where
\( m_+ \) and \( m_- \) are the peak gradient intensities of the +ve going and –ve going candidate edges respectively.

In addition, where a potential central light reflex is present (i.e. an intervening pair of peak gradients is present between candidate edges), the light reflex must not be excessively prominent compared to the vessel edges i.e. the following must be satisfied:

\[
\frac{|m_+| + |m_-|}{|m_{+r}| + |m_{-r}|} > k_r \quad \{5-11\}
\]

and

\[
\frac{x_{-r} - x_{r+}}{x_{+r} - x_{-r}} > k_x \quad \{5-12\}
\]

where

\( m_{+r} \) and \( m_{-r} \) are the peak gradient intensities of the +ve going and –ve going components of the candidate central light reflex respectively;

\( x_+ \) and \( x_- \) are the mid-positions of peaks in gradient intensity corresponding to the +ve going and –ve going candidate edges respectively, and

\( x_{+r} \) and \( x_{-r} \) are the mid-positions of peaks in gradient intensity corresponding to the +ve going and –ve going components of the candidate central light reflex respectively.

The constants \( k_a \), \( k_r \) and \( k_x \) have been set empirically to 1.0, 0.67 and 2.0 respectively.

To be accepted as a candidate vessel, all of the relevant criteria in equations \{5-10\} to \{5-12\} above must be satisfied. If more than one candidate vessel remains, then a final selection is made taking into account the proximity to the expected centre, and the expected vessel diameter. Deviations from the expected values for centre position and vessel width are combined into a cost function with empirically determined
weighting factors 2.0 and 1.0 respectively, and the most favourable candidate vessel adopted.

5.5 Noise Tolerance

Due to the nature of red-free retinal images, tolerance to image noise (e.g. from photographic grain) is of major importance in making reliable measurements of retinal vascular diameters. Accordingly, an evaluation was performed to examine the effect of intensity noise on the measurement of synthesised vessel profiles. The evaluation was first performed by fitting a double Gaussian profile (as described in Chapter 4) and then for comparison using the SLRF method. The synthesised vessels were themselves of double Gaussian form with $a_1 = 1.5$ and with the addition of normally distributed pixel intensity noise giving a standard deviation in intensity of 0.1 corresponding to 6.7% of the maximum height of the vessel profile. This level of noise was selected to give a similar subjective effect to the grain noise observed in typical film images. Three different widths of synthesised profiles were evaluated with $a_3 = 5$, 10 and 20 pixels, and in the largest vessel both without and with ($a_7 = 6$ pixels) a central light reflex of different depths ($a_5=0.45$ and 0.75), yielding five different synthesised profiles. For each profile, a total of 250 noisy cross-sections were evaluated. From this exercise, it was found that the additive noise gave rise to an rms error in estimating the vessel width of just 0.87 pixels using the SLRF method, compared to 2.35 pixels using double Gaussian fitting. Accordingly, it appears that the SLRF method offers substantially improved tolerance to noise than the double Gaussian fitting technique.

5.6 Evaluation on Clinical Images

A further evaluation of the SLRF method was undertaken to assess its behaviour when applied to clinical retinal images, as opposed to the synthesised images reported above. Foregoing analysis suggests that the SLRF method might be expected to offer superior performance to competing techniques, but it is necessary to confirm independently that this remains the case in images exhibiting characteristics typically encountered in a clinical setting.
This evaluation was carried out using a software tool implementing the SLRF technique, together with other methods of computer assisted vascular diameter measurement, including a facility to perform a purely manual measurement. This software was developed entirely by the author of this thesis, and can be considered as an early forerunner of the more comprehensive tool described in Chapter 7. A clinician experienced in viewing retinal images (Chapman) performed operator directed measurements on clinical images using the tool, and collated the results, which are reported in [15].

5.6.1 Objectives

The objective of the evaluation was to validate the performance of the SLRF method by capturing measurements of arteriolar diameters from red-free clinical images using three different automated techniques:

- the SLRF method described in this chapter
- the double Gaussian fitting method, as described in Section 4.4
- Sobel masks (a well known elementary method for detecting edges in images)

and comparing them in each case with manual measurements of the same vessels performed by a skilled clinician.

5.6.2 Methods

The evaluation was performed on 60 arteriolar vessel segments selected by the operator from a set of 11 red free clinical retinal images from male and female subjects aged 30-80 years. The images were originally captured on 35mm photographic film and were subsequently scanned into digital form of 2800 x 2400 pixels in size. All measurements were made using the aforementioned software tool, in each case within a region of interest of 512 x 512 pixels displayed to the operator on a 15” CRT monitor.

Manual diameter measurements were achieved from each arteriolar vessel segment by operator selection of two points corresponding to the opposite edges of the vessel, using a mouse driven cursor. The software tool then computed the diameter as the
linear distance between the points. Each vessel was measured manually in this way five times, and the median value adopted.

Measurements by the three computer aided techniques were made across an operator selected cross section normal to the vessel, defined by dragging a line across the vessel using a mouse driven cursor. The software tool then performed measurements across a total of five parallel cross-sections (two either side of that input by the operator) all two pixels apart. Again the median across the five measurements was adopted for the vessel.

Additionally, a further 21 vessel segments were measured on two separate occasions to test repeatability.

5.6.3 Results

The differences (mean ± sd) between manual and automated measurements for the three latter techniques are reported in Table 5-1 below, together with the coefficient of repeatability (2.77 x within subject standard deviation [9]) for each technique.

<table>
<thead>
<tr>
<th>Automated Technique</th>
<th>Difference (mean ± sd) Manual – Automated (pixels)</th>
<th>Coefficient of Repeatability (pixels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLRF</td>
<td>-2.97 ± 3.7</td>
<td>3.89</td>
</tr>
<tr>
<td>Double Gaussian</td>
<td>0.75 ± 4.6</td>
<td>9.22</td>
</tr>
<tr>
<td>Sobel mask</td>
<td>2.82 ± 6.8</td>
<td>4.82</td>
</tr>
</tbody>
</table>

Table 5-1: Results of independent evaluation of SLRF on clinical images [15]

5.6.4 Conclusions

It was found that “of the three automated methods compared, the SLRF method was the most consistent (defined as the method producing diameter estimations with the least scatter) and the most repeatable in measurements of retinal arteriolar diameter” [15]. A Bland-Altman plot [9] illustrating the difference between manual and SLRF diameter measurements against the mean diameter of both measurements is reproduced in Figure 5.5.
It may be observed from the Bland-Altman plot that the SLRF method tends to produce greater diameters than the manual measurement. This should not necessarily be unexpected. Virtually all the alternative techniques for the measurement of retinal diameters discussed in Chapter 4 as well as the SLRF method apply essentially arbitrary criteria for determining the locations of vessel edges. Hence there is an implied scaling constant for any such method relating the measured diameter to the actual diameter. Analysis by linear regression of the SLRF diameters against the mean diameters of both automated and manual measurements (as in the Bland-Altman plot) suggests that the difference may be attributed predominantly to a scaling factor (SLRF diameters are typically 9% larger than the mean diameters) but with a bias of less than 0.4 pixels. In the context of non-dimensional measures which are the focus of this work, a scaling factor may be immaterial, such as in a ratio of diameters advocated in Chapter 6.

It was also noted, based on practical experience of the SLRF method, that it becomes “unreliable for total vessel widths of less than 10 pixels” [15], which is consistent
with the same observation made earlier from consideration of the impact of window size $W$.

For completeness, it should be noted that the early version of the SLRF method evaluated by Chapman employed a different technique for estimating the vessel diameter for the purpose of deriving the window size $W$, and so would not necessarily have utilised an identical window size for a given vessel as the current algorithm described in this chapter. Nevertheless, given the comparative insensitivity of the diameter measurement made by the SLRF method to the window size, the results obtained by Chapman are considered to be representative of the performance of the SLRF method as currently specified.

### 5.7 Extension of the SLRF method to Vessel Segments

The foregoing description has considered the application of the SLRF method to a single intensity profile, but in practice it is necessary to apply the method along an entire vessel segment, either to obtain an aggregate measure of diameter, or else to identify local features of interest such as focal narrowing. In principle this could be achieved simply by taking consecutive profiles along the vessel a certain distance apart, and combining the diameter measurements from individual profiles. However, the question is raised as to whether any additional advantage, such as improved noise immunity, might be gained by adapting the method so as to achieve a measurement from multiple adjacent intensity profiles simultaneously.

The SLRF principle may be readily extended so that a plane is fitted to the observed intensity data along a section of a vessel by a least squares process, taking into account a window of data adjusted to reflect the size of the vessel, in an analogous form to that described earlier where a line is fitted over a single profile.

An alternative, simpler technique is also possible, in which a plane is fitted to the intensity data by a convolution mask of fixed size. The latter technique is computationally simpler than extending the SLRF technique to fit planes, but does not offer the benefit of being able to adapt the window size.
In order to compare these alternative techniques they were each applied to a 
synthesised vessel based on a single Gaussian intensity profile (a3=5 pixels) with 
additive noise as described in Section 5.5. It was found that:

- The standard deviation (sd) in diameter estimates produced by the SLRF method 
  (using a window size of 5 pixels) from individual consecutive cross-sections at 
  two pixel intervals, was 0.67 pixels.

The following alternative techniques for aggregating measurements over adjacent 
cross-sections were then considered:

- The sd in SLRF diameters averaged over five adjacent cross-sections was 0.31 
  pixels

- The sd in diameter measurements from extended SLRF fitting of planes to five 
  adjacent cross-sections was 0.28 pixels

- The sd in diameter measurements from fitting planes by a fixed 5x5 convolution 
  mask (equivalent in size to the SLRF window) was 0.27 pixels.

The principal question of interest is whether there is any evidence that a significant 
reduction in scatter of diameter measurements is achieved by any one of the latter 
three aggregation techniques. Under a null hypothesis that the scatter is the same from 
all three techniques, a Bartlett test for equivalence of variance was performed, 
indicating that there is no evidence to reject the null hypothesis at the 5% level 
($p=0.097$).

A drawback was observed with the use of the fixed convolution mask in that when 
applied to synthesised vessels of different sizes between 12 and 47 pixels in width, the 
linearity was noticeably inferior to the SLRF based methods which adapt the window 
size to the size of the vessel under measurement. For a 7x7 convolution mask, the rms 
error from the expected linear response was 0.34 pixels compared to 0.14 pixels with 
the SLRF method, and for an 11x11 convolution mask (giving better noise immunity 
in larger vessels) the rms error was 0.98 pixels.
These findings from synthesised vessels appear to suggest that there is no significant advantage to fitting planes to vessel edges, as opposed to averaging diameter estimates from individual cross-sections. This conclusion was reinforced by informal application to clinical retinal images, where it was found that fitting planes to vessel edges appeared to increase variability in diameter estimates along vessels. Accordingly, techniques involving fitting planes were not considered further, and the simpler approach of averaging the SLRF diameter estimates from adjacent cross-sections has been retained.

5.8 Vessel Tracking

The process of obtaining SLRF diameter measurements along a vessel segment proceeds as described below. Typically a vessel segment will join consecutive bifurcations in the vascular tree.

First of all, a candidate vessel track is established in the form of a cubic spline derived from a sequence of points along the vessel centre line. Such points may in principle be obtained from manual input, a pattern matching and/or tracking algorithm, or else segmentation of the image.

Prior to application of the SLRF method, a series of initial estimates of the vessel diameter are obtained to set the window size $W$, as described earlier. Normally, a total of 12 estimates are made at regular intervals along the vessel, and the median value is adopted as the basis for setting the window size, thus avoiding excessive influence of any outlying estimates.

A series of cross-sections are then derived along the candidate track, each being oriented orthogonally to the local direction vector of the track. The first cross-section is offset from the beginning of the track by typically 10 pixels, to avoid disturbance from a bifurcation at the beginning. Subsequent cross-sections are separated by distance $d$ which is nominally 2 pixels, as illustrated in Figure 5-6.

Each cross-section is centred at its intersection with the spline representing the candidate vessel track, a point referred to as the trial centre at which the value of $x$ is defined to be 0. The vector of $\hat{x}$ values representing linear distance along the cross
section, and the corresponding intensity values $\hat{z}$ are then extracted from the image as described in Section 5.1. The SLRF method is applied to the $\hat{x}$ and $\hat{z}$ vectors to produce a measurement of the vessel diameter, and the measured centre of the vessel.

The SLRF measurement from an individual cross-section is subjected to a credibility check and may be rejected if a potential error or inconsistency in the measurement (for example at vessel crossings) is indicated. Specifically, a measurement may be rejected for any of the following reasons:

- The robustness check of the SLRF method (Section 5.4 refers) may fail to identify any eligible candidate vessels.

\textbf{Figure 5-6: The SLRF method is applied to consecutive cross-sections along a cubic spline representing the candidate vessel track}

The SLRF measurement from an individual cross-section is subjected to a credibility check and may be rejected if a potential error or inconsistency in the measurement (for example at vessel crossings) is indicated. Specifically, a measurement may be rejected for any of the following reasons:

- The robustness check of the SLRF method (Section 5.4 refers) may fail to identify any eligible candidate vessels.
• The measured centre may deviate from the trial centre by an excessive amount. If $x_c$ is the value of $x$ at the measured centre, then if the following is not satisfied

$$|x_c| < k_c w_{eq} \quad \{5-13\}$$

where $k_c$ is a constant set empirically to 0.3, the measurement is rejected.

• The measured width may show a sudden deviation from an established trend. This is evaluated using a simple box filter which produces a running filtered diameter estimate $f_n$ after the $n$th measurement. If the measured diameter at the $n$th cross-section is $w_n$, then if the following is not met

$$|w_n - f_{n-1}| < k_f f_{n-1} \quad \{5-14\}$$

where $k_f$ is a constant set empirically to 0.3, then the measurement is not retained. The filtered diameter is in any case updated according to

$$f_n = f_{n-1} + \min\left(|w_n - f_{n-1}|, w_{\text{box}}\right) \text{sgn}(w_n - f_{n-1}) \quad \{5-15\}$$

where $w_{\text{box}}$ is a constant representing the size of the box, set to 1.5 pixels.

A situation may arise in which a sequence of measured centres may deviate systematically from the trial centres along the candidate vessel track, indicating the track to be inaccurate. In such cases, a risk exists that the cross-section may not in fact be perfectly orthogonal to the vessel, potentially leading to an error in the diameter measurement. In order to overcome this risk, following completion of all the measurements along the vessel, the actual vessel direction at each cross-section is determined by least squares linear regression applied to a total of thirteen measured centres; six in front and six behind the one in question. In order to be acceptable, at least half of the measured centres in the top and bottom four must be valid, so as to minimise the possibility that a spurious direction may be derived. If the actual direction derived from measured centres at a given point is represented by the unit vector $\vec{D}_{\text{meas}}$, and the direction of the vessel indicated by the candidate track is given
by the unit vector $\hat{D}_{cand}$, then a multiplicative factor to correct the diameter for the
deviation between the two is given by

$$fac = \frac{\hat{D}_{meas} \cdot \hat{D}_{cand}}{= \cos \theta}$$ \hspace{1cm} \{5-16\}

where $\theta$ is the angle between the candidate and measured directions.

Finally, an overall measure of diameter for the whole vessel segment is determined by
a measure of central tendency from all valid measurements along the vessel.
Ordinarily, the mean diameter is taken, but if the deviation between the mean and the
median exceeds 10%, then the median is taken instead, since this would tend to
suggest the exceptional influence of some outlying measurements.
Chapter 3 highlighted the difficulty of deriving absolute measurements from retinal photographs due to the impact of individual optical characteristics of the subject’s eye. To take account of such characteristics would require additional optical measurements on each subject. Such measurements are unlikely to be available in many existing retinal image sets. Based on measurements in the Blue Mountain Eye Study, Wong et al [143] estimated that in the absence of correction for the effects of ocular refraction, variability of up to 20% may arise in measurements of absolute vascular diameter from retinal fundus photographs. Accordingly, this work focuses particularly on non-dimensional parameters characterising the retinal vasculature, typically involving a ratio of distances. This approach avoids the confounding effects of variations in the refractive characteristics of the eye.

The geometry of the retinal vasculature may be characterised by a number of features that are intrinsically insensitive to absolute scale, of which this work will concentrate in particular on:

- optimality of diameter relationships at bifurcations
- internal angles at bifurcations
- length/diameter ratios of vascular segments
- tortuosity of vascular segments.

This chapter addresses the definitions of these features and the procedures needed to derive robust quantitative parameters representing them. In general, such parameters suffer from the effect of measurement noise (e.g. from vascular diameters) and the potentially confounding effects of imperfections in the images themselves. Accordingly, special attention has been given to techniques that may minimise the
impact of such effects and improve the robustness of the parameters when measured from clinical images.

6.1 Diameter Relationships at Bifurcations

Conventionally, the relationship of vascular diameters at bifurcations has been described by the junction exponent $x$, as introduced in Chapter 2, and defined as follows

$$d_0^x = d_1^x + d_2^x \quad \{6-1\}$$

where $d_0$ and $d_1, d_2$ are the diameters of the parent and daughter vessels respectively. Murray’s Law predicts that $x$ takes the value 3 under optimal conditions.

However, the junction exponent is not a practically convenient parameter to express the optimality of the diameter relationships at a bifurcation. Given measurements of the parent and daughter diameters (e.g. from the SLRF method described earlier) it is not possible to immediately derive the junction exponent by an analytical method. Typically an iterative approach is employed, or else a look-up table might be used in conjunction with interpolation.

Perhaps of even greater importance, it has been found that the junction exponent exhibits bias in the presence of random measurement noise in vascular diameters. This effect may be illustrated by a Monte-Carlo simulation in which an optimal symmetrical bifurcation (i.e. with $x = 3$) is considered, with the addition to each vessel of independent normally distributed noise in the measurement of diameter, having zero mean, and standard deviation corresponding to 5, 10 and 15% of the vessel diameter. In each trial, the junction exponent was calculated iteratively from the vascular diameters, including the measurement noise. After 2000 trials, the mean bias between the true and ‘measured’ junction exponent as well as the standard deviation was recorded, and is shown in Table 6-1. It should be noted that the apparent reduction in bias for increased measurement noise of 15% of vessel diameter is likely due to the failure of the iteration to produce a valid result for extreme cases where the daughter diameter exceeds that of the parent vessel.
<table>
<thead>
<tr>
<th>Std dev of measurement noise (% of vessel diam)</th>
<th>Mean difference between true and measured junction exponent</th>
<th>Std dev of difference between true and measured junction exponent</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.311</td>
<td>1.42</td>
</tr>
<tr>
<td>10</td>
<td>0.982</td>
<td>3.76</td>
</tr>
<tr>
<td>15</td>
<td>0.687</td>
<td>5.23</td>
</tr>
</tbody>
</table>

Table 6-1: Effect of measurement noise on measured junction exponent

The iterative process to determine the measured junction exponent in the above simulation was continued until the following criterion was satisfied

\[
\left| \left( \frac{d_1}{d_0} \right)^x + \left( \frac{d_2}{d_0} \right)^x - 1 \right| \leq T \quad \{6-2\}
\]

where the threshold \( T \) was set to \( 10^{-6} \). By differentiating the above, it may be shown that the influence of the threshold in terminating the iteration may give rise to a variation in the junction exponent of

\[
\Delta x = \frac{2T}{\left( \frac{d_1}{d_0} \right)^x \ln \left( \frac{d_1}{d_0} \right) + \left( \frac{d_2}{d_0} \right)^x \ln \left( \frac{d_2}{d_0} \right)} . \quad \{6-3\}
\]

In the case of a symmetrical optimum bifurcation (where \( x = 3 \)) the variation in junction exponent \( \Delta x \) arising from the termination criteria is less than \( 10^{-5} \), in other words insignificant compared to the observed bias and standard deviation from the simulation. Hence it can be concluded that these latter phenomena arise from the fundamental behaviour of the junction exponent, rather than being an artefact of the iterative procedure to measure it. Accordingly, an alternative measure has been sought to characterise the optimality of diameter relationships at vascular bifurcations. This measure must be better behaved in the presence of measurement noise.
For convenience, non-dimensional variants of the daughter diameters at a vascular bifurcation are defined as follows

\[ \zeta_1 = \frac{d_1}{d_0}, \quad \zeta_2 = \frac{d_2}{d_0} \quad \{6-4\} \]

Hence, from \{6-1\} and \{6-4\} the definition of junction exponent may be re-stated as

\[ \zeta_1^* + \zeta_2^* = 1 \quad \{6-5\} \]

Insight may be gained into the behaviour of the junction exponent (\(x\)) by plotting it against the non-dimensional daughter diameters \(\zeta_1\) and \(\zeta_2\) as shown in Figure 6-1. In the \(\zeta_1, \zeta_2\) plane, the line \(\zeta_1 = \zeta_2\) represents the set of perfectly symmetrical bifurcations, and departures from this are associated with increasing asymmetry. In general, excursions along the line \(\zeta_1 = \zeta_2\) from the origin represent an increase in the sum \(\zeta_1 + \zeta_2\), which is proportional to the mean daughter diameter, non-dimensionalised by the parent diameter. For further convenience, the latter is defined as the mean diameter ratio

\[ \gamma = \frac{d_1 + d_2}{2d_0} = \frac{\zeta_1 + \zeta_2}{2} \quad \{6-6\} \]

and the set of bifurcations of constant \(\gamma\) (but varying asymmetry) is represented by a line orthogonal to \(\zeta_1 = \zeta_2\), passing through the point \((\zeta_1, \zeta_2)\).
An evident feature of the illustrated surface in Figure 6-1 is that the relationship between mean diameter ratio ($\gamma$) and junction exponent ($x$) appears largely insensitive to bifurcation asymmetry. Accordingly, a parameter based on $\gamma$ offers the prospect of a robust, statistically well-behaved alternative to the junction exponent. Nevertheless, detailed examination of plots of $\gamma$ against $x$ for a range of symmetrical and asymmetrical bifurcations reveal a small dependency on asymmetry concentrated in the region of optimality ($x = 3$), and since small deviations are of particular interest in this region, it is desirable to correct for this.

![Figure 6-1: Relationship between non-dimensional daughter diameters ($\zeta_1$, $\zeta_2$) and junction exponent ($x$)](image)

**Figure 6-1: Relationship between non-dimensional daughter diameters ($\zeta_1$, $\zeta_2$) and junction exponent ($x$)**

constant $\gamma = (\zeta_1 + \zeta_2) / 2$
Conventionally, the asymmetry factor ($\alpha$) of a bifurcation is defined [151] as follows

$$\alpha = \left( \frac{d_1}{d_2} \right)^2 = \left( \frac{\xi_1}{\xi_2} \right)^2$$

where $d_1, d_2 > 0$ and $d_1 \leq d_2$ \hspace{1cm} \{6-7\}

so that a perfectly symmetrical bifurcation is described by $\alpha = 1$.

By algebraic manipulation of equations \{6-5\}, \{6-6\} and \{6-7\} it may be shown that for a general bifurcation

$$\gamma = \frac{1 + \alpha^{\frac{1}{2}}}{2\left(1 + \alpha^{\frac{1}{2}}\right)^{\frac{x}{k}}}.$$ \hspace{1cm} \{6-8\}

Under optimum conditions (i.e. $x = 3$) the value of mean non-dimensional daughter diameter is given by

$$\gamma^* = \frac{1 + \alpha^{\frac{1}{2}}}{2\left(1 + \alpha^{\frac{1}{2}}\right)^{\frac{3}{k}}}$$ \hspace{1cm} \{6-9\}

that is dependent only on asymmetry, and for a symmetrical bifurcation (i.e. $\alpha = 1$) takes the constant value

$$\gamma^{**} = \frac{1}{2^{\frac{3}{k}}} = 0.7937.$$ \hspace{1cm} \{6-10\}

It is therefore proposed to characterise the optimality of the diameter relationships at a bifurcation by the optimality ratio defined as

$$\Gamma_{ratio} = f(\alpha)\gamma$$ \hspace{1cm} \{6-11\}

where the correction factor
\[ f(\alpha) = \frac{\gamma''}{\gamma} = \frac{4(1 + \alpha^{\frac{1}{4}})}{(1 + \alpha^{\frac{1}{2}})^{\frac{3}{2}}} \]  \hspace{1cm} \{6-12\}

adjusts the measured mean diameter ratio, to take account of the measured asymmetry, yielding the approximate value that would have been obtained for a symmetrical bifurcation. For a bifurcation obeying Murray’s Law, the correction is exact.

By algebraic simplification of \{6-6\}, \{6-11\} and \{6-12\}

\[ \Gamma_{\text{ratio}} = \left( \frac{d_1^3 + d_2^3}{2d_0^3} \right)^{\frac{1}{2}} \]  \hspace{1cm} \{6-13\}

Thus, the optimality ratio \( \Gamma_{\text{ratio}} \) exhibits the desirable property that under optimal conditions predicted by Murray’s Law it equals the constant \( 2^{-\frac{3}{4}} \), given by \{6-10\}, irrespective of asymmetry.

The dependence of the correction factor \( f(\alpha) \) on the asymmetry factor is illustrated in Figure 6-2 below. It can be seen that for reasonably symmetrical bifurcations, say \( \alpha > 0.4 \), the correction factor has only a small effect on the overall non-dimensional daughter diameter, affecting it by less than 5%. However, for less symmetrical bifurcations, the effect of the correction factor is amplified, and reliable derivation of the corrected parameter may then be compromised by measurement noise, particularly in the diameter of the smaller daughter vessel. Therefore, wherever possible, exclusion of highly asymmetrical bifurcations \( (\alpha < 0.4) \) from consideration of vessel diameter optimality is advocated. In practice this also avoids difficulties in measuring very small vessel diameters.
The behaviour of the corrected optimality ratio may be illustrated by plotting the relationship with the junction exponent for various asymmetry factors, as shown in Figure 6-3. This confirms that for a junction exponent of 3 the value of optimality ratio remains constant irrespective of asymmetry, and also shows a negligible sensitivity to asymmetry for small departures from optimal conditions. However, for substantial departures from optimal conditions, there remains a residual dependency on asymmetry. In such cases, asymmetry causes the optimality ratio to slightly underestimate the extent of the deviation from optimality.
For convenience we also define the optimality index

\[ \Gamma_{ind} = 2^{\frac{1}{\Gamma_{ratio}}} \]  \{6-14\}

that possesses similar characteristics to the optimality ratio, except that it takes the value of 1 under optimum conditions.

Finally, we define the optimality deviation, a measure of the extent of deviation from the optimum conditions

\[ \Gamma_{dev} = \left| \Gamma_{ratio} - \frac{1}{2^{\frac{1}{\Gamma_{ratio}}}} \right| \]  \{6-15\}
In order to evaluate the robustness of the new parameters above, they were calculated using the same Monte-Carlo simulation of measurement noise described earlier, for a symmetrical bifurcation conforming to Murray’s law and the results compared to those from the junction exponent (Table 6-2). To facilitate comparison, the simulation results (bias and standard deviation) relating to the junction exponent are expressed in units of optimality ratio through multiplication by the scaling factor

\[
\frac{\Delta \Gamma_{\text{ratio}}}{\Delta x} = 0.06113 \quad \{6-16\}
\]

derived by analytical differentiation based on equations \{6-5\}, \{6-6\} and \{6-11\}.

<table>
<thead>
<tr>
<th>Std dev of measurement noise (% of vessel diam)</th>
<th>Mean bias (standard deviation) between true and ‘measured’ value, expressed in units of optimality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>From junction exponent</td>
</tr>
<tr>
<td>5</td>
<td>0.019 (0.087)</td>
</tr>
<tr>
<td>10</td>
<td>0.060 (0.23)</td>
</tr>
<tr>
<td>15</td>
<td>0.042 (0.32)</td>
</tr>
</tbody>
</table>

Table 6-2: Effect of measurement noise on junction exponent and optimality ratio

The simulations were also run for a range of bifurcations with optimality ratios between 0.6 and 1.0, and with asymmetry ratios between 0.4 and 1.0 to explore the behaviour of the optimality ratio under a wider range of conditions. The results of addition of noise of zero mean and standard deviation of 10% of vessel diameter are shown in Table 6-3 below.

<table>
<thead>
<tr>
<th>Asym ratio (α)</th>
<th>Mean bias (standard deviation) between true and ‘measured’ optimality ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Gamma_{\text{ratio}} = 0.6$</td>
</tr>
<tr>
<td>1.0</td>
<td>0.008 (0.075)</td>
</tr>
<tr>
<td>0.8</td>
<td>0.008 (0.077)</td>
</tr>
<tr>
<td>0.6</td>
<td>0.008 (0.077)</td>
</tr>
<tr>
<td>0.4</td>
<td>0.011 (0.082)</td>
</tr>
</tbody>
</table>

Table 6-3: Effect of measurement noise on optimality ratio for a range of bifurcations
It is evident from Table 6-2 that the optimality ratio is not as severely affected by measurement noise as is the junction exponent. For simulated noise with standard deviation of 10% of the true vessel diameter, the bias in optimality ratio is less than 15% of that arising from the junction exponent, and the scatter in measured data is less than half that observed in the junction exponent. The data presented in Table 6-3 also confirm that the optimality ratio remains well behaved over a wide range of bifurcation geometries that are likely to be encountered in the measurement of clinical images. Accordingly, the expression of the optimality of diameter relationships through parameters based on optimality ratio appears to offer improved robustness compared to the junction exponent.

6.2 Internal Angles at Bifurcations

The total internal bifurcation angle was introduced in Chapter 2 as

$$\psi = \theta_1 + \theta_2 \quad \{6-17\}$$

where $\theta_1$ and $\theta_2$ are the angles by which the daughter vessels deviate from the extended centre line of the parent vessel (illustrated in Figure 2-1). Given tracks of the centre line of the vessels, either from manual input or else from an automated process, measurement of these angles should in itself be straightforward. However, the path of these vessels will in general be tortuous. This raises the question of the length of the segment of the vessel over which the angle measurement should be made.

Gao et al [36] and later Martinez-Perez et al [74] sought to address this question, albeit with some differences in detail between their techniques. Both approaches defined the bifurcation centre by a circle of radius $r$ fitting exactly inside the bifurcation boundary, and used the dimension $r$ to represent the scale of the bifurcation. Martinez-Perez followed the principle that the angle of the daughter vessels should be measured along a length proportional to the scale of the bifurcation, and applied a length averaging process to the pixelated vessel skeleton over segments of length $5r$. 

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A similar principle has been adopted in the work reported here, albeit with some adaptation. A circle of radius $r$ is used to define the centre and scale of the bifurcation as described by the previous approaches, and a larger concentric circle of radius $5r$ is used to define the distal limit of the vessel segment over which the angle is calculated. The angle of the vessel (with respect to the horizontal) is then taken to be that of a straight line joining the pair of points where the vessel centre line intersects the inner and outer circles, as illustrated in Figure 6-4. The total internal angle of the bifurcation $\psi$ can then be calculated as the difference between the angles of the daughter vessels, expressing the result in the range from 0 to $2\pi$ radians.

The approach adopted here has the benefit of simplicity, but with the desirable feature that the position of the inner circle defining the bifurcation centre, which is likely to be more prone to error, has only a minor impact on the measurement of the vessel angle.

Figure 6-4: Measurement of bifurcation angle
6.3 Conventional Length/Diameter Ratios

The length to diameter ratio (L/D) of vascular segments was also introduced in Chapter 2 as a non-dimensional parameter that has been measured in previous studies, and is sensitive both to rarefaction and generalised arteriolar narrowing, both of which are associated with hypertension. It is calculated as the overall path length (\(L\)) of a vascular segment joining two bifurcation centres, divided by the best estimate of diameter (\(D\)) over the same segment, as illustrated in Figure 6-5. The path length is calculated from a cubic spline representing the vessel centre line and the diameter is determined from a set of SLRF measurements along the vessel segment, all as described in Chapter 5.

![Figure 6-5: Measurement of conventional L/D ratio](image)

In a practical measurement system for L/D ratio, it is necessary to establish criteria for the calibre of daughter vessels that are admitted as termination criteria for a vessel segment giving rise to an L/D ratio. Extremely small vessels bifurcating from a much...
larger parent are sometimes encountered in retinal vascular trees, and raise the question of whether they should be regarded as a termination for a vascular segment for which an L/D ratio is sought. Such highly asymmetrical bifurcations may make a minor contribution to efficient blood transport, but nevertheless make an important contribution to perfusion of local tissue. Accordingly, it could be argued that the presence of such vessels represents a reduction in rarefaction, and hence that they should be admitted into the termination criteria so as to be reflected in reduced L/D ratios.

Nevertheless, inclusion of vessels as terminating criteria that are so small as to be at or close to the detection threshold is extremely undesirable, since this is likely to introduce a dependency of L/D measurements on image quality. An image of particularly good focus and definition would be likely to exhibit more vessels of small diameter, and thus reduced L/D ratios, compared to a poor image of an anatomically similar vascular tree.

Furthermore, features in the image that offer the appearance of very small retinal vessels may in fact be either retinal nerve fibres or else choroidal vessels. Clearly, erroneous classification of such features would have a significant confounding effect on measurement of L/D ratios, and should be eliminated. Purely subjective evaluation of such small vessels would be expected to give poor reproducibility in semi-automated operator directed measurement tools (such as the one used to evaluate the Beaver Dam images as reported in Chapters 7 and 8).

Taking into account the competing considerations outlined above, criteria have been sought to determine whether or not a small vessel should be accepted as terminating its parent vascular segment for the purpose of L/D measurement. Following a number of reproducibility tests between graders making subjective judgements on such vessels, it was concluded that a ‘scoring’ scheme based on balancing points in favour of including a bifurcation, against points in favour of disregarding it, gave the best reproducibility and were judged most likely to reduce the dependence on image quality.
Accordingly, the following points were established in favour of accepting a bifurcation for the purposes of L/D measurement:

- the daughter vessel is clearly connected to the parent
- the daughter vessel appears continuous over a distance of several diameters
- the edges of the daughter vessel are distinct
- there is a discrete and sustained change of direction of the parent vessel at the site of the bifurcation

alongside the following points in favour of disregarding the bifurcation:

- there is discontinuity in the daughter vessel in the region of the connection to the parent vessel
- the daughter vessel appears excessively fuzzy
- the internal angle between the parent and daughter vessels is greater than 90 degrees
- the diameter of the daughter vessel is less than 5 pixels.

The bifurcation is considered eligible to terminate an L/D measurement if the number of points in favour of including it exceed the number of points in favour of disregarding it.

A further consideration affecting the practical measurement of L/D ratios is the region of a vascular tree over which L/D of individual segments should be measured, and a measure of central tendency derived. The generalised theoretical approach of Kassab [53] to the geometry of the vascular tree, introduced in Chapter 2, suggests a complex progression in L/D ratios over different orders of the tree. This is supported by earlier measurements by Schröder et al [103] from histological preparations of the human retina which show that increasing lengths of vessel segments towards the proximal
regions can be approximated by a second order polynomial, and that increasing diameters can be approximated by an exponential.

Accordingly, it is necessary to ensure that L/D ratios are measured over a consistent region of the vascular tree to avoid bias in an overall measure of central tendency. In the measurement protocols employed in this study, emphasis has been placed on measuring the entire vascular tree within a peripheral limit of fixed radius, centred at the optic disc. Measurement of the tree commences preferably at a clearly visible bifurcation within the optic disc (or else from the disc boundary), and continues along each connected branch until the diameter decreases below 10 pixels (the limit at which measurement by SLRF is considered reliable), or the tree crosses the peripheral limit. This process is illustrated in Figure 6-6.

Vessels commencing at a proximal bifurcation within the peripheral limit and terminating in an eligible bifurcation outside it are measured, but no measurements are made on vessels commencing outside the limit.

In the event that a vessel traverses the entire region from the optic disc to the peripheral limit but does not terminate in an eligible bifurcation (according to the criteria described above), then the L/D ratio is calculated taking the path length as far as the peripheral limit to avoid introduction of bias from exclusion of exceptionally long vessels.

The total path length of the vessel is derived from the cubic spline representing the centre line. The SLRF algorithm provides the best estimate of vessel segment diameter. Calculation of L/D is readily achieved from these two measures.
6.4 Length/Diameter Ratios between Symmetrical Bifurcations

The conventional L/D ratio described above suffers a number of drawbacks for the practical characterisation of retinal vascular trees. Most notably, the question of whether to accept a small apparent vessel as terminating a segment for the measurement of L/D presents difficulties that are likely to increase scatter in measured data and impair reproducibility.

Furthermore, although accepting such small vessels as terminating an L/D segment may be expected to increases the sensitivity of the L/D parameter to rarefaction, it may overlook other important topological features of the vascular tree, such as whether it tends towards a dichotomous \textit{i.e. balanced symmetrical) structure or else
towards a herringbone \((i.e.\) elongated asymmetrical) form [68]. Figure 6-7 illustrates extreme examples of these topological forms [130].

![Herringbone Asymmetric](image)

![Dichotomous Symmetric](image)

**Figure 6-7: Extreme forms of vascular tree topology [130]**

Yet another issue with the conventional L/D measure is that in interventional studies that may give rise to vascular dilatation, a risk exists that the diameter of some very small vessels may be increased beyond the detection threshold, giving rise to a confounding step change in the measured L/D ratio.

For all these reasons, a variant of the L/D ratio is proposed here with the aim of offering better objectivity and improved robustness, as well as sensitivity to the topological character of the tree. The proposed variant measures the path length to diameter ratio of vascular segments that join bifurcations that exceed a threshold of symmetry. This will be referred to as L/D_{sym}. For consistency with the preference for evaluation of symmetrical bifurcations discussed in Section 6.1 earlier, the same criterion has been adopted for accepting a bifurcation as symmetrical for the purposes of terminating the L/D_{sym} measurement, namely that asymmetry ratio \(\alpha > 0.4\).
Unlike the conventional L/D ratio, which can be calculated over a single vascular segment at a time, the L/D_{sym} is calculated as a post-process only after the entire tree has been measured. All connections between vascular segments are established, so that the asymmetry ratio can be calculated at each bifurcation.

As for the conventional L/D ratio, a circular limit is implemented, centred on the optic disc, so that any vascular segment that traverses from the optic disc to the limit without terminating in a symmetrical bifurcation can be included in an overall measure of central tendency to avoid bias against long L/D_{sym} ratios. In the context of the L/D_{sym} measurement algorithm, this limit is referred to as the selection limit, and may be configured to be of any radius, without being constrained to coincide with the peripheral limit used for the conventional L/D measurement. In addition, as for the conventional L/D parameter, a diameter threshold is established at 10 pixels, below which the vessel is considered too small to produce a reliable measurement.

Working from the most proximal vessel segment, commencing at the optic disc, each vascular segment between a pair of bifurcations is processed in accordance with the algorithm summarised in Figure 6-8, with the objective of calculating L/D_{sym} over a sequence of such segments linking bifurcations meeting the symmetry criterion.

The application of the algorithm is illustrated in Figure 6-9.
if vessel commences at optic disc or commences new L/D_{sym} sequence

set \( i = 1 \) and sequence as incomplete

while L/D_{sym} sequence is incomplete

record length and diameter of \( i^{th} \) segment

if diameter of \( i^{th} \) segment falls below allowed threshold

record L/D_{sym} sequence as complete but invalid

elseif \( i^{th} \) segment terminates with single connection

record L/D_{sym} sequence as complete but invalid

elseif \( i^{th} \) segment terminates without connections

if vessel sequence commences at optic disc and crosses selection limit

record L/D_{sym} sequence as complete and calculate L/D_{sym} to intersection with selection limit

else

record L/D_{sym} sequence as complete but invalid

end

elseif \( i^{th} \) segment terminates in asymmetrical bifurcation

record current L/D_{sym} sequence as incomplete, continuing to next segment along daughter vessel with diameter closest to parent setting \( i = i+1 \)

record new L/D_{sym} sequence commencing at opposite daughter vessel

elseif \( i^{th} \) segment terminates in symmetrical bifurcation

record L/D_{sym} sequence as complete and calculate L/D_{sym} from recorded segments

record new L/D_{sym} sequence commencing at each daughter vessel

end

end

end

Figure 6-8: Algorithm for construction of L/D_{sym} sequences between symmetrical bifurcations
The length to diameter ratio is calculated over the sequence of all individual segments as follows

\[
\frac{L}{D_{\text{sym}}} = \frac{\left( \sum L_i \right)^2}{\sum L_i D_i}
\]  \{6-18\}

where \( L_i \) and \( D_i \) are the path length and best diameter estimate of the \( i^{\text{th}} \) vessel segment respectively. These values are determined in the same way as for the conventional L/D ratio.
If the final segment of the L/D_{sym} sequence does not terminate in a symmetrical bifurcation, but the sequence is eligible to terminate at the selection limit, then the point of intersection with the limit is found from the cubic spline representing the centre line of the vessel segment, by searching for the proximal pair of points along the spline that straddle the limit, and interpolating to give the exact point of intersection. The path length from the start of the segment, along the spline to the intersection with the limit is then readily calculated.

6.5 Vascular Tortuosity

Vascular tortuosity in the retina has been qualitatively assessed by physicians for over two centuries, but quantitative measurement of tortuosity of retinal vessels has received attention only recently. A range of computational techniques have been proposed for the measurement of vascular tortuosity, and Hart et al [43] have compared the characteristics of seven different measures, although without conclusively recommending any particular approach.

A simple and widely used technique compares the path or arc length ($L_a$) of a vascular segment to its direct chord length ($L_c$) in the following form

$$\tau_{simple} = \frac{L_a}{L_c} - 1 \quad \{6-19\}$$

thus giving the property that a perfectly straight vessel yields a tortuosity value of zero.

Evidently, this measure is sensitive to the increase in path length in a tortuous vessel, but doesn’t necessarily reflect the extent or rate at which a vessel changes direction. However, alternative measures of tortuosity offering sensitivity to the latter characteristic can be derived from the curvature of the vessel ($\kappa$), defined [43] as

$$\kappa(t) = \frac{d\alpha}{ds}(t) \quad \{6-20\}$$

where $\alpha$ is the angle of the tangent (radians) and $s$ is the path length, measured at point $t$. This may be shown to be equivalent to the inverse of the radius of curvature.
Hart et al [43] have considered the following measure based on curvature, normalised by the arc length

$$\tau_{\text{curve}} = \frac{1}{L_a} \int_{l_{\text{start}}}^{l_{\text{end}}} \left[ \kappa(l) \right]^2 dl \tag{6-21}$$

where $l$ represents the distance along the vessel, noting that (unlike simple tortuosity) it benefits from a property referred to as ‘compositionality’, meaning that if a vessel is composed of two smoothly connected segments, then the tortuosity of the entire vessel must lie between those of the adjoining segments. Measures having such a property have been considered a priori to correspond more closely to the tortuosity judged qualitatively by ophthalmologists [43]. However it should be noted that the curvature based measure ($\tau_{\text{curve}}$) has dimension of $l^{-2}$ and so is not invariant with scale.

Differences in the behaviour of these alternative measures may be appreciated by reference to Figure 6-10, which illustrates three vessels, all constructed from concatenated semi-circles, allowing different measures of tortuosity to be calculated analytically. All three vessels are of equal arc and chord length, but with different numbers of inflection points. Taking into account that curvature based tortuosity is not dimensionless, for the purposes of illustration, this measure has been computed assuming an arbitrary chord length of 200 pixels, corresponding to an arc length of 314 pixels, which is comparable with the length of typical vessels observed in retinal images from digitised film. As may be seen, simple tortuosity is the same in all three cases, due to the constant arc and chord length, whereas curvature tortuosity progressively increases with the number of inflection points.

Calculation of simple tortuosity is readily achieved, since the total path (arc) length is available from the L/D computation, and direct (chord) length is easily calculated from the start and end points of the vessel. The curvature tortuosity is measured from analytical treatment of curvature at each point of the cubic spline representing the vessel centre line, with numerical integration along the vessel and normalisation by the path length in accordance with equation \{6-21\}.
Figure 6-10: Comparison of simple and curvature tortuosity in vessels of equal arc and chord length, but differing number of inflection points.

<table>
<thead>
<tr>
<th></th>
<th>Simple Tortuosity</th>
<th>Curvature Tortuosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.57</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10 x 10^-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.40 x 10^-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.90 x 10^-3</td>
</tr>
</tbody>
</table>
To facilitate analysis of the vascular geometry, an operator directed tool was developed, incorporating the SLRF technique (Chapter 5) to measure vascular diameters, together with computation of the parameters described in Chapter 6. The tool was developed to run within the MATLAB (The MathWorks Inc, MA) environment, initially on Release 13 under Windows (Microsoft Corp.), but is compatible with later versions supported on a range of industry standard platforms. The tool makes extensive use of the Graphical User Interface (GUI) features of this environment, allowing the user to interact predominantly via clicking and dragging a mouse driven cursor.

The main screen that the tool presents to the operator is illustrated in Figure 7-1. The window at the left hand side presents a series of buttons allowing a particular measurement operation to be selected by positioning the cursor on the button and clicking. The buttons are context-sensitive, so that only those offering a valid operation at any particular time are capable of being activated, whereas the remaining buttons are greyed-out and do not respond to a click. The image under analysis is shown in a separate window normally located to the right, through which the user selects the particular bifurcation or vessel to be measured, and the tool in turns indicates where measurements have been made.

7.1 Description of Tool

The steps involved in the analysis of a retinal image using the tool are as follows, where for clarity the name of a particular button is shown in bold.

Upon starting the tool, only the **Start Result File** button is available. On clicking it, the user is prompted to enter their initials into a dialogue box, from which they are then incorporated into the filename used to record results to facilitate traceability. The filename is also date/time stamped, and is shown at the bottom of the window containing the control buttons.
In the event of any error opening the result file, the error is notified to the user and no analysis is permitted until a result file has been created.

![Figure 7-1: Screenshot of the grading and analysis tool](image)

Figure 7-1: Screenshot of the grading and analysis tool

Once a result file has been successfully created, the **Select Image** button becomes active, allowing an image file to be selected for analysis through a file browser window. Once the image file has been loaded, a monochrome representation of the image is shown in the right hand window. The tool expects either a monochrome negative red-free image, which is presented directly to the user with bright vessels against a dark background (as required by the SLRF method), or else a colour reversal image, in which case the green layer is extracted automatically (Chapter 3) and the image reversed to give the required bright vessels. If any other type of image is loaded (e.g. monochrome red-free positive) then the vessels may be presented as dark against a light background, in which case the image must be reversed by the user through clicking on the **Reverse Image** button.
In order to ensure that vascular arcades are analysed over a consistent region of the image, a circular grid is superimposed on the image before measurements commence, centred on the optic disc, as can be seen in Figure 7-1. The distal circle of the grid represents the peripheral limit for measurement of length/diameter ratios described in Chapter 6. The dimension of the grid is established a priori by measurements on a sample of images; in the case of the Beaver Dam images, the boundary of the optic disc (represented by a dashed red line) is set at a radius of 249 pixels, and the radii of the proximal and distal grid circles within which vascular measurements take place are set at 1.2 and 4.0 times the disc radius respectively. A vertical line through the centre of the optic disc is also included to define the point at which measurement commences. To position the grid, the user clicks on the Add Grid button, whereupon a circle representing the boundary of the optic disc is dragged by the mouse so as to overlay the image of the disc.

In general, the monochrome presentation of bright vessels has been found to give the user the most favourable view of the vessels for the purpose of selecting those to be subjected to measurement, but on occasions it has been found useful to be able to refer to an original colour image, where available. For example, this might aid in distinguishing between arteries and veins. For this reason, whenever a colour image is available, two additional buttons, Colour Image and Mono Image, are provided to toggle the presentation between the two options with a single click.

While conducting measurements of vascular parameters, it is generally easier to magnify the image to allow closer examination of a particular region. A click on the Zoom Centre button allows the user to identify the centre of the region of interest with a further click on the image, and additional clicks on the Zoom In or Zoom Out buttons then expand or contract the region, by a factor of approximately 60% per click.

Grading a vascular tree commences with identification of eligible bifurcations, according to the criteria described in Section 6.3. In order to facilitate judgement of bifurcations that are excluded by virtue of the diameter of a daughter vessel, the tool provides a cursor in the form of a circle of 10 pixels in diameter. Only bifurcations centred between the proximal and distal grid circles are considered. To identify an eligible bifurcation, the user clicks on either the Arterial Bif or the Venous Bif button as appropriate, and drags a circle with the mouse, representing the inner circle enclosing the bifurcation centre as defined in Section 6.2. The
tool then displays, in addition, the outer circle (Section 6.2) defining the intersect with the daughter vessels at which the vascular angles are calculated. Following identification of the bifurcation, the Reject Bif button becomes available, offering the user the option to reject it with a single click on this button. If the user is content, then clicking on any other button to commence another measurement will cause the bifurcation to be accepted, and the option to reject it is cancelled.

Once all eligible bifurcations within a particular tree have been identified by the process above, the vascular segments linking them are measured, starting at the optic disc, and progressing to the distal regions of the tree. A vascular segment is measured by first clicking on the Track Artery or Track Vein button as appropriate. In the version of the tool applied to the Beaver Dam study, the vessel is measured by the user clicking on a sequence of points along the vessel centre line, through which the tool then fits a cubic spline. When a vessel starts or commences at a previously identified bifurcation, the vessel is automatically associated with the bifurcation as a daughter or parent vessel respectively. Otherwise, the user is prompted by the tool to identify the start or end point as necessary. Starting at the optic disc, the first vessel is measured starting at a clearly visualised bifurcation within the optic disc if one is evident; otherwise the measurement commences at the dashed red grid circle representing the disc boundary. The tool prompts the user to identify the vessel as commencing either at a ‘Disc Bifurcation’, the ‘Disc Boundary’ or an ‘Undefined’ point. Measurements continue along the tree until at the distal end a vessel crosses the peripheral grid circle. If such a vessel terminates at a clearly visualised bifurcation beyond the grid it is terminated at that bifurcation, otherwise it is terminated at the peripheral grid circle itself. The user is prompted to identify the end point of the vessel as a ‘Peripheral Bif’, the ‘Peripheral Grid’ or an ‘Undefined’ point. A daughter vessel of an eligible bifurcation that neither crosses the peripheral grid, nor terminates in an eligible bifurcation is terminated at an ‘Undefined’ point. Once a measurement of a vessel has been made, the tool displays the cubic spline centre line derived from the user entered points, together with a line of dots representing the vessel edges at individual cross-sections determined by the SLRF method. These features can be seen in Figure 7-2 which illustrates the grading of an arterial tree, distinguished by red centre lines. The veins are omitted for clarity in this figure, but are displayed by the tool with blue centre lines. Once the vascular track and edges have been displayed, the Edit Track and Reject Track buttons also become available. Clicking on the former button allows the user to drag the cursor over a small region within which any
individual diameter measurements are discarded. This feature is employed where the tool has clearly returned an incorrect measurement (e.g. where an artefact in the image has been detected rather than a vessel edge). Alternatively, a click on the Reject Track button cancels the entire measurement. If the user is content with the vessel measurement, then commencing another measurement signals its acceptance.

Figure 7-2: Arterial vessels measured by grading and analysis tool
A set of additional features are also provided to support purely manual measurements of distance (between two points), length (along a sequence of points), angle (between two lines) and SLRF diameter over a user defined cross-section. These are invoked by clicking on the **Manual Distance**, **Manual Length**, **Manual Angle** and **Manual Slope** buttons respectively. These are not used routinely in the grading process, except that rarely, in a challenging vessel, the SLRF method may fail to return any valid measurements that meet the acceptance criteria described in Chapter 5. In such circumstances, the user is prompted to make manual measurements of vessel diameter using the **Manual Distance** button. Three individual manual measurements are accepted to form an L/D measurement, but are not considered sufficiently reliable to contribute to a measurement of bifurcation optimality.

In order to aid compliance with a grading protocol (such as described in Section 8.2) requiring a minimum number of eligible bifurcations and informative vessels to be graded, the tool displays counters of these entities, updated as grading progresses, at the bottom of the image window. Following completion of grading an image, the tool writes all results relating to individual bifurcations and vessels to the Result file for subsequent post processing and analysis as described in Section 7.2 below. In addition a User file is also produced which records all measurement actions undertaken by the user. This allows the opportunity to replay the grading process through an updated version of the tool, for example that may incorporate an improved version of the measurement algorithm, thus protecting the investment in the manual effort spent on the grading process.

### 7.2 Post Processing of Results

The Result files produced by the grading and analysis tool contain measurements of all parameters relating to each individual vessel or bifurcation. In order to calculate a measure of central tendency for each parameter within a single subject, the Result files are run through a post processor, specially prepared for this purpose, and also written to operate within the MATLAB (The MathWorks Inc, MA) environment. Additionally, the post processor performs reconstruction of the vascular trees to calculate the $L/D_{sym}$ parameter, described in Section 6.4.
7.3 Further Enhancements of Tool

The foregoing sections describe the version of the tool that was employed for the exploratory study of the Beaver Dam cohort discussed in the following chapter. Subsequent to that analysis, however, a number of enhancements and additional features have been incorporated to facilitate its application to further retinal image sets, as mentioned in Chapter 9.

Most noteworthy has been the incorporation of a vessel tracking algorithm to allow the grader to identify an individual vessel merely by clicking its start and finish points. The tool then tracks the centre line of the vessel automatically using a template matching technique, rather than requiring the grader to click along intermediate points. The tracking algorithm incorporates two sets of idealised two dimensional templates of a vessel’s image intensity, one set for arteries and the other for veins. Each set comprises five individual templates of progressively increasing size, all based on a double quadratic curve, blurred by a Gaussian function. The most appropriate template is selected for each vessel segment to be tracked, by application of a cross correlation process.

The automatic vessel tracking significantly reduces the effort required to grade a retinal image for vascular geometry, and also improves the reproducibility of measurements of vessel path length, since minor scatter arising from different placements of user points along the vessel is avoided. However, the grader remains responsible for selecting vessels for measurement, which is a potential source of inconsistency. Furthermore, the tracking algorithm may fail to track a vessel correctly in certain situations, such as crossing arteries and veins, or vessels running alongside each other. In such situations, the grader may reject the automatic track and define the vessel’s centre line manually, as described earlier.

A further enhancement to the tool has been the introduction of a feature to allow the grader to delete an entire tree by pressing a Delete Tree button, in the event of realising that a significant grading error has been made. Upon selecting this option a pull-down menu is then presented to identify the tree to be deleted. In earlier versions of the tool, the grader had to make a separate note of such errors, requiring manual editing of the Result file prior to post processing, which was inconvenient and time consuming.
Finally, an **Add Note** button has been incorporated, allowing a grader to add a textual note, commenting on any noteworthy aspect of the image or grading. The note is then embedded in the Result file, and can be examined during post processing.
CHAPTER 8
THE BEAVER DAM EYE STUDY

The City of Beaver Dam occupies an area of 6.6 square miles and sits on the south eastern shore of the Beaver Dam Lake in Dodge County of the state of Wisconsin, USA. The 1980 census recorded a relatively stable population of 17,179 individuals, with outmigration in the county over the preceding five years being just 0.32% per year [59]. The stable population in a well defined geographical area, coupled with an excellent response rate of 96% in a previous study of diabetic retinopathy in the area [58], led to the selection of the city by the Department of Ophthalmology at the University of Wisconsin for a prospective population-based study into ocular and cardiovascular health, known as the Beaver Dam Eye Study, and described in detail by Klein et al [59].

Based on a private census performed in 1987-8, eligibility criteria for entry into the study were established as follows:

- living in the city or township of Beaver Dam, and
- age of 43 to 84 years at time of the census.

A total of 5925 eligible individuals were identified, of which 4926 participated in the study (83.1%). The average age of participants was 60.6 years, and 33.0% were male, with the population being predominantly (99%) white.

Participants underwent a baseline examination, performed within a 30 month period commencing on 1st March 1988, mostly at a specially designed facility at Beaver Dam Hospital, with small numbers examined instead at nursing homes (47) or at home (20). The examination included [59][138]:

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• a questionnaire addressing personal and family medical history, lifestyle, and sociodemographic factors, including household income, a history of cardiovascular disease (CVD), use of medication, and cigarette smoking,

• measurement of blood pressure from the average of two measurements with a random-zero sphygmomanometer, with hypertension being defined as systolic blood pressure (SBP) ≥ 140 mmHg, diastolic blood pressure (DBP) ≥ 90 mmHg, or the combination of self-reported high blood pressure and use of hypertensive medication at the time of examination,

• measurement of height, weight, and anthropometry, with body mass index (BMI) being defined as weight(kg)/height(m)²,

• collection of non-fasting blood samples and measurement of blood glucose, glycosylated haemoglobin (HbA1c), total cholesterol and high density (HDL) cholesterol, with diabetes mellitus being defined as having a history of diabetes, or else exhibiting elevated glycosylated haemoglobin with a random blood sugar measurement > 200 mg/dL,

• measurement of standardized refraction and visual acuity,

• capture of 30 degree field-of-view stereoscopic retinal photographs, in both eyes, centred on the optic disc (Field 1) and the macula (Field 2) (see Chapter 3) on colour reversal film, in accordance with a modification of the standard Early Treatment Diabetic Retinopathy Study (ETDRS) protocol.

Participants were subsequently followed-up with an interim examination after five years, and a final examination after ten years. Mortality over the ten year period was recorded using death certificate data, in conjunction with contact with relatives and family physicians. A total of 417 deaths related to CVD occurred during follow-up, of which 258 were specifically categorised as due to ischemic heart disease (IHD) (ICDA codes 410-414) and 73 to stroke (ICDA codes 430-438).
8.1. Exploratory Study of Vascular Geometry

Retinal images from the Beaver Dam Eye Study represent a valuable data set in which to examine the association between retinal abnormalities and subsequent cardiovascular disease.

A previous case-control study of Beaver Dam images reported by Wong et al in 2003 [138] showed that in younger subjects (aged under 75 years at baseline) 10 year cardiovascular mortality was predicted significantly and independently of other risk factors, by the presence of retinopathy, focal arteriolar narrowing, arteriovenous nicking and generalised arteriolar narrowing in baseline retinal photographs. In older subjects, only retinopathy was significantly predictive of CVD related death. This study considered all cases of CVD related death within 10 years of the baseline examination for which retinal photographs were gradeable (n=413), with age and sex matched controls (n=1202) selected by a stratified random sampling strategy. A target ratio of 1:3 cases to controls was achieved in the younger subjects (aged under 75 years), although this target could not be met for older subjects. Retinopathy, focal arteriolar narrowing and arteriovenous nicking were judged subjectively by certified graders (blinded to participant and case/control status), whereas generalised arteriolar narrowing was measured by means of the A/V ratio, calculated by the Parr and Spears approach, as described earlier in Chapter 2.

Based on the results obtained in the earlier study by Wong, an exploratory case-control study of broadly similar design, based on the Beaver Dam images, has been performed to evaluate the power of the parameters characterising retinal vascular geometry described in Chapter 6 to predict cardiovascular mortality. Taking into account that stronger associations between microvascular abnormalities and subsequent disease were observed in younger subjects, it was elected to restrict the study reported here to subjects under 75 years of age at baseline. Furthermore, since the retinal parameters of particular interest, such as the optimality of bifurcations, and L/D ratios, are hypothesised to be particularly associated with atherogenesis and hypertension respectively (Chapter 2), it was considered more appropriate to include cases only where cause of death was coded specifically as ischemic heart disease (IHD) (n=142 in the younger cohort) or stroke (n=31 in the younger cohort).
Computations of statistical power are reported in [129]. In the absence of accepted data on the distribution of the retinal geometrical parameters of interest, it is assumed that they are dichotomised at the median value in the control population, and furthermore that exposure in the cases is uncorrelated with that in controls. With 142 IHD cases and a 1:3 ratio of cases to matching controls, the study should be capable of detecting a logistic coefficient ratio of 0.4 (odds ratio 1.5) with 5% significance (i.e. probability of Type I error) and 80% power (i.e. 20% probability of Type II error). This compares with observed odds ratios of between 1.9 and 3.3 for prediction of CVD related death by microvascular abnormalities, in the earlier study reported by Wong.

For the 31 cases of death arising from stroke, under the same assumptions, but using the same control population (without age and sex matching) as for IHD cases, representing a matching ratio of approximately 1:13, the study should be capable of detecting a coefficient ratio of 0.9 for 80% power (odds ratio 2.5). In this regard the study would have to be considered as somewhat underpowered for prediction of death from stroke, but is nevertheless of interest for exploratory purposes.

Cases of IHD and stroke in the desired age range were identified by the University of Wisconsin, together with randomly selected controls matched on 5 year age intervals and sex to cases of IHD and stroke. Disc centred retinal images (Field 1 of left and right eyes) of selected subjects were scanned at Wisconsin using a commercial photographic scanner based on an RGB line sensor (Nikon LS1000) at a resolution of 2700dpi, yielding digital images of approximately 2900x2500 pixels over the retinal field. The digital images were then transferred electronically to London for analysis of the vascular geometry, after transformation of the image identifiers to mask the subject identity and case/control status during analysis.

Controls were selected from participants who did not die from a CVD related cause within the 10 year period following baseline examination. No further information was provided on endpoints of the controls, and it cannot be ruled out that some may have suffered from atherosclerosis, potentially weakening the power of the study.
The Field 1 (disc centred) images were analysed by means of the semi-automated computer based grading tool, described in the previous chapter, operated by two graders, in accordance with a protocol summarised in Section 8.2. The graders were blinded to the case/control status during the grading process.

A total of 829 subjects were identified by Wisconsin for which a Field 1 retinal image was available from at least one eye. Of these, 45 subjects were excluded because the images were of insufficient quality to allow reliable measurements to be made. In addition, 102 potential controls were excluded because the required quota of controls had been completed. Accordingly the results reported are based on, 126 cases of IHD death, 28 cases of stroke death, and 528 controls.

8.2. Grading Protocol

Grading procedures were established for analysis of the images and both graders were trained to follow them. The procedures are summarised as follows.

- Subjects were allocated to graders by a random process.
- For consistency of analysis, one grader undertook measurements on both left and right eyes of each subject. Graders were not blinded to the commonality of images from left and right eyes.
- Reproducibility of measurements (inter and intra-observer) was monitored throughout the study by allocation of a randomly selected subset of images to all graders.
- Grading of a subject normally commenced with the image of the Right eye, identified by an image file number of the form xxxxx101.tif.
- In the event that this image exhibited obvious defects, such as poor focus or restricted field of view, then the analysis commenced with Left eye (image file of the form xxxxx111.tif), if it was found to show superior image quality.
• In the event that images of both eyes exhibit obvious defects, then no grading was performed, and the subject was noted for subsequent review.

• Analysis commenced with the first arterial tree crossing the disc boundary in the clockwise direction from the 12 o’clock position.

• Analysis proceeded over complete vascular trees; in each tree all qualifying bifurcations and vessel segments were measured in accordance with the criteria (as described in Chapter 6 of this document).

• After completing the first vascular tree, the analysis proceeded to the next tree crossing the boundary of the optic disc in the clockwise direction.

• In the event that a particular tree was judged by the grader to be poorly visualised, or it was not possible to determine with reasonable certainty whether it was an artery or vein, then that tree was skipped, and analysis continued with the next tree in the clockwise direction.

• Measurements continued until the current tree had been measured in its entirety and the following totals had been achieved:
  
  o a minimum of five bifurcations yielding measurement of internal angle, and

  o a minimum of seven vessel segments yielding conventional L/D measurements between two bifurcation, or between the disc boundary and a bifurcation, or else between the distal and proximal (peripheral) grid circles.

• After completing measurements on arterial trees as described above, the procedure were repeated in an identical fashion on the venous trees, commencing at the 12 o’clock position and proceeding in a clockwise direction.

• In the event that the image of the Right eye did not yield the minimum number of bifurcations and vessel segments specified above, the analysis continued in an identical fashion in the Left eye, commencing again at the 12 o’clock position,
proceeding in a clockwise direction until the minimum number of measurements was achieved in both arterial and venous arcades.

8.3. Statistical Analysis

All the retinal vascular parameters of interest were found to exhibit skewed distributions, and so it was judged most appropriate to use the geometric mean as a measure of central tendency over all vessel segments or bifurcations within each subject. This measure was also generally found to give superior reproducibility.

Subsequent statistical analysis was performed using STATA 11.0 (StataCorp).

Mean values of baseline characteristics and retinal vascular geometry parameters were compared by outcome (IHD death, stroke death or control). Analysis of variance (ANOVA) was used to detect significant differences between the three groups for continuous variables, correcting for age and sex, and the $\chi^2$ test for categorical variables. For the retinal parameters where ANOVA indicated a significant difference, Fisher’s Least Significant Difference (LSD) test [80] was applied separately to age and sex adjusted variables comparing IHD cases against controls, and stroke cases against controls.

An underlying assumption of ANOVA is that variance is homogenous between groups [80]. In order to guard against the possibility of Type I error, Levene’s test, as modified by Brown and Forsythe (replacing the mean with the median), was applied to retinal parameters exhibiting significant differences between groups, to test for homogeneity of variance using the W50 statistic produced by the robvar programme [18].

Multivariate logistic regression was used to assess the independence of associations between the retinal parameters and outcome, from other cardiovascular risk factors evident from the baseline data.
8.4. Reproducibility

Inter-observer reproducibility was assessed initially during a pilot study to gain confidence in the measurement protocols, and the consistency of the graders’ judgement, prior to commencing the full study. This reproducibility analysis focussed particularly on the conventional L/D ratio between arterial bifurcations, since these embody both vascular length and diameter measurements, and also rely on the graders’ application of the eligibility criteria for terminating an L/D segment, as discussed in Chapter 6. Ten subjects were graded by each grader using the grading tool in accordance with the study protocol. The differences in the geometric mean of L/D ratio between graders for each subject were derived, and are illustrated in the form of a Bland-Altman [9] diagram in Figure 8-1. The mean difference in L/D ratio between the two graders was 0.84 and the standard deviation of the difference was 2.07.

Analysis was performed to identify the predominant origin of differences in L/D measurements between the graders. Both graders measured a total of 58 L/D vessel segments between bifurcations over the ten subjects, but only 50 vessels were measured in common. In two common vessels, the SLRF method rejected the diameter measurement as unreliable in one or both gradings. A further Bland-Altman plot is shown in Figure 8-2 showing the difference in 48 L/D measures of individual vessel segments measured by both graders in common. As can be seen, the reproducibility was noticeably superior, with a mean difference in L/D of just 0.12, and a standard deviation of 1.59. One significant outlier is evident in Figure 8-2 which arose because one grader terminated the vessel segment earlier than the other because of inclusion of a terminating bifurcation rejected by the other grader. If this single outlier is excluded, then the mean and standard deviation of difference in L/D ratio falls to −0.09 and 0.63 respectively.

Based on these findings it was concluded that the predominant contribution to differences in L/D measures was the difference in subjective judgement by the graders, as opposed to any underlying issue with the measurement techniques.
Figure 8-1: Bland-Altman plot showing reproducibility of overall L/D measurements

Figure 8-2: Bland Altman plot showing reproducibility of L/D of individual vessel segments measured in common by both graders
As highlighted in the description of the grading protocol in Section 8.2, reproducibility both inter and intra observer was also monitored during the conduct of the study by the inclusion of additional subjects into the workflow of the graders. A summary of the reproducibility for the geometrical parameters of interest in arteries through this mechanism is given in Table 8-1. Measurements within a single subject were aggregated by the geometric mean as described earlier, and reproducibility expressed as the mean and SD of the differences between overall results of the same subjects. The mean observed value of each parameter is also given.

<table>
<thead>
<tr>
<th>Retinal Arterial Parameter</th>
<th>Inter-Observer (31 subjects)</th>
<th>Intra-Observer (57 subjects)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Obs</td>
<td>Mean Diff</td>
</tr>
<tr>
<td>Conventional L/D</td>
<td>14.77</td>
<td>-1.21</td>
</tr>
<tr>
<td>Diameter (pixels)</td>
<td>20.71</td>
<td>0.04</td>
</tr>
<tr>
<td>Length (pixels)</td>
<td>282.7</td>
<td>-19.6</td>
</tr>
<tr>
<td>Simple tortuosity (x10²)</td>
<td>0.696</td>
<td>-0.154</td>
</tr>
<tr>
<td>Curve tortuosity (x10⁵ pixels⁻²)</td>
<td>0.614</td>
<td>-0.097</td>
</tr>
<tr>
<td>Optimality ratio over all bifis.</td>
<td>0.863</td>
<td>-0.012</td>
</tr>
<tr>
<td>Optimality ratio over sym. bifis.</td>
<td>0.893</td>
<td>0.013</td>
</tr>
<tr>
<td>Bifurcation angle (deg)</td>
<td>73.36</td>
<td>-1.71</td>
</tr>
</tbody>
</table>

¹ Intra-Observer reproducibility of Symmetrical L/D was assessed over 56 subjects, since one subject did not yield a valid measurement.

**Table 8-1: Reproducibility of retinal arterial parameters (geometric mean within subject) measured during conduct of study**

Reproducibility of parameters involving vessel length (e.g. L/D and length) observed during the study exhibit slightly larger scatter, although of comparable magnitude to that of L/D in the pilot study, and it appears reasonable to conclude that this originates from the same cause, namely different subjective selection of vessel segments by graders. Possibly scatter inherent in manual tracking of vessel length also adds a contribution.
It is noteworthy that parameters based exclusively on diameter measurements (e.g. diameter and optimality ratio) appear to exhibit better reproducibility, giving confidence in the consistency of the SLRF measurement technique.

The reproducibility of tortuosity parameters was disappointing, and the origins of this are considered more fully in Chapter 9.

### 8.5. Study Results

Table 8-2 shows the baseline parameters of the three groups, namely the controls, cases of death by IHD, and cases of death by stroke. Unsurprisingly, comparisons between the groups by ANOVA shows significant differences for all the recognised cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=528)</th>
<th>IHD cases (n=126)</th>
<th>Stroke cases (n=28)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.4 ± 7.4</td>
<td>64.4 ± 7.1</td>
<td>66.0 ± 7.1</td>
<td>0.48</td>
</tr>
<tr>
<td>Male sex (n(%))</td>
<td>336 (63.5)</td>
<td>77 (61.1)</td>
<td>18 (64.3)</td>
<td>0.83</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>133 ± 19</td>
<td>138 ± 24</td>
<td>148 ± 26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>77 ± 11</td>
<td>78 ± 12</td>
<td>81 ± 15</td>
<td>0.070</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.9 ± 4.9</td>
<td>29.8 ± 5.5</td>
<td>29.9 ± 5.6</td>
<td>0.14</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>233 ± 39</td>
<td>252 ± 47</td>
<td>242 ± 50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51.2 ± 18.5</td>
<td>45.2 ± 16.4</td>
<td>50.9 ± 24.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>6.03 ± 1.33</td>
<td>6.68 ± 2.39</td>
<td>6.86 ± 2.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Below median income (n(%))</td>
<td>237 (46.4)</td>
<td>67 (56.3)</td>
<td>16 (59.3)</td>
<td>0.007</td>
</tr>
<tr>
<td>Diabetes (n(%))</td>
<td>52 (9.9)</td>
<td>24 (19.5)</td>
<td>9 (32.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No proteinuria (n(%))</td>
<td>469 (88.8)</td>
<td>99 (79.8)</td>
<td>21 (75.0)</td>
<td>0.004</td>
</tr>
<tr>
<td>Current smokers (n(%))</td>
<td>90 (17.1)</td>
<td>39 (31.0)</td>
<td>8 (28.6)</td>
<td>0.011</td>
</tr>
<tr>
<td>Hypertension (n(%))</td>
<td>202 (38.4)</td>
<td>71 (56.3)</td>
<td>20 (71.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of CVD (n(%))</td>
<td>89 (17.0)</td>
<td>47 (37.9)</td>
<td>12 (42.9)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are mean ± SD. p values were calculated by ANOVA for continuous data and by \( \chi^2 \) for categorical data. Categorical data show the number of occurrences (n), with proportion (%) of group for which data is available.

**Table 8-2: Baseline characteristics of Beaver Dam cohort by outcome**
Table 8-3 indicates the unadjusted values of all retinal parameters measured during the course of the current study, again categorized by the three groups of cases and controls. Table 8-4 shows the retinal measurements age and sex adjusted to the mean values over all cases and controls (64.44 years and 63.20% male), together with \( p \) values from ANOVA identifying significant differences between groups. In addition, Tables 8-5 and 8-6 similarly show values for the arteriovenous diameter ratio (AVR) previously measured by Wong [140], and supplied by the University of Wisconsin for the present subjects for purposes of comparison. AVR is considered as a continuous variable.

<table>
<thead>
<tr>
<th>Retinal parameter</th>
<th>Control ((n=528))</th>
<th>IHD cases ((n=126))</th>
<th>Stroke cases ((n=28))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial conventional L/D</td>
<td>15.01 ± 4.91</td>
<td>14.37 ± 5.31</td>
<td>17.14 ± 7.29</td>
</tr>
<tr>
<td>Arterial symmetrical L/D</td>
<td>22.05 ± 8.40</td>
<td>20.55 ± 8.55</td>
<td>23.78 ± 10.4</td>
</tr>
<tr>
<td>Arterial diameter (pixels)</td>
<td>20.02 ± 3.26</td>
<td>20.34 ± 3.59</td>
<td>18.69 ± 3.68</td>
</tr>
<tr>
<td>Arterial length (pixels)</td>
<td>292.1 ± 83.5</td>
<td>283.4 ± 90.7</td>
<td>305.1 ± 105.1</td>
</tr>
<tr>
<td>Arterial simple tortuosity ((x10^5))</td>
<td>0.888 ± 1.54</td>
<td>0.529 ± 0.665</td>
<td>0.819 ± 1.47</td>
</tr>
<tr>
<td>Arterial curve tortuosity ((x10^5) pixels))</td>
<td>0.849 ± 1.41</td>
<td>0.534 ± 0.631</td>
<td>0.824 ± 1.44</td>
</tr>
<tr>
<td>Opt. ratio over all arterial bif.</td>
<td>0.868 ± 0.067</td>
<td>0.867 ± 0.061</td>
<td>0.848 ± 0.061</td>
</tr>
<tr>
<td>Opt. dev. over all arterial bifs.</td>
<td>0.078 ± 0.063</td>
<td>0.078 ± 0.055</td>
<td>0.061 ± 0.054</td>
</tr>
<tr>
<td>Opt. ratio over sym. arterial bifs.</td>
<td>0.878 ± 0.095</td>
<td>0.898 ± 0.106</td>
<td>0.855 ± 0.073</td>
</tr>
<tr>
<td>Opt. dev. over sym. arterial bifs.</td>
<td>0.097 ± 0.083</td>
<td>0.114 ± 0.096</td>
<td>0.070 ±0.064</td>
</tr>
<tr>
<td>Arterial bifurcation angle (deg)</td>
<td>71.86 ± 10.68</td>
<td>72.35 ± 10.36</td>
<td>69.28 ± 8.28</td>
</tr>
<tr>
<td>Venous conventional L/D</td>
<td>9.69 ± 3.46</td>
<td>10.23 ± 3.67</td>
<td>9.05 ± 2.76</td>
</tr>
<tr>
<td>Venous diameter (pixels)</td>
<td>25.13 ± 4.86</td>
<td>25.00 ± 5.28</td>
<td>25.69 ± 4.73</td>
</tr>
<tr>
<td>Venous length (pixels)</td>
<td>234.3 ± 74.1</td>
<td>245.8 ± 80.3</td>
<td>227.8 ± 68.2</td>
</tr>
<tr>
<td>Venous simple tortuosity ((x10^5))</td>
<td>0.419 ± 0.801</td>
<td>0.545 ± 0.808</td>
<td>0.405 ± 0.527</td>
</tr>
<tr>
<td>Venous curve tortuosity ((x10^5) pixels))</td>
<td>0.674 ± 1.47</td>
<td>0.882 ± 1.48</td>
<td>0.609 ± 0.796</td>
</tr>
<tr>
<td>Venous bifurcation angle (deg)</td>
<td>73.78 ± 9.14</td>
<td>74.13 ± 8.62</td>
<td>72.60 ± 13.28</td>
</tr>
</tbody>
</table>

Data are unadjusted mean ± SD. Arterial symmetrical L/D is measured over 525 controls, 126 IHD cases and Symmetrical Bifurcation optimality parameters in symmetrical bifurcations are calculated over 520 controls, 125 IHD cases and 28 stroke cases.

Table 8-3: Unadjusted retinal parameters by outcome status measured in current study
Retinal parameter Control (n=528) IHD cases (n=126) Stroke cases (n=28) ANOVA p
Arterial conventional L/D 15.02 ± 0.22 14.38 ± 0.45 16.94 ± 0.95 0.048
Arterial symmetrical L/D 22.06 ± 0.37 20.58 ± 0.76 23.58 ± 1.60 0.12
Arterial diameter (pixels) 20.03 ± 0.14 20.33 ± 0.30 18.71 ± 0.63 0.067
Arterial length (pixels) 292.3 ± 3.6 283.3 ± 7.5 301.3 ± 15.8 0.45
Arterial simple tortuosity (x10<sup>2</sup>) 0.888 ± 0.062 0.530 ± 0.13 0.807 ± 0.27 0.041
Arterial curve tortuosity (x10<sup>2</sup> pixels<sup>-2</sup>) 0.848 ± 0.056 0.537 ± 0.12 0.821 ± 0.25 0.056
Opt. ratio over all arterial bifs. 0.868 ± 0.003 0.867 ± 0.006 0.849 ± 0.012 0.31
Opt. dev. over all arterial bifs. 0.077 ± 0.003 0.078 ± 0.005 0.062 ± 0.011 0.40
Opt. ratio over sym. arterial bifs. 0.878 ± 0.004 0.898 ± 0.009 0.854 ± 0.018 0.040
Opt. dev. over sym. arterial bifs. 0.097 ± 0.004 0.114 ± 0.008 0.070 ± 0.016 0.022
Arterial bifurcation angle (deg) 71.85 ± 0.46 72.35 ± 0.94 69.51 ± 1.99 0.43
Venous conventional L/D 9.69 ± 0.15 10.21 ± 0.31 9.00 ± 0.65 0.16
Venous diameter (pixels) 25.13 ± 0.22 25.00 ± 0.44 25.67 ± 0.94 0.81
Venous length (pixels) 234.5 ± 3.2 245.2 ± 6.5 226.3 ± 13.9 0.26
Venous simple tortuosity (x10<sup>2</sup>) 0.419 ± 0.034 0.541 ± 0.070 0.405 ± 0.149 0.29
Venous curve tortuosity (x10<sup>2</sup> pixels<sup>-2</sup>) 0.674 ± 0.063 0.878 ± 0.130 0.611 ± 0.275 0.35
Venous bifurcation angle (deg) 73.78 ± 0.40 74.16 ± 0.82 72.58 ± 1.75 0.71

Data are age and sex adjusted mean ± SEM. p values were calculated by ANOVA, followed by Fisher’s LSD test for comparisons with controls if ANOVA indicated significant differences between groups. Bifurcation optimality parameters in symmetrical bifurcations are calculated over 520 controls, 125 IHD cases and 28 stroke cases. Highlighted data exhibits p < 0.05.

Table 8-4: Adjusted retinal parameters by outcome status measured in current study

<table>
<thead>
<tr>
<th>Retinal parameter</th>
<th>Control (n=527)</th>
<th>IHD cases (n=124)</th>
<th>Stroke cases (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVR (continuous)</td>
<td>0.887 ± 0.076</td>
<td>0.869 ± 0.082</td>
<td>0.870 ± 0.075</td>
</tr>
</tbody>
</table>

Data are unadjusted mean ± SD.

Table 8-5: Unadjusted retinal AVR by outcome status measured by Wong [140]

<table>
<thead>
<tr>
<th>Retinal parameter</th>
<th>Control (n=527)</th>
<th>IHD cases (n=124)</th>
<th>Stroke cases (n=28)</th>
<th>ANOVA p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVR (continuous)</td>
<td>0.887 ± 0.003</td>
<td>0.869 ± 0.007</td>
<td>0.871 ± 0.015</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Data are age and sex adjusted mean ± SEM. p values were calculated by ANOVA followed by Fisher’s LSD test for comparison with controls. Highlighted data exhibits p < 0.05.

Table 8-6: Adjusted retinal AVR by outcome status measured by Wong [140]
Tests for homogeneity of variance on parameters exhibiting significant differences between groups were performed as discussed earlier. Under a null hypothesis of homogeneity of variance, the following probability $p$ values were obtained for the W50 statistic [18]:

<table>
<thead>
<tr>
<th>Retinal parameter</th>
<th>W50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial conventional L/D</td>
<td>0.085</td>
</tr>
<tr>
<td>Arterial simple tortuosity ($10^2$)</td>
<td>0.031</td>
</tr>
<tr>
<td>Opt. ratio over sym. arterial bifs.</td>
<td>0.53</td>
</tr>
<tr>
<td>Opt. dev. over sym. arterial bifs.</td>
<td>0.35</td>
</tr>
<tr>
<td>AVR (continuous)</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Table 8-7: Levene, Brown and Forsythe test of homogeneity of variance

Based on the above, the assumption of homogeneity of variance may be maintained, except in the case of arterial simple tortuosity, where the findings may be compromised by differences in variance between groups.

When multiple statistical tests are performed on data from a study, the risk increases of a Type I error, and to reduce such a risk, the Bonferroni correction is sometimes advocated to adjust the significance criterion ($p$) value to reflect the number of tests performed [85]. However, this procedure has been criticized [85] because it is mainly applicable to a universal null hypothesis (requiring that all null hypotheses of individual tests are true), which is frequently not the principal focus of research. Otherwise, the correction increases the risk of a Type II error, potentially leading to dismissal of important differences, and implies that interpretation of a given comparison is dependent on the number of other tests performed, which is contrary to common sense [85]. Accordingly the Bonferroni correction remains controversial, and Armstrong [3] has advised that it should not be used routinely except under specific conditions, and in particular that it should not be applied where “it is the results of the individual tests that are important”. The latter consideration is judged to be pertinent to the current study, and hence the Bonferroni correction has not been applied.
Following the ANOVA and Fisher LSD tests to the retinal parameters, the following were found to be significantly associated with subsequent disease:

- Increased arterial conventional L/D predicts death due to stroke.
- Reduced simple tortuosity predicts death due to IHD, although this finding must be treated with caution due to lack of homogeneity of variance.
- Increased optimality deviation in arterial bifurcations predicts death due to IHD.
- Reduced AVR predicts death due to IHD

No other retinal parameters differed significantly between cases and controls.

Application of the multivariate model to test for independence of these findings from recognised cardiovascular risk factors did not attenuate the negative association between arterial tortuosity and IHD mortality, nor the positive association between optimality deviation and IHD death. Following adjustment for age, sex, systolic BP, serum total cholesterol, smoking, antihypertensive therapy, past history of CVD, glycosylated haemoglobin, serum HDL cholesterol, BMI, and income, the following unadjusted and adjusted $\beta$ coefficients $\pm$ SE were found:

- **Arterial optimality deviation**
  - Unadjusted $\beta$ coefficient $= 2.17 \pm 1.08$ ($p=0.044$)
  - Adjusted $\beta$ coefficient $= 2.61 \pm 1.28$ ($p=0.042$) (odds ratio $= 13.6$)

- **Arterial simple tortuosity**
  - Unadjusted $\beta$ coefficient $= -30.2 \pm 11.7$ ($p=0.010$)
  - Adjusted $\beta$ coefficient $= -35.7 \pm 12.9$ ($p=0.005$) (odds ratio $= 3.0 \times 10^{-16}$)

- **Arterial-venous ratio (AVR)**
  - Unadjusted $\beta$ coefficient $= -2.99 \pm 1.36$ ($p=0.024$)
  - Adjusted $\beta$ coefficient $= -3.47 \pm 1.54$ ($p=0.024$) (odds ratio $= 0.031$)
To further verify the potential effect of diabetes, subjects with diabetes were excluded from the analysis, but this was found to yield similar logistic coefficients to the group as a whole.

Arterial conventional L/D ratio was found to predict stroke death, which was not affected by adjustment for age and sex. However, the multivariate model showed this parameter to be also strongly associated with systolic BP, and after adjustment for this, the relationship between conventional L/D and stroke mortality ceased to be significant:

- Unadjusted $\beta$ coefficient = $0.073 \pm 0.034$ ($p=0.032$)
- Adjusted (for age and sex) $\beta$ coefficient = $0.069 \pm 0.34$ ($p=0.049$)
- Adjusted (for age, sex and systolic BP) $\beta$ coefficient = $0.047 \pm 0.36$ ($p=0.187$)
9.1. Study of Vascular Geometry in Beaver Dam Cohort

The most compelling finding reported in the previous chapter was the significant association of altered arterial bifurcation optimality (when measured over symmetrical bifurcations) with death from ischemic heart disease (IHD). This is consistent with the underlying hypothesis that altered bifurcation geometry is indicative of endothelial dysfunction, raising the risk of atherogenesis and cardiovascular disease (CVD). The optimality deviation appears to exhibit greater statistical power than the optimality ratio, most probably because it reflects absolute deviation from the optimal geometry, as opposed to the aggregate deviation recorded by optimality ratio.

It is generally held [124] that the tone of vascular smooth muscle is maintained by a balance between vasoconstrictive influences (e.g. endothelin and angiotensin II) and vasodilatory effects, particularly arising from nitric oxide released by the endothelium, the synthesis of which is modulated by shear stress sensed at the interface with flowing blood. It might be presumed that a functional endothelium would lead these competing actions to balance such as to regulate arterial diameters in conformity with the optimum conditions (in small vessels) predicted by Murray’s Law. In contrast, endothelial dysfunction would be expected to lead to disruption of that balance, and departures from Murray’s Law to arise. This is consistent with observations by Griffiths et al [42] that departures from optimal junction exponents arose in arterioles of a rabbit ear when nitric oxide synthesis was inhibited.

It is noteworthy that in the multivariate model, the association between altered bifurcation optimality and IHD was independent of other recognised risk factors (age, gender, history of CVD, BP, serum cholesterol, HbA1c, diabetes, BMI and smoking) suggesting that it may provide additional prognostic value. Optimality deviation was positively associated with BMI ($\beta = 0.002 \pm 0.001; p = 0.001$) but not with any other conventional risk factor.
The association between bifurcation optimality and IHD was present when measured over symmetrical bifurcations (with asymmetry factor $\alpha \geq 0.4$), as advocated in Chapter 6, but was not present when measured over all bifurcations. This is suggestive that reliable measurement of bifurcation optimality is difficult to achieve in highly asymmetrical bifurcations, for the reasons discussed previously. Over all cases and controls a mean of 6.7 arterial bifurcations were graded per subject (compared to a minimum of 5 required by the grading protocol), but only 3.5 of these met the symmetry criterion. Accordingly, a modest increase in statistical scatter might be expected in the overall measure of optimality per subject when restricted to symmetrical bifurcations, although the underlying measurements are believed to be more reliable. It would appear prudent in future measurement projects to give emphasis to grading a minimum number of symmetrical bifurcations, since these appear to be more informative.

It’s also noteworthy that in the assessment of reproducibility during the study (reported in the previous chapter), the bifurcation optimality ratio (and hence also the optimality deviation) was amongst the most reproducible of the retinal parameters of interest.

The values of arterial bifurcation optimality ratio exhibited are somewhat greater than those expected in optimal bifurcations compliant with Murray’s Law. Bearing in mind that the derivation of the optimality ratio was tailored to minimise the impact of bifurcation asymmetry around the Murray optimum, and that some residual dependence on asymmetry exists away from the optimum, it is prudent to consider whether any differences in asymmetry between IHD cases and controls might have had a confounding effect on the findings in bifurcation optimality.

Unlike other retinal vascular parameters bifurcation asymmetry ratio is not long tailed, and so the mean is an appropriate measure of central tendency within each subject. Aggregated over subjects, asymmetry ratio was found to be:

- in controls $0.664 \pm 0.102$ (mean ± sd)
- in IHD cases $0.677 \pm 0.092$ (mean ± sd)

and the age and sex adjusted ANOVA model shows no suggestion of a significant association with outcome ($p = 0.46$). Accordingly, there is no evidence to suggest that asymmetry has had a confounding effect on the findings related to bifurcation optimality.
A significant association was also observed between increased arterial conventional L/D ratio and death from stroke. Since no significant increase in length was observed between stroke cases and controls, this increase in L/D appears to arise predominantly from arterial narrowing in the stroke cases. This is consistent with the Atherosclerosis Risk in Communities Study (ARIC) which found an association between arteriolar narrowing (assessed by AVR) with incident stroke, after adjustment for other risk factors including BP and diabetes [134].

In the multivariate model reported here, the conventional L/D ratio did not maintain significance after adjustment for systolic BP. This may be a consequence of the small number of stroke cases in the present study, which can be considered to be underpowered in respect of stroke. Alternatively, it is possible that the changes in L/D ratio also reflect damage to the microvasculature arising from prior elevated BP.

The arterial symmetrical L/D ratio was also elevated in stroke cases compared to the controls, but did not reach statistical significance ($p = 0.12$). The rationale for the symmetrical variant of this parameter was that the underlying measurements may be more reliable, and that the parameter is likely to reflect topological features of the vascular tree (Chapter 6). In the present study, over all cases and controls, the mean number of arteries yielding a conventional L/D measurement was 8.6 per subject (compared to a minimum of 7 required by the grading protocol), whereas the number between symmetrical bifurcations was only 4.3. For this reason alone, the overall within subject measurement of symmetrical L/D would be expected to exhibit greater statistical scatter than for the conventional parameter, and this is indeed exhibited both in the reproducibility findings and study results. It is possible that with more measurements of symmetrical L/D over a greater number of trees per subject, the symmetrical L/D may have proved more informative.

A further finding reported in the previous chapter was that reduced simple tortuosity was significantly associated with death from IHD, although this must be treated with some caution due to the lack of heterogeneity of variance between the ANOVA groups. This was unexpected; Michelson et al [76] have previously reported that increased retinal arteriolar tortuosity assessed by fundoscopy was indicative of more severe coronary artery disease (together with abnormal light reflex and decreased calibre), which is not consistent with the finding from the Beaver Dam cohort. However, the work reported by Michelson is based on
subjective assessment of tortuosity rather than quantitative measurement, and did not correct for factors such as age, hypertension or diabetes which may have had a confounding effect.

In the multivariate model of the Beaver Dam cohort, only a weak positive relationship between arterial tortuosity and systolic BP was observed, which is consistent with a study of 715 subjects by de Margerie et al [23], and so this would not appear to explain the association of reduced tortuosity with IHD.

It has been speculated [129] that reduced microvascular tortuosity may be linked with endothelial dysfunction, possibly explaining the association with IHD. This might also explain observations reported by Yamakawa et al [148] of straightened precapillary retinal arterioles in rats with inherited hypercholesterolemia, compared to controls, taking into account that hypercholesterolemia is typically accompanied by endothelial dysfunction [124]. In a computational study, decreased tortuosity of the microvasculature has been predicted to impair oxygenation [38]. Given that endothelial cells play an important role in the regulation of flow and angiogenesis, it is conceivable that reduced tortuosity may arise from an impaired endothelial response to inadequate oxygenation of local tissue.

It must also be noted that the reproducibility of simple tortuosity assessed during the study of the Beaver Dam cohort was disappointingly poor, which invites closer examination of the behaviour of this parameter. Like other retinal parameters, measurements of simple tortuosity from individual vessel segments exhibit a long tailed distribution, and hence the geometric mean was employed as a measure of central tendency to derive an overall result for each subject. However, there appears to be a concentration of very small values of tortuosity measured from individual vessels, with the result that the distribution of log transformed simple tortuosity is bi-modal, and likely to distort the geometric mean. Accordingly, minor differences in grading an image may give rise to large changes in simple tortuosity. Against that background, the median might be considered a more appropriate measure of central tendency within subjects. This does indeed yield considerably superior reproducibility (intra-observer reproducibility over 57 subjects yields a mean observation of 0.0160 with mean ± SD of difference = 0.0012 ± 0.0078) compared to that reported in Section 8.4. It also exhibits reduced values in IHD cases (0.012 ± 0.002 SE) compared to controls (0.015 ± 0.001 SE) but is not statistically significant ($p = 0.35$ in the ANOVA model).
A further consideration in the behaviour of simple tortuosity is that there appears to be a strong underlying association with vessel length. This is illustrated in the following Figures 9-1 to 9-3, which are based on measurements of individual vessel segments (*i.e.* not aggregated within subjects) over all images graded from the Beaver Dam cohort. Vessels are divided into bins based on their path length to yield a conventional bar histogram shown in Figure 9-1.

![Figure 9-1: Histogram of individual vessel segment path length in Beaver Dam cohort](image)

It is evident from inspection that the path length of a majority of vessel segments fall in the range from 100 to 400 pixels. The following Figure 9-2 illustrates for each bin of path length, the median simple tortuosity, as well as the middle and upper/lower quartiles. From this it can be appreciated that the distribution of simple tortuosity is highly skewed, with the maximum value close to a factor of 10 times the 75 percentile value. It is also evident that the largest...
range of simple tortuosity values is found in the region of most commonly occurring path length. Figure 9-3 shows on an expanded vertical axis the median and middle quartile values, clearly illustrating a strong underlying trend for simple tortuosity to increase with vessel path length. The latter figure exhibits some inconsistencies relative to the overall trend in longer vessels, but greater statistical scatter would be expected here due to the comparatively small number of vessels in each bin in the tail of the distribution of vessel length.

Figure 9-2: Median and quartiles of simple tortuosity by bin of path length
These figures offer some insight into the eccentric behaviour of simple tortuosity, such as the poor reproducibility and different outcomes from alternative measures of central tendency with subjects. In seeking a measure of central tendency in a highly skewed distribution, small values may be particularly influential. In a lengthy vessel, a small value of tortuosity may have physiological significance, but in a very short vessel, may serve only to distort the overall measure.

Accordingly it would appear to be advantageous to derive an alternative measure of tortuosity that takes into account the vessel length, whilst ideally remaining dimensionless. This might for example represent the excess (or deficit) of tortuosity compared to the expected value for a vessel of the same length. This is outside the scope of the present work, but is proposed for future attention.

Curvature based tortuosity exhibits a very similar trend to the simple tortuosity discussed above, with lower values in IHD cases compared to controls, but without quite reaching
statistical significance \((p = 0.056 \text{ in the ANOVA model})\). The same discussion points raised about the simple tortuosity also apply to the curvature based variant.

Taking into account all of the above, the finding of an association between reduced tortuosity and IHD should be treated with some caution.

Kalitzeos et al [52] have noted that a number of studies report findings from measurements of retinal vessel tortuosity, but remarked that a lack of consensus on an appropriate measurement technique frustrates comparison of results. The findings from the Beaver Dam cohort support the view that further attention to such measurement techniques is warranted.

Strengths and limitations of the exploratory study of vascular geometry in the Beaver Dam cohort have been noted [129]. The strengths are:

- the prospective design of the study
- a community based population
- quantitative measurement of the retinal vascular geometry by graders blinded to the participants and their outcome.

Most notable amongst the limitations are:

- cause of death was identified from death certificates and not validated against medical records
- hypertension and diabetes are likely to have had substantial effects on the associations reported, and may not have been fully eliminated by the statistical adjustments of the multivariate model
- no adjustment was made for the use of medication which may have affected the vascular geometry; although a previous study of the Beaver Dam cohort suggested that this had little or no effect on retinal microvascular diameters, with the exception of antiglaucoma medication [144]. Elevated intraocular pressure has been associated with increased BP [57], so it cannot be ruled out that this may have affected the relationship between
L/D and systolic BP, but bifurcation optimality and tortuosity are likely to have been influenced less severely.

9.2. Subsequent Studies of Retinal Vascular Geometry

Subsequent to the analysis of the Beaver Dam cohort discussed above, the same measurement methods and grading tool (enhanced as described in Section 7.3), have been applied by other researchers at Imperial College London to measure retinal vascular geometry in a number of further cohorts.

The author of this thesis has supported these subsequent studies by:

- advising on the specification of the grading protocol to incorporate prior lessons learnt (including those from the Beaver Dam cohort)
- configuring the grading tool to specify the structure and size of the grid to reflect the scale and field of the retinal images (Section 7.1)
- configuring the post processor (Section 7.2) to apply criteria for acceptance of retinal measurements in accordance with the grading protocol, and running the software to deliver retinal measurements aggregated over each subject.

The most notable such studies are discussed in the following paragraphs.

The Avon Longitudinal Study of Parents and Children (ALSPAC) followed a cohort of children born in the region of Bristol, England during a period from April 1991 to December 1992 [118]. A study was performed including the first 263 children screened at age 12 years of which 166 had a gestation of ≥ 37 weeks with complete data, to test the hypothesis that small birth size may be associated with variations in the retinal vasculature. Retinal photographs were captured (45° field with a non-mydriatic camera), and retinal vascular geometry measured. Increased arterial bifurcation optimality deviation and simple tortuosity were found to be associated with lower birth weight in linear regression modelling, after adjustment for age, gender, and BP. These findings suggest that factors in early life (such as impaired fetal growth) may adversely affect the retinal vascular structure, possibly through impaired endothelial function.
The Brent Study covered a bi-ethnic population based cohort aged 40-69 years of British Caribbean migrants and White Europeans in North West London [120]. The study included capture of retinal photographs (45° field, non-mydriatic camera but with dilation of pupils) in 683 subjects. After rejection of poor quality images, vascular geometry was measured in 538 subjects (215 African Caribbean and 323 European) to explore variation between these ethnic groups, recognising that people of African origin suffer an increased risk of stroke and microvascular disease. Arterial bifurcation optimality deviation was significantly elevated in African Caribbeans compared to Europeans after adjustment for age and gender, and was not explained by other conventional risk factors. Arterial L/D ratio was also greater in African Caribbeans, but the association ceased to be significant following adjustment for systolic BP. Arterial L/D ratio was strongly associated with systolic BP in non-diabetics, but not in those with diabetes. Accordingly British African Caribbeans appear to exhibit less effective endothelial function than White Europeans.

The Anglo Scandinavian Cardiac Outcome Trial (ASCOT) was a multicentre, randomized controlled clinical trial of alternative antihypertensive therapies (amlodipine-based regimen versus atenolol-based regimen) over 19,342 subjects, aged 40 to 79 years [119]. In a substudy, digital retinal photography (30° field with mydriatic camera) was undertaken in 743 subjects on two centres in London and Dublin, after a median of 4.5 years of follow up when therapies and BP were stable in the two groups. Retinal vascular geometry was measured in 720 evaluable images (373 amlodipine, 347 atenolol). The large scale study showed more advantageous outcomes with the amlodipine-based treatment, compared to the traditional atenolol-based regimen, and the substudy objective was to evaluate whether a similarly favourable impact was evident in the retinal microvasculature. Amlodipine-based treatment was associated with smaller arterial L/D ratios than atenolol-based treatment, and significance was maintained after adjustment for age, gender, cholesterol, systolic and diastolic BP, BMI, smoking and statin treatment. The effect appears attributable to shorter arterial segments in the amlodipine group, most probably due to vasodilation causing the emergence of visible branching vessels terminating L/D segments.

Southall and Brent Revisited (SABRE) is a population-based cohort of three ethnic groups (Europeans, South Asians and African Caribbeans) [48]. Analysis was performed over 1185 subjects, aged 63 to 75 years (77% male), comprising 438 South Asians, 178 African Caribbeans, with the remainder being European. These participants underwent retinal
photography (30° field with mydriatic camera), as well as MRI cerebral imaging. Retinal images were subject to both retinopathy grading in accordance with the NHS Diabetic Eye Screening Programme, and measurement of retinal vascular geometry. MRI images were graded for stroke or large infarct, and also presence of white matter hyperintensities (WMH). Smaller retinal arterial diameters, fewer symmetrical arterial bifurcations, increased venular tortuosity, as well as lower AVR were all significantly associated with cerebral infarct, stroke, as well as WMH, after adjustment for age, gender and ethnicity. The association was maintained after adjustment for confounding factors (hypertension, BMI, HbA1c, diabetes, total cholesterol, high density lipoprotein cholesterol, C-reactive protein, coronary artery disease, years of education and smoking habit). The above associations with retinal vascular geometry were also independent of retinopathy. Increased arterial optimality deviation was also associated with stroke and cerebral infarcts after adjustment for age, gender and ethnicity, but was attenuated after adjustment for other risk factors, which included coronary artery disease.

Along with the exploratory study in the Beaver Dam cohort, the findings from the subsequent studies mentioned above reinforce the utility of retinal geometric parameters, including the optimality of arterial bifurcations, in understanding and quantifying cardiovascular risk.

Contemporaneous work elsewhere on associations between the retinal vasculature and cardiovascular disease has focussed predominantly on retinal vessel calibre. McKeegan et al [70] undertook a systematic review and meta-analysis in 2009, based on 22,159 middle to older aged subjects from six population based studies, who suffered 2,219 coronary heart disease (CHD) events (fatal and non-fatal). They reported that reduced calibre in arterioles and wider venules were each associated independently with increased risk in women, but not in men, suggesting that microvascular dysfunction makes a greater contribution to pathogenesis of CHD in women. The CHD risk associated with these retinal changes was elevated in women without diabetes or hypertension. The same authors also reported on stroke [71] based on six cohort studies, including 20,798 subjects and 945 incident events, finding that wider retinal venules predicted stroke, but no association with retinal arteriole calibre. In a later meta-analysis in 2014, Ding et al [26] reported on 10,229 subjects from six population-based studies, without prevalent hypertension, cardiovascular disease or diabetes, and found that narrower retinal arterioles and wider venules were associated independently with increased risk of hypertension over 5 years, but with little evidence that these
associations were affected by sex, race, smoking status or BMI. In the context of diabetes, Sabanayagam et al [100] reported in 2015 on 18,771 participants over five population-based studies, in which 2,581 cases of incident development of diabetes developed. Wider retinal venules were found to be associated with an increased risk of diabetes, after adjustment for demographic, lifestyle and clinical factors. The association was stronger in men than in women. No association was found between retinal arteriole calibre and diabetes.

It is noteworthy that these studies into retinal vascular calibre are generally based on calculation of equivalent central retinal arterial diameter (CRAE) and similarly for veins (CRVE) as described in Chapter 2. As further discussed there, it appears credible to suggest that the calculation of central equivalent diameters may also reflect deviations from optimal bifurcation geometry, which may contribute to the association with cardiovascular disease.

### 9.3. Methodology and Tool

Measurement of retinal vessel diameters from fundus photography is challenging for the reasons discussed previously (Chapters 3 and 4), and derivation of parameters describing bifurcation optimality place additional demands on the underlying measurement method, since they combine three diameter measurements, leading to accumulation of scatter. Accordingly it is encouraging to find that the diameter measurements performed by the SLRF method (Chapter 5) appear to demonstrate interesting associations between disordered arterial bifurcation optimality with IHD in the Beaver Dam cohort, and with low birth weight in the ALSPAC study.

One of the limitations of the SLRF method is that it doesn’t yield a reliable estimate of vessel diameter in vessels smaller than 10 pixels. However, given the trend towards direct digital imaging with increasing spatial resolution, the impact of this is expected to decline.

The SLRF method has been subjected to comparison with manual measurements of vascular diameter performed by an experienced clinician (Chapter 5) over 60 arterial vessel segments, but this does not inform us about the accuracy nor linearity of the measurement compared to the actual vessel lumen, since the manual measurements are also likely to be imperfect. It might be tempting to consider comparing the performance of the measurement method against a theoretical model of the intensity profile across a vessel in a red-free fundus image. However, as discussed previously (Chapter 3) the formation of a red-free image of a retinal
vessel involves a complex interaction of scattering, reflection and attenuation processes in biological tissue, and the development, let alone validation of such a model would be a challenging task in itself. The currently accepted gold standard for visualisation and measurement of retinal vessels is fluorescein angiography (FA), but this is an invasive technique involving a number of clinical risks. An emerging alternative approach is Optical Coherence Tomography Angiography (OCTA) [22], which is non-invasive and produces rapid high resolution 3-dimension scans over a small field of view. Accordingly it would be interesting to consider a comparison of retinal vascular diameters measured from fundus images using techniques such as the SLRF method with measurements of the same vessels from OCTA scanners, with the aim of gaining confidence in the accuracy of measurements from fundus images.

The challenge of measuring retinal vessels from fundus images has led many groups to propose other novel measurement techniques, either in parallel with or subsequent to the development of the SLRF technique. Examples include the technique proposed by Xu et al [147] using a graph based approach, followed by Kumar et al [61] who proposed an alternative based on Unsupervised Linear Discriminant Analysis. More recently Lupascu et al [67] have proposed an algorithm based on fitting a parametric surface model of the cross-sectional model, followed by a decision tree to estimate the vessel width from the parameters, which is claimed to offer superior accuracy to previous techniques. Another technique, also based on fitting a model to the intensity profile has been proposed by Fraz et al [31], which has been incorporated into the QUARTZ retinal analysis tool [32], aimed at highly automated analysis of large databases. The first three techniques mentioned above have been evaluated using the publically available REVIEW image [1] set consisting of 16 images and 193 annotated vessels, manually measured by three observers. Since manual measurements of retinal vessels may also be imperfect, this form of validation suffers from similar limitations as discussed above in connection with the SLRF method. Moreover, while a common reference image set is of value in comparing certain performance aspects of different algorithms, there is a possibility that algorithms may be optimised to match the manual measurements in what is a limited training set of images. This heightens the value of considering an alternative gold standard, such as might be achieved from OCTA as mentioned earlier.
Scatter may also arise in vascular diameters irrespective of the measurement technique used, due to the impact of the cardiac cycle. In work performed at Imperial College London, the capture of retinal images was synchronised with the R-wave of an ECG signal, and images captured throughout the cycle in steps of 100ms to quantify this effect both in arteries and veins [128]. The vessel diameters were measured by the grading tool and SLRF method. Aggregated over 15 subjects it was found that arterial diameters increased by 2.8% compared to that at the R-wave, peaking 300ms afterwards. Venous diameters increased by 1.9%, peaking at 600ms after the R-wave. These variations would be expected to affect L/D ratios and AVR more severely than measures of bifurcation optimality which are based on ratios of diameters. It appears unrealistic to synchronise retinal image capture with ECG in a routine clinical setting, but in interventional studies where only small vascular changes are involved, it might be beneficial.

It is evident that there is considerable scatter in the measurements within a subject from individual vessel segments and bifurcations. Accordingly it is desirable to measure as many trees as possible to gain the most reliable measure of central tendency. However, it would also be of considerable interest to identify where in a retinal vascular tree the most informative vessels and bifurcations can be expected. In the distal regions of a tree, typically found in the peripheral area of a retinal image, the vessels are smaller, the contrast is less favourable, and hence measurements are expected to be less precise. On the other hand, these regions of the tree may be more informative of the condition of the microvasculature.

The grading tool described in Chapter 7 is semi-automated, requiring a grader to manually identify the vessel segments and bifurcations to be measured, which incurs a cost in the graders’ time required to complete a measurement campaign. For this reason, the grading protocol applied to the Beaver Dam cohort (Section 8.2) limited the number of trees graded per subject in order to complete the grading exercise within a practicable time frame. The enhancements to the tool described in Section 7.3, particularly the introduction of automated vessel tracking, have substantially reduced the time required to grade an image, but the manual effort remains significant. In order to maximise the power of the techniques that have been described, it would be desirable to automate the measurement process of retinal vascular geometry to a much greater extent. This could be achieved in principle by using an existing segmentation method to locate the vessels, in conjunction with an algorithm to classify
vessels as either arteries or veins, and a technique such as SLRF to measure the vessels, allowing quantitative measures to be derived.

9.4. Recent Approaches

The geometric features discussed earlier are examples of retinal image biomarkers for prediction of cardiovascular disease. MacGillivray et al [69] opined in 2014 that retinal imaging represents an “unexplored landscape” of candidate biomarkers with potential to offer significant additional insight to disease prediction, and longitudinal studies in this field are likely to remain active and clinically relevant for many years to come.

The alteration in arterial bifurcation optimality found to predict death from IHD in the exploratory Beaver Dam study is noteworthy since it can be related directly to the physiology of blood flow, vascular dysfunction and increased risk of atherogenesis. A similar retinal marker (alteration in arterial junction exponent) was found to predict progression to proliferative diabetic retinopathy (PDR) in type 2 diabetics in a small study of 30 PDR cases and the same number of subjects without retinopathy [20]. More recently, altered retinal arterial branching coefficient (a parameter related to bifurcation optimality but without correction for asymmetry) was found to be associated with a higher risk of nephropathy in a cohort of 181 type 1 diabetics [92].

Another biomarker in receipt of recent attention is the fractal dimension of the retinal vascular network, which aims to quantify the complexity or density of the vasculature [69]. It is typically derived following segmentation of the vessels, by counting the number of square boxes of varying scales required to cover the vasculature, with the fractal dimension being given by the gradient of log(count) against log(scale) [45]. Alteration of fractal dimension of the retinal vasculature has been found to predict increased risk of coronary heart disease mortality independently of conventional risk factors in a prospective population-based cohort of 3303 subjects [65]. Additionally, in 1187 subjects of the Australian Heart Eye Study (at high risk of CVD) reduced retinal fractal dimension (as well as reduced arterial bifurcation angle) was found to be associated with the number of vessels exhibiting obstructive (>50% stenosis) lesions in the coronary arterial tree [125]. Wu et al have reported an association between reduction in retinal vascular fractal dimension and stroke in a meta-analysis of 20,659 subjects [146]. Several studies have pointed to associations between fractal dimension and the presence of diabetic retinopathy compared to healthy controls, and also progression
during follow-up of diabetic patients [45][88], but there are conflicting findings [45]. Popovic et al [88] found (in 37 images of the STARE database) reductions in fractal dimension were associated with both hypertensive and diabetic retinopathy, and that additional consideration of lucunarity (a measure of geometric diversity) could distinguish between these pathologies. Generally, studies have been based on a single measure of fractal dimension (monofractal) although the retinal vasculature displays multifractal properties, possibly reflecting different structural characteristics in different regions of the retina, which may yield further information [117][88]. However, Huang et al [45] caution that the fractal dimension is very sensitive to variations in image characteristics, such as resolution, noise, type of camera, as well as the performance of the segmentations process, making it a potentially “deceptive” biomarker. Wang et al [125] reinforce the potential influence of variations in image quality and particularly the impact of media opacity.

A recent study by Klein et al [60] has explored relationships between a large number of retinal vascular geometrical parameters and the incidence and progression of diabetic retinopathy in subjects (996 with type 1 DM and 1370 with type 2 DM) from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR). After adjusting for a range of established risk factors including severity of retinopathy, as well as arterial and venous calibre (CRAE and CRVE respectively), only four geometrical parameters were significantly associated with outcomes over a 15 year period:

- arterial simple tortuosity was associated with incidence of any DR
- venular branching angle was associated with progression of diabetic retinopathy and incidence of clinically significant macular oedema
- venular curvature tortuosity was associated with incidence of proliferative diabetic retinopathy.

However, taking into account the number of model runs, these may have been chance findings, and it was concluded that provided CRAE and CRVE are evaluated, other geometric parameters add little additional information in the context of DR.

Increasingly, work on retinal image analysis has been applied to the application of artificial intelligence and particularly deep learning techniques [102]. Early classical machine learning systems were based on a set of hand-engineered biomarkers or features which the system learns to recognise from a training set of examples. In contrast, deep learning systems
(typically implemented as multi-layer convolutional neural networks) learn both to extract relevant features and classify them from the training images labelled with relevant clinical information. Whereas the performance of classical systems tends to plateau after exposure to a certain amount of training data, the performance of deep learning systems continues to grow with greater training.

Deep learning techniques have been applied successfully in retinal fundus images to tasks such as segmenting the vasculature [109] and classification of arteries and veins [126], but the principal focus has been to identify and classify retinal pathologies. In a very recent review, Ting et al [121] summarised the performance of a number of deep learning systems evaluated between 2016 and 2018. The predominant objective (18 evaluations of four distinct systems) has been detection of referable diabetic retinopathy (DR), although glaucoma, age related macular degeneration, and retinopathy of prematurity have also received attention. Several systems addressing DR exhibit good diagnostic performance on images within public databases, but their behaviour with images captured by different cameras and from different ethnicities remains uncertain [121]. One system (IDx DR) based on a dedicated retinal camera was approved by the US Food and Drug Administration in April 2018 for assessment of DR in routine clinical settings [94].

Poplin et al have recently reported [87] on a deep learning model, trained on fundus images from 284,335 patients from the UK Biobank and EyePACS, aimed at prediction of cardiovascular risk. When applied to independent datasets of 12,026 and 999 images from these resources respectively, the model demonstrated the ability to predict a range of factors including age, gender, smoking status, systolic BP, and major adverse cardiac events, all significantly better than the average values over the dataset. Whilst the model is able to identify through a “heat map” the regions of the image contributing to a prediction (e.g. vessel, optic disc or non-specific), it does not reveal the actual features that are most important.

A number of challenges are evident for AI and deep learning based systems to gain widespread acceptance in clinical practice [102][121]. Existing training sets are comparatively homogenous and may not be representative of real populations, nor the variability in image characteristics in a wider clinical setting. They may also lack cases of rare diseases or co-morbidities. For all these reasons, performance in everyday clinical practice may not reflect that found in research studies. Acquisition of sufficiently large and
comprehensive training databases of images may also raise confidentiality issues, since retinal images cannot be truly anonymised [102]. Furthermore, interpretation of the outputs from deep learning algorithms (such as heat maps) may not provide clinicians (more experienced in examining particular features of a pathology) with sufficient information to make clinical decisions with confidence. For these reasons, applications of AI based systems in the clinic are likely to fall initially in the role of screening until more experience has been gained.

Accordingly, studies aimed at parameter estimation and identification of biomarkers of disease, such as the exploratory study reported in this thesis, should be considered as complimentary to AI technology. Findings in respect of geometrical features (e.g. bifurcation optimality) based on consideration of flow physiology have potential to provide additional insight to the aetiology of cardiovascular disease, and thus contribute to the interpretability of findings from AI and deep learning based systems.
CHAPTER 10
CONCLUSIONS AND FUTURE WORK

It is widely recognised that the structure and function of the microvasculature is implicated in the development of cardiovascular disease, and the eye offers a unique window through which the retinal microvasculature can be readily imaged and evaluated. Physicians have made qualitative observations of the retina for centuries to aid in diagnosis of disease, but retinal photography by fundus cameras allows computer aided measurement of quantitative parameters characterising vascular form and function.

10.1. Measurement of Retinal Vascular Geometry

The gold standard in imaging the retinal vasculature is fluorescein angiography (FA) but this is an invasive technique not without clinical risks. Hence for routine fundus photography, either red-free or colour photography is preferred. However, the measurement of vessel diameters from red-free (or the green layer from colour) images is challenging for a number of reasons. The intensity profile across vessels in such images is the product of a complex interaction of scattering, reflection and attenuation processes, leading to indistinct edges. The central light reflex often observed along the centre of vessels may also frustrate the measurement process. Image noise is also a potential problem, particularly in older images captured on photographic film which features prominent grain.

In an attempt to improve the precision of vascular diameter measurements from red-free retinal fundus images, a novel technique known as a Sliding Linear Regression Filter (SLRF) has been proposed in order to overcome deficiencies perceived in earlier approaches. This involves passing a sliding window over the intensity profile of the vessel to detect the points of greatest intensity gradient. The size of the window is adapted to an initial estimate of the size of the vessel, which has been found to improve performance. Confidence in the behaviour of the SLRF method has been gained by comparing its measurements over 60 arterial vessels with manual measurements by a skilled clinician.

This work has focussed on the derivation of non-dimensional parameters characterising the vascular geometry since these are robust against variation in refractive characteristics of the
eye. Of particular interest is the optimality of diameter relationships at arterial bifurcations, based on the hypothesis that departure from optimal conditions suggested by Murray’s Law is indicative of endothelial dysfunction, which is associated with atherogenesis and cardiovascular disease. The optimality of bifurcations is frequently expressed by the junction exponent, but simulation studies have shown that this is poorly behaved in the presence of measurement noise. Alternative parameters have been proposed, namely the optimality ratio and optimality deviation which overcome this drawback. This work also suggests that bifurcation optimality is best evaluated in comparatively symmetrical bifurcations (asymmetry ratio $\alpha \geq 0.4$) where the impact of measurement noise is minimised.

Other non-dimensional parameters of interest include bifurcation angles, length/diameter (L/D) ratios of vessel segments, and vascular tortuosity. A variant of the length/diameter parameter measured between vessel segments joining symmetrical bifurcations has also been proposed, since this would be expected to be more robust, and also reflective of different tree topologies.

A computer based semi-automatic grading and analysis tool has been developed to measure vessel segments and bifurcations over entire retinal vascular trees, using the SLRF method to measure diameters, and to derive the non-dimensional parameters mentioned above.

10.2. Retinal Vascular Geometry in the Beaver Dam Cohort

The grading and analysis tool has been applied to retinal images captured in the Beaver Dam Eye Study, a prospective population-based study into ocular and cardiovascular health. Retinal images were captured at baseline and participants followed up over a 10 year period. An exploratory case-control study in younger subjects (< 75 years at baseline) from this cohort was performed to explore the hypothesis that disorders in retinal vascular geometry are predictive of incident cardiovascular disease. Cases of death from IHD (n=142) and stroke (n=31) were selected, together with age and gender matched controls. After rejecting images which were of inadequate quality to measure parameters of interest, results were obtained from 126 IHD and 28 stroke cases, together with 528 controls. Measurements were performed by graders blinded to the identity and outcome of the subjects.

It was found that disordered arterial bifurcation optimality measured over symmetrical bifurcations was associated with death from IHD, which is consistent with the underlying
hypothesis that such geometrical disorder is indicative of endothelial dysfunction. Furthermore, this association appears independent of other recognised risk factors, suggesting that it adds additional prognostic value.

Elevated conventional arterial L/D ratio was associated with death from stroke after correction for age and gender, but this association was not maintained after adjustment for systolic blood pressure (BP).

An interesting association between reduced simple tortuosity and death from IHD was found, which was unexpected. It has been suggested speculatively that reduced tortuosity may also be associated with endothelial dysfunction. However, the finding regarding tortuosity should be treated with some caution, as the behaviour of this parameter exhibits a number of eccentricities as discussed in Chapter 9, and demonstrated poor reproducibility both inter and intra-observer.

10.3. Subsequent Studies and Approaches

A number of subsequent studies have been performed by other researchers within Imperial College London using the same grading tool and methodologies, as discussed in Chapter 9. In the ALSPAC study, disordered arterial bifurcation optimality was associated with lower birth weight, suggesting that factors in early life may adversely affect vascular health. In a substudy of the ASCOT trial, it was found that lower L/D ratios were associated with amlodipine-based anti-hypertensive treatment, compared with an atenolol-based regimen, most likely arising from vasodilatation.

It is encouraging to find that the diameter measurements performed by the SLRF method (Chapter 5) appear able to demonstrate interesting associations between disordered arterial bifurcation optimality with IHD in the Beaver Dam cohort, and with low birth weight in the ALSPAC study. Furthermore, assessment of reproducibility during the exploratory study in the Beaver Dam cohort indicated that bifurcation optimality was among the most reproducible parameter examined, in spite of combining three individual diameter measurements.

Contemporaneous studies elsewhere of retinal vascular geometry have concentrated predominantly on measurement of vascular diameters, expressed as central retinal arterial and
venous equivalent values (CRAE and CRVE), which have also shown associations with cardiovascular disease as discussed in Chapter 9.

These geometrical features are examples of retinal image biomarkers of systemic disease, and growing interest may be expected from researchers over coming years in exploring other candidate retinal biomarkers. One such biomarker that has received recent attention is the fractal dimension of the retinal vasculature, which has also shown associations with cardiovascular disease, although sensitivities to variations in image quality may confound findings (Chapter 9).

Growing interest has arisen recently in the application of artificial intelligence (AI) and in particular deep learning to retinal image analysis. This has focussed particularly on the identification of retinal pathology such as diabetic retinopathy (DR), but has also been applied to prediction of cardiovascular risk. However, several challenges are evident in applying such techniques to both research and clinical settings, not least in interpreting outputs from such algorithms, as discussed in Chapter 9. Hence studies aimed at parameter estimation and identification of biomarkers (such as described in this thesis) should be seen as complimentary to the development of AI based techniques.

10.4. Future Work

The work described in this thesis points to a number of areas of future interest.

Confidence in the SLRF method was initially gained by comparison with manual measurements, but the latter are also likely to be imperfect, and so cannot inform us of the accuracy of the measurement compared to the actual lumen of the vessel. Fluorescein angiography is invasive and is not risk-free, so is unhelpful in the context of validating measurement methods. An emerging alternative is Optical Coherence Angiography (OCTA) which is non-invasive and provides high resolution images over a small field of view. It would be interesting to examine diameter measurements obtained from fundus images in comparison with those obtained from OCTA in the same vessels. This might aid in refining the measurement techniques and providing a more meaningful comparison between alternative approaches.
Mention has been made above of the eccentricities in the behaviour of the simple tortuosity parameter in the Beaver Dam cohort, as discussed more fully in Chapter 9. It would be desirable to formulate a parameter that is less strongly associated with vessel length, whilst ideally remaining dimensionless. In particular, it appears important to distinguish between long vessels which exhibit low tortuosity with potential physiological significance, as opposed to short vessels exhibiting low tortuosity for that reason alone.

It is evident that there is considerable scatter in the values of parameters over bifurcations and vessel segments within a subject, and hence it would be helpful to identify whereabouts in a retinal vascular tree, the most informative vessels and bifurcations are expected. Analysis of the scatter and power of measurements in different generations of vessels in the Beaver Dam cohort might provide valuable information on where measurements should be concentrated, allowing protocols to be optimised.

One of the limitations of the semi-automated grading tool described in this document is the manual effort required over a large data set. Enhancements to the tool introduced after the Beaver Dam study, particularly automatic vessel tracking, have eased the effort required, but it remains significant. Accordingly an important goal of future work should be to achieve a significantly greater degree of automation, to allow larger image sets to be measured with minimal effort. Possibly deep learning techniques have a role to play in this goal.
BIBLIOGRAPHY


