Computer-aided Diagnosis of Plus Disease via Quantitative Analysis of the Vascular Architecture in Retinal Fundus Images of Preterm Infants

by

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Abstract

Retinopathy of prematurity (ROP) is a disorder of the eye that may develop in preterm-born infants. If left untreated, ROP may lead to retinal detachment and ultimately blindness. A warrant for treatment of ROP is the detection of plus disease, which is clinically diagnosed by the presence of certain levels of increase in the tortuosity and thickness of retinal vessels. The openness of the major temporal arcade (MTA), the thickest branch of the venules, has also been observed to decrease in the presence of plus disease. It has been shown that there is interexpert disagreement on diagnosis of plus disease, even among expert ophthalmologists and retinal specialists, which calls for development of image processing techniques to detect and diagnose plus disease more accurately by analysis of retinal fundus images of preterm infants.

This work presents image processing methods for the extraction of diagnostic features from retinal fundus images of preterm infants, for the purpose of computer-aided diagnosis (CAD) of plus disease. The features include measures of the thickness and openness of the MTA as well as tortuosity of retinal vessels. The methods include directionally sensitive Gabor filters for the detection and extraction of vessels, as well as morphological techniques for segmentation, binarization, and skeletonization of the vasculature. Methods are proposed for tracking and segmentation of only the MTA. Using geometrical modeling and analysis, the openness and thickness of the MTA are quantified. Tortuosity of vessels is quantified by analyzing the variation in the dominant orientation of vessels obtained by the application of Gabor filters.

Receiver operating characteristic (ROC) analysis of the diagnostic performance of the measures of thickness, openness, and tortuosity resulted in area under the ROC curves of 0.75, 0.70, and 0.98, respectively. The methods may be used in a clinical or teleophthalmological setting for CAD of plus disease.
Acknowledgments

I thank Dr. Anna L. Ells for providing me with indispensable clinical expertise and guidance, as well as access to the TROPIC database, without which this work would not have been possible.

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<td>$A_z$</td>
<td>area under the binormal ROC curve</td>
</tr>
<tr>
<td>$\delta_E(X)$</td>
<td>dilation of $X$ using $E$</td>
</tr>
<tr>
<td>$\varepsilon_E(X)$</td>
<td>erosion of $X$ using $E$</td>
</tr>
<tr>
<td>AN</td>
<td>actually negative</td>
</tr>
<tr>
<td>AP</td>
<td>actually positive</td>
</tr>
<tr>
<td>AUC</td>
<td>area under the discrete ROC curve</td>
</tr>
<tr>
<td>AVT</td>
<td>angle-variation-based tortuosity</td>
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<tr>
<td>BW</td>
<td>birth weight</td>
</tr>
<tr>
<td>CA</td>
<td>chronological age</td>
</tr>
<tr>
<td>CAD</td>
<td>computer-aided diagnosis</td>
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<tr>
<td>CRA</td>
<td>central retinal artery</td>
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<tr>
<td>CRV</td>
<td>central retinal vein</td>
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<tr>
<td>DR</td>
<td>diabetic retinopathy</td>
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<tr>
<td>DRIVE</td>
<td>Digital Retinal Images for Vessel Extraction</td>
</tr>
<tr>
<td>FNF</td>
<td>false-negative fraction</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>FPF</td>
<td>false-positive fraction</td>
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<tr>
<td>GA</td>
<td>gestational age</td>
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<td>GHT</td>
<td>generalized Hough transform</td>
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<tr>
<td>GUI</td>
<td>graphical user interface</td>
</tr>
<tr>
<td>HEI-MED</td>
<td>Hamilton Eye Institute Macular Edema Dataset</td>
</tr>
<tr>
<td>IAA</td>
<td>inferior arcade angle</td>
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<tr>
<td>INA</td>
<td>inferior nasal arcade</td>
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<tr>
<td>ITA</td>
<td>inferior temporal arcade</td>
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<tr>
<td>JPEG</td>
<td>Joint Photographic Experts Group</td>
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<tr>
<td>LoG</td>
<td>Laplacian of Gaussian</td>
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<td>LR</td>
<td>logistic regression</td>
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<td>LTI</td>
<td>local tortuosity index</td>
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<td>LUT</td>
<td>look-up table</td>
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<td>MAD</td>
<td>median absolute deviation</td>
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<td>MLP</td>
<td>multilayer perceptron</td>
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<tr>
<td>MNA</td>
<td>major nasal arcade</td>
</tr>
<tr>
<td>MTA</td>
<td>major temporal arcade</td>
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<tr>
<td>NPDR</td>
<td>nonproliferative diabetic retinopathy</td>
</tr>
<tr>
<td>OCT</td>
<td>optical coherence tomography</td>
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<tr>
<td>ONH</td>
<td>optic nerve head</td>
</tr>
<tr>
<td>ONHH</td>
<td>ONH height</td>
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<tr>
<td>ONHW</td>
<td>ONH width</td>
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<tr>
<td>PDR</td>
<td>proliferative diabetic retinopathy</td>
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<tr>
<td>PDF</td>
<td>probability density function</td>
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<tr>
<td>RGB</td>
<td>red, green, and blue (image components)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RISA</td>
<td>Retinal Image multiScale Analysis</td>
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<td>receiver operating characteristics</td>
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<td>retinopathy of prematurity</td>
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<td>RNFL</td>
<td>retinal nerve-fiber layer</td>
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<td>SAA</td>
<td>superior arcade angle</td>
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<tr>
<td>SNA</td>
<td>superior nasal arcade</td>
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<tr>
<td>SLO</td>
<td>scanning laser ophthalmoscopy</td>
</tr>
<tr>
<td>SOA</td>
<td>sum of angles</td>
</tr>
<tr>
<td>STA</td>
<td>superior temporal arcade</td>
</tr>
<tr>
<td>STD</td>
<td>standard deviation</td>
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<td>standard error (statistical analysis)</td>
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<td>SE</td>
<td>structuring element (morphological processing)</td>
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<td>temporal arcade angle</td>
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<td>TN</td>
<td>true negative</td>
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<td>TNF</td>
<td>true-negative fraction</td>
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<tr>
<td>TP</td>
<td>true positive</td>
</tr>
<tr>
<td>TPF</td>
<td>true-positive fraction</td>
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<td>TROPIC</td>
<td>telemedicine for ROP in Calgary</td>
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<td>VST</td>
<td>vascular structure tree</td>
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Chapter 1

Introduction

1.1 Anatomy of the Eye and Retina

The eye is one of the important sensory organs that provides the ability to gather and process visual information from the surroundings. The eye and the visual system as a whole are made up of several different segments and mechanisms that are all crucial in creating a meaningful vision [1]. The retina and the optic nerve head (ONH) are outgrowths of the forebrain and are the only parts of the nervous system that can be studied and analyzed noninvasively [1][2].

The eyeball is made up of three different layers that provide protective, nutritive, and receptive functionalities to the eye. The cornea, the anterior-most part of the eye, is a transparent membrane that acts as a lens which focuses the incoming light to create a clear picture at the back of the eye [1]. The iris, part of the vascular layer of the eye, is responsible for adjusting the amount of incoming light to provide adaptive visual acuity in various lighting conditions by narrowing or widening the pupil. The choroid, also part of the vascular layer of the eye, provides nutriment to the outer part of the retina [1].

The retina is the most important part of the nervous layer of the eye with functionality that is analogous to that of a film or sensor in a camera. The retina is covered with highly specialized neurons called rod and cone photoreceptor cells, which transduce the incoming visual stimuli to electrical signals called photoresponses [3]. Photoresponses are transmitted and processed through the outer retina via a complex system of electrical and chemical synapses [4]. The visual information is further processed and encoded in the inner retina through ganglion cell-output pathways and finally passed on to the brain through the visual cortex for interpretation [5].
All neurons transmitting the visual information from the retina to the brain pass through the ONH, which appears as a bright oval-shaped spot in the retina \[2\]. Considering the coronal plan of the eye going through the center of the ONH, the area towards the temples is referred to as the temporal side and the opposite area is called the nasal side of the retina \[1\]. The average ONH width (ONHW) is about \(1.6 \, mm\) \[6\] in adults and about \(1.05 \, mm\) \[7\] in preterm infants. The ONH height (ONHH) is about \(1.41 \, mm\) in preterm infants. The macula is a dark region (in comparison to the rest of the retina) situated about two average ONHWs away temporally from the center of the ONH and has the highest concentration of photoreceptors with an avascularized region at its center called the fovea \[1,2\]. The line connecting the center of the ONH to the fovea is called the retinal raphe. The areas above and below the retinal raphe are referred to as the superior and inferior sides of the retina \[1\]. The ONH is the point of entry of nerves and blood vessels into the retina. Figure 1.1 shows a macula-centered retinal fundus image with some of the important features labeled.

All ocular vessels are derived from the ophthalmic artery and vein, which are branches of the carotid artery and jugular vein, respectively \[8\]. The central retinal artery and vein (CRA and CRV) branches, which supply blood to various layers of the retina, enter the optic nerve about \(10-15 \, mm\) behind the eye and converge to its center \[8\]. Both the central retinal vessels and nerves diverge away from the ONH and follow roughly half-parabolic paths as they spread into the four quadrants of the retina. The CRA and CRV divide into four main branches per quadrant of the retina as they extend toward the periphery of the eye \[8\]. The four main branches are identified based on their spatial localization and vessel type as superior/inferior temporal/nasal artery/vein branches \[8\]. The major superior/inferior temporal venular branches are commonly referred to as arcades (STA and ITA) and together referred to as the major temporal arcade (MTA) (see Figure 1.1). Similarly, the terms superior and inferior nasal arcades (SNA and INA) and major nasal arcade (MNA) refer to the venular branches on the nasal side of the retina \[1,8\]. Retinal vessels follow dichotomous
and side-arm branching patterns and the resulting child branches ultimately turn into a two-layer capillary network that nourishes the retinal nerve-fiber layer (RNFL) and the ganglion-cell layer, as well as the inner-nuclear layer [8].

Figure 1.1: Image 24 of the DRIVE database (see Section 3.2 for details on the database) with the main anatomical features annotated. The circle near the middle of the image approximates the macular region. The green dot in the middle of the circle indicates the fovea. The center of the ONH, the point of convergence of the blood vessels, is marked by a blue dot on the right-hand side of the image. The ITA and the STA are the upper and lower parts of the major venule, respectively. The inferior major vein/artery pair is labeled. This is a macula-centered retinal fundus image.
1.2 Diagnostic Imaging of the Retina

The first documented retinal fundus image of a human eye goes back to the end of the nineteenth century using an early version of the Helmholtz ophthalmoscope (fundoscope), with an albocarbon burner and a $2.5$-minute exposure time [9]. The image showed only the ONH as a bright spot, but also displayed another large bright spot (an artifact) with the retinal vessels not visible at all. By the mid-twentieth century, the availability of $35$-mm film cameras and electronic flashes gave rise to the field of retinal fundus imaging [10, 11].

A fundus imaging system is essentially a low-powered specialized microscope with a camera attached to it; see Figure 1.2 for one such setup. Fundus photography uses the same optical principles as an indirect fundoscope that is capable of producing a real image [12]. Such imaging systems contain a light source to illuminate the retina as well as a series of lenses and prisms to capture the reflected light and form an image of the retina [13]. The illuminating light rays are formed into an annular shape; such a presentation of the illuminating light along with the refractive properties of the cornea allow the reflected light to come back through the pupil, cornea, and the middle of the annulus without interfering and crossing over the illuminating light beam [12]. The operator of the imaging system is required to adjust the lenses to focus the incoming light [12]. The image capturing screen could be a film or a charge-coupled device [14] that allows capture of a digital image of the retinal fundus. The present work is concerned and deals with analysis of retinal vasculature in optical fundus photographs (digital images) of the retina. Chapter 3 presents examples of digital optical retinal fundus images taken from adults and preterm infants. Figure 1.2 demonstrates a patient’s retinal image being taken with a digital fundus camera. Figure 1.1 shows a typical macula-centered fundus image.

The same imaging techniques as optical fundus photography could be used with fluorescein dyes to capture the retinal vasculature with high contrast through fluorescein angiography [15, 16]. In more recent years, advancements in various imaging modalities have
allowed for more accurate and application-specific imaging of the retina, its features, and the associated physiological changes. Adaptive optics and scanning laser ophthalmoscopy (SLO) [17–20], as well as optical coherence tomography (OCT) [21–25], can be used to visualize the retina and its nervous layers, including the ONH, at the cellular level with high resolution and detail. Various forms of the Doppler imaging technique have been developed for assessment of blood flow in the retinal vasculature [26–28]. Spectral and photoacoustic imaging methods allow for assessment of the levels of oxygenation and absorption of light by the retina, respectively [9]. The details of the physical principles of the various mentioned image-acquisition modalities are beyond the scope and objective of this work and are not discussed here, but can be found in different works in the literature [16, 18, 20, 24, 29].

Maamari et al. [30] recently introduced a retinal fundus camera called the Ocular CellScope that attaches to a smart phone and provides images similar in terms of quality, contrast,
and field of view (FOV) as compared to stationary retinal fundus cameras. Pinello and Mazzarolo [31] showed that, in case direct fundoscopy of an uncooperative infant is not possible, analysis of the retina and diagnosis of various pediatric retinal diseases are possible using digital retinal fundus images obtained using the RetCam II camera, as shown in Figure 1.3. Remote observation and analysis of retinal fundus images of preterm infants by experts in a teleophthalmological setting can facilitate detection and diagnosis of sight-threatening diseases such as ROP, as shown by initiatives such as the Karnataka Internet Assisted Diagnosis of Retinopathy of Prematurity (KIDROP) [32,33] and the Stanford University Network for Diagnosis of Retinopathy of Prematurity (SUNDROP) [34,35].

Considering the small size of the retinal vasculature and the fact that, in a given retinal fundus image with a 45° FOV, only about 13% of the surface of the retina is covered with visible vessels (see Section 4.2.1), it becomes clear that objective and precise analysis of the vasculature using a fundoscope is not feasible. Such a drawback justifies the use of digital retinal fundus images to allow for objective and temporal observation, analysis, and detection of vascular changes in the presence of various diseases and disorders.

1.3 Manifestations of Diseases in the Retina

With the recent advancements in the field of retinal imaging and with digital retinal fundus images being easily acquired, more research is being conducted to understand the effects that various diseases may have on the retina. Most retinal manifestations of diseases fall into two main categories of vascular and neuronal (related to the RNFL) changes.

It has been observed that the retina is the most metabolically active tissue in the human body and can be easily affected by changes to the oxygen demand and supply [36,37]. Many disorders manifest themselves via vascular and/or neuronal retinal changes; such disorders include, but are not limited to, retinopathy, venular dilation, venular beading, arteriolar narrowing, increased vessel tortuosity, neovascularization, decreased openness of the MTA,
Figure 1.3: Various steps involved in retinal fundus imaging of a preterm infant using the RetCam II camera: (a) topical anesthesia is applied, (b) coupling gel is applied to the camera tip, (c) camera tip is placed in position, (d) and image capture is performed.

venular/arteriolar occlusion, varying venular-arteriolar ratio, exudates, hemorrhages, macular edema, thickness changes to the RNFLs, cellular-level changes of the photoreceptors, and changes to the blood-retina barriers [38–45]. The disorders and adverse conditions that exhibit such manifestations in the retinal vasculature and RNFL include diabetes, hypertension, congestive heart failure, nephropathy, cerebral white-matter lesion and atrophy, stroke, obesity, diabetic retinopathy (DR), large waist-hip ratio, tobacco smoking, coronary heart disease, Crohn disease, renal disease, cat-scratch disease, Parkinson's disease, glaucoma, myopia, retinopathy of prematurity (ROP), and plus disease, to name a
The following sections provide clinical details on plus disease, an indicator of the active phase of ROP requiring treatment as well as DR. However, the present work is focused only on computer-aided diagnosis (CAD) of plus disease.

1.3.1 Diabetic Retinopathy

DR is the leading cause of preventable blindness among people of working-age in developed countries and is generally divided into the classes of nonproliferative DR (NPDR) and proliferative DR (PDR), with NPDR further broken down into four stages.

Even though there are many screening programs in effect for the detection and diagnosis of NPDR, only early signs of PDR and severe NPDR warrant preventive measures to stop the progression of PDR into later stages which can ultimately lead to blindness. The number of patients who develop PDR can be significant even in a developed country such as the United States (US). As shown by the results of a screening program for DR, performed by the California HealthCare Foundation as part of the state’s health care safety net, out of the 53,188 people who were screened for DR over a period of three years, approximately 25% (13,297 people) were diagnosed with NPDR (all stages) and 2% (more than 1,000 people) were diagnosed with PDR. Approximately 9% of the screened population had some level of sight-threatening retinopathy (severe NPDR, PDR, and macular edema) that required referral for treatment.

Pathogenesis of DR

The exact reasons behind the pathogenesis of DR are not well understood. However, there are several different physiological factors that could lead to DR. It has been suggested that DR may be a neurovascular disease rather than a purely vascular one. The neuronal and vascular changes may be concurrently present; however, the exact associations between the two changes are still unknown. An increase in the vascular endothelial growth factor...
(VEGF) is cited as a probable cause for neovascularization and proliferation of vessels in PDR, which could consequently lead to tractional retinal detachment \[65, 66\]. It is possible that there are multiple factors involved in the pathogenesis of DR.

Clinical Diagnosis of DR

Signs of NPDR include exudates, microaneurysms, cotton wool spots, hemorrhages, macular edema, presence of venular beading, and occasional changes in the tortuosity and width of retinal vessels \[43, 59, 67, 70\]. The presence of such features has been shown to correlate with the progression and severity of NPDR \[71, 72\], which is broken down into four stages of mild, moderate, severe, and very severe \[73\]. The characteristic intraretinal lesions that primarily define the nonproliferative stage are not always present when new vessels are first observed, because of the temporary nature of such lesions \[54\].

PDR has been observed to affect the structure of the retinal vasculature and to cause neovascularization within the ONH and elsewhere \[51, 54\]. The narrowing (or straightening) of the MTA has been used as an indicator of compromised integrity of the macular region, which is responsible for visual acuity; such changes may result in loss of vision \[51, 54, 69\].

CAD of DR

There has been an extensive amount of research conducted on CAD of DR \[25, 56, 59, 68, 74-95\]. The most common approach for CAD of DR is through detection of intraretinal lesions. For this purpose, it is crucial to first detect and segment the vasculature and then to exclude it from any further analysis involving lesion detection.

1.3.2 Retinopathy of Prematurity and Plus Disease

ROP is a complex disease that affects the process of development of the vascular architecture in the retina of preterm infants and is the leading cause of preventable childhood blindness worldwide \[40\]. It is estimated that at least 50,000 children worldwide are suffering from blindness caused by ROP \[96\]. Based on a study conducted in 1993 in the US, it was
reported that there are about 30,000 preterm babies born each year with a birth weight of 500 to 1249 g, out of whom 1,000 are estimated to progress to a level of ROP that requires treatment (threshold disease) \[97\]. An analysis \[98\] of a New York state patient database from 1996 to 2000 indicated that the incidence of ROP among newborn infants was 1 in 511. Out of the 6,998 premature infants screened during a two-year period for the Early Treatment for ROP study in the US, 68% developed ROP \[99\]. Gunn et al. \[100\] reported an incidence of 81% of ROP in premature infants based on a screening study conducted over an 18-year period in Australia. The governmental cost of visual impairment due to ROP in the US was estimated at about $38 to $65 million per year in 1993 \[97\], which is about $63 to $107 million per year after adjusting for inflation in 2015.

**Plus Disease**

Because ROP can advance rapidly in the first 8 to 12 weeks of life, timely diagnosis and identification of signs of ROP are important for clinical management of the affected infants. In the past decade, it has been established that early detection and treatment of ROP are highly correlated with the presence of plus disease, which is an indicator of a severely progressing phase of ROP \[57, 58, 101-106\]. An active shunt at the junction of the vascularized and nonvascularized parts of the retina is associated with the presence of plus disease. Vascular manifestations of plus disease include increased vessel thickness and tortuosity in the posterior part of the retina \[107\], defined as a circular area centered on the ONH and reaching up to the macula \[108\]. A change in the openness of the MTA has also been observed as a sequela of ROP as well as an indicator of compromised structural integrity of the macular region \[40, 41, 44, 96, 109, 110\].

**Pathogenesis of Plus Disease**

There are several theories that attempt to explain the underlying mechanisms that cause morphological vascular changes in the presence of plus disease. It was first theorized that such changes occur as a result of a reduction in capillary resistance \[111\], the fact that
venules are more distensible than arterioles [112], and an increase in blood flow to the retina in the presence of plus disease [113]. The increase in blood flow is thought to be caused by an increase in the angiogenic stimulus that leads to a larger arteriovenous shunt [113]. However, it has been shown that not only does blood flow not increase in the presence of plus disease [114] but that it could actually decrease in the CRA of infants with plus disease [115]. Another theory relates the changes in the blood vessels to an increase in VEGF in the presence of plus disease [116–118]. Both prematurity and supplemental oxygen provided in the neonatal intensive care unit are factors in the suppression of vessel growth in premature infants [118]. The lack of a completely developed retinal vasculature causes an increase in VEGF, which, in turn, causes neovascular proliferation of blood vessels that ultimately leads to tractional retinal detachment and blindness [118]. It has been postulated that an increase in VEGF could be associated with changes in pericytes and smooth muscle cells, which lead to a decreased ability to regulate blood flow, and as a result, to susceptibility to fluctuations in oxygen levels in the vascular bed [119]. It is possible that pathological mechanisms, such as an increase in VEGF and neovascular proliferation of blood vessels, cause straightening or narrowing of the MTA. Regardless of the underlying mechanism, plus disease, and ultimately ROP, has an effect on the characteristics and architecture of the retinal vessels.

Clinical Diagnosis of Plus Disease

The Early Treatment for ROP Cooperative Group suggested that the presence of Type 1 ROP should warrant treatment [58]. One of the conditions that defines Type 1 ROP is the presence of any stage of ROP in zone I (the area within a circle centered on the ONH with a radius of two times the distance from the ONH center to the fovea) accompanied by plus disease. The presence of plus disease is now considered to be the main indicator for the need for treatment. Even though the staging and zone of ROP are important markers of the severity of the disease, plus diagnosis is almost always associated with cases of acute ROP requiring treatment [57,101–103].
Plus disease is clinically diagnosed by visual qualitative comparison (using a fundoscope) to a standard retinal fundus photograph [40] that exhibits abnormal levels of thickness and tortuosity; the standard photograph is shown in Figure [1.4]. However, the standard photograph is believed to be atypical because it shows more vascular dilation and less tortuosity as compared to most cases with plus disease [57]. Also, the image possesses a narrow FOV, and does not reveal possible tortuous vessels in the periphery of the retina. Furthermore, the range of vascular changes is smaller and more challenging to distinguish in premature infants in the presence of plus disease even for experts, since the retinal vasculature is not fully developed as compared to similar pathological changes in adults.

Figure 1.4: The standard image showing minimum venular dilation and minimum arteriolar tortuosity required for the diagnosis of plus disease. Image reprinted with permission from Springer Science + Business Media.

The current clinical method for diagnosis of plus disease is subjective. As shown by Chiang et al. [101], among 22 recognized ROP experts who performed diagnosis of plus disease using 34 fundus images of preterm infants based on three-level classification (plus,
preplus, and neither), the experts agreed on the diagnosis of only 12% of the images (four out of 34). With two-level classification (plus and no-plus), the experts agreed on the diagnosis of 21% of the images (seven out of 34). It is likely that no optimal visual reference standard exists for the diagnosis of plus disease, as shown by disagreement even among recognized experts [101, 103, 120]. Considering such facts, it can be concluded that there is a great need of methods for CAD of plus disease, which can lead to timely diagnosis and effective treatment of ROP.

CAD of Plus Disease

Various semiautomated computer-aided procedures have been designed to perform diagnosis of plus disease by quantification of changes in the tortuosity and thickness of retinal vessels [102, 103, 112, 121, 127]. Such studies have demonstrated that methods for CAD are capable of discriminating between cases with and without plus disease as accurately as experts. To the best of the author’s knowledge, all studies that have performed diagnosis of plus disease via quantification of vascular changes have employed methods that involve manual marking of vessel segments to be analyzed, or manual selection and correction of parts of automatically detected vessels to include only the desired or selected vessels for further analysis, which may not be feasible in a clinical or teleophthalmological setting. Considering the measurement of tortuosity, all studies seen in the literature have limited the area of analysis to the posterior of the retina; however, peripheral-vessel tortuosity has been shown to be more correlated to the presence of plus disease as compared to posterior-vessel tortuosity [105].

Even though a direct relationship appears to exist between increasing venule thickness and increasing severity of ROP, the detection of small changes in venule thickness may require high-resolution imaging as these changes are at, or below, the spatial resolution of typical retinal imaging systems [42]. Arteriolar tortuosity has shown higher correlation with the presence of plus disease in some studies, but such a correlation has not been consistent across
all trials [57, 121, 127]. Moreover, the detection of arterioles presents an image processing challenge; arterioles have lower contrast as compared to venules and their thickness can be below the resolution limit of even high-resolution retinal fundus images of premature infants [128]. It has also been observed that the distinction between arterioles and venules becomes difficult in the presence of zone I disease [129]. About 20% of the time, even experts cannot distinguish between arterioles and venules in retinal images of preterm infants [130].

Despite the clinical importance of abnormal changes in the openness of the MTA, it has been quantified, in various manners, in only three studies reported in the literature: a study dealing with myopia [55] and two studies dealing with ROP [41, 110]. The angle of insertion of the MTA, also referred to as the temporal arcade angle (TAA), has been loosely defined as the angle between the STA and the ITA as they diverge from the ONH and extend towards the periphery of the retina [109, 110]. A change in the angle of insertion of the MTA has also been featured in the classification of retrolental fibroplasia [131]; it has also been used in the evaluation of structural changes following cryotherapy [109]. Such factors and limitations indicate a need for automated, precise, and specific methods for quantification of the vascular changes described to perform CAD of plus disease in a clinical and/or teleophthalmological setting, which is the motivation for the present work.

1.3.3 Other Diseases

There are several other pathologies that can be diagnosed by computer-aided analysis of retinal fundus images. Changes in the properties of the ONH can indicate the presence of certain pathologies, including glaucoma. Changes in the measured area or RFNL thickness of the avascular macular region can indicate the severity of various diseases including DR and age-related macular degeneration (AMD) [132]. Furthermore, certain lesions that are indicative of the presence of retinal pathology appear specifically within the macular region [64, 70]; two such common diseases are glaucoma [49, 50, 133, 137] and AMD [88, 90, 138, 140]. Both of these diseases manifest themselves in terms of both vascular and neuronal changes and...
can be detected by analysis of images acquired using digital fundus imaging, SLO, or OCT in different manners.

1.4 Scope of the Thesis

The main aim of the present work is CAD of plus disease via development of digital image processing techniques to first detect and segment retinal blood vessels, and then to quantify the related changes in the thickness of the MTA, the openness of the MTA, and tortuosity of vessels in retinal fundus images of preterm infants. The contents of the thesis are organized in nine chapters and a list of references.

Chapter 2 presents a brief overview of image processing methods for the detection of retinal blood vessels as well as quantification of the thickness and openness of the MTA and tortuosity of vessels.

Chapter 3 provides details of the database used in the present work (and other publicly available databases) and the methods used for statistical analysis and evaluation of the results as well as pattern classification.

Chapter 4 presents an overview of image processing techniques that have been developed and implemented in the present work for detection, segmentation, and modeling of vessels.

Chapter 5 presents detailed descriptions of the procedures designed and developed in the present work for detection, segmentation, and extraction of the MTA skeleton as well as detection and interpolation of the edges of the MTA, computation of the width of the MTA, and ultimately CAD of plus disease in retinal fundus images of preterm infants.

Chapter 6 provides detailed descriptions of the procedures developed and realized in the present work for detection, segmentation, and quantification of the openness of the MTA via parametric modeling and CAD of plus disease.

Chapter 7 presents detailed descriptions of the procedures designed and developed in the present work for detection, segmentation, and computation of the tortuosity of retinal
vessels towards CAD of plus disease.

Chapter 8 provides details on a graphical user interface (GUI) designed and implemented for clinical application of the proposed methods.

Chapter 9 presents a concluding discussion, remarks, and notes on potential future work on CAD of plus disease.
Chapter 2

Computer-aided Detection and Quantitative Analysis of Retinal Vasculature

2.1 Detection of Retinal Vasculature

The problems of detection and segmentation of vasculature in retinal images have been considered since fundus photographs were scanned to digital formats and became available for computer-aided analysis. There has been an extensive amount of research performed on development of methods for enhancement, detection, and segmentation of retinal vessels by considering various physiological properties of vessels; these properties include being piecewise-linear oriented features, possessing a negative contrast with respect to the background, possessing an inverted-Gaussian cross-sectional intensity profile, and presenting a branching tree pattern. In addition to enabling quantitative analysis of the vasculature, vessel detection facilitates localization of certain additional retinal features, such as the macula and the ONH, in relation to the vascular structure. Even though vessel detection is a major step in the present work as a prerequisite to quantitative measurement of vascular features, vessel detection is not the focus of the present study; the author’s M.Sc. thesis and related publications addressed this topic in detail. The following review of the state-of-the-art of vessel detection methods published in the literature provides a general overview of the available methods.

The vessel detection methods available in the literature fall within several different categories that include, but are not limited to, the use of

- template matching via matched filters,
- vessel ridge, edge, or contour detection and tracking.
• directional filters [163–165],

• gray-scale variation and local thresholding [166–170],

• morphological operators [171–173],

• Hessian-based gray-scale gradient information [174, 175],

• multifeature analysis [176–178], and

• probabilistic models [179–180]

Other available methods in the literature for the detection of blood vessels in retinal images include segmentation using tram-line filtering [181], multiconcavity modeling [182], the use of amplitude-modified second-order Gaussian filters [183], fractal analysis [184], and vessel models and the Hough transform [185]. Several techniques have been proposed to model and analyze the structure of retinal vasculature, including fractals [184, 186] and geometrical models and analysis of topological properties [142, 143, 187]. Some procedures attempt to combine several of the above-mentioned methods into one algorithm; Roychowdhury et al. [188] combined global image enhancement and local adaptive thresholding with region growing in an iterative manner to detect retinal vessels. Roychowdhury et al. [189] also combined morphological operators with pixel training and classification schemes using local gradient information for detection of retinal vessels.

Analysis of oriented structures is not limited to biomedical images and has been used in diverse applications such as detection of roads and waterways in satellite images [190–193], analysis of geophysical maps [194], and measurement of fiber orientation in paper [195].

In the present work, five vessel detection methods [141, 164, 174, 175, 196] were implemented and quantitatively compared against one another. Detailed explanation and comparative analysis of these methods are presented in Section 4.1.
2.2 Quantitative Analysis of Retinal Vasculature

2.2.1 Measurement of the Thickness of Vessels

Methods for measurement of retinal vessel thickness have evolved from the use of manually operated devices on photographs [197] to sophisticated computer methods for various other clinical and research applications. Methods used for measurement of vessel thickness typically fall into two main categories. The first category of methods initially attempt to detect and segment vessels, then obtain pixels associated with vessel center-lines and edges, and finally compute a thickness measure based on the distance between the associated edges and center-line pixels [121, 198, 199]. The second category of methods employ vessel detection techniques via a modeling approach. The models are mainly Gaussian-based since the intensity profile of a vessel at a given vessel center-line pixel resembles an inverted Gaussian curve [42,122,143,168]. As a result, the spread or standard deviation (STD) of the Gaussian model used to detect the vessel could be used to estimate the width of the vessel.

Using projection micrometry [197], Kristinsson et al. [198] showed that the differences in the width of retinal vessels of normal (adult) cases as compared to cases with diabetic macular edema are statistically highly significant. Projection micrometry employs a mechanical device that magnifies the retinal fundus photograph using a modified transparency viewer. The observer aligns a fixed vertical pointer (a wire) at the center of the magnified vessel. The wire is then manually moved using a knob until it is aligned with one of the edges of the vessel and its position is recorded. The same procedure is repeated to obtain the position of the remaining edge of the vessel. The width of the vessel is taken as the distance between the two marked positions.

Given the center-lines of the vessels in a retinal image, Lowell et al. [143] estimated the cross-section of the intensity profile of the vessels using a Gaussian model. The parameters of the model were optimized using a variable-metric method. The STD of the optimized Gaussian model at each pixel of the center-lines was taken as the width of the corresponding
vessel. The method was not evaluated in a clinical application. Wilson et al. [42] employed the same technique using Gaussian models and a contrast measure based on the magnitude response of Laplacian-of-Gaussian (LoG) models obtained at vessel center-line pixels. Based on tracking and extraction of skeletons of manually selected vessels via multiscale ridge detectors, Wallace et al. [122] determined a vessel width estimate at each detected ridge point using the spread of a LoG model.

Using semiautomated methods, Heneghan et al. [121] obtained a binary image of retinal vessels via multiscale analysis of the second-order derivative of the original intensity images combined with preprocessing and postprocessing morphological methods, as well as a two-level thresholding strategy. Heneghan et al. measured the vessel width by extending a line from the two opposite sides of a given vessel pixel until it reached the boundary of the vessel and noting the length of the line. This procedure was repeated for lines of various orientations originating from the same pixel. The vessel width at the given pixel was taken as the minimum distance obtained for all orientations.

Martínez-Pérez et al. [166, 187] developed a semiautomated image analysis software called Retinal Image multiScale Analysis (RISA) for detection, segmentation, and measurement of vessel width in retinal images. RISA uses a region-growing algorithm in order to segment (binarize) blood vessels based on two features of edge-strength and ridge-strength. The edge-strength feature is derived as the gradient of the intensity image. The ridge-strength feature is based on the eigenvalues of the Hessian matrix of the intensity image. The resulting binary image is skeletonized and broken into several segments based on the detected branching points. RISA estimates the width of each vessel segment as the total area (in pixels) of the segment, divided by its length. RISA requires manual user correction at the stage of detection of branching points, as well as manual input regarding the vessel segment to analyze and to distinguish between venular and arteriolar branches.

Fiorin and Ruggeri [199] used a web-based software package to draft manually the center-
line of a retinal vessel segment. Canny’s edge detection method was used to obtain a set of vessel-edge pixels around the selected center-line. Two edge curves were then estimated on either side of the selected center-line by fitting a cubic spline to the previously detected edge pixels. The width of the selected vessel segment at each pixel was defined as the distance along the normal at a center-line pixel between the two estimated edge curves.

Niemeijer et al. [168] detected retinal vessels using likelihood estimation of vessel presence based on the outputs of multiscale Gaussian filters and a $k$-nearest-neighbor classifier in high-resolution images of the retina. Magnitude responses of multiscale gradient operators were used to define homogeneous regions in the vessel-likelihood image. A splat map was generated based on the homogeneity criterion. Niemeijer et al. assumed that each splat is either inside or outside a vessel; however, they noted that the assumption fails for low-contrast and narrow vessels. Each splat was then assigned the median likelihood value of the pixels belonging to that specific splat. The technique was used to refine the edges of the vessels in the likelihood response image at the binarization step. The vessel width at each center-line pixel was measured as the distance between a pair of edge pixels that would fall on the normal to the local vessel direction, obtained as the eigenvector with the largest eigenvalue of the covariance matrix of the coordinates of the center-line pixels in a $15 \times 15$-pixel neighborhood centered on the pixel under consideration.

Xu et al. [200] used boundary detection and region growing algorithms based on graph-based theory on high-resolution retinal images for vessel width measurement. More recently, specialized algorithms have been developed for the analysis of the ratio of arteriolar to venular width [201], analysis of temporal variation of vessel width in video images [202], and measurement of vessel width in hand-drawn binary maps of retinal vasculature [203]. The methods referred to in the present paragraph may not be applicable to retinal fundus images of preterm infants due the associated low quality and poorly developed nature of the vessels.

Locating vessel edges based on binarization of the intensity image obtained using a vessel
detection algorithm is prone to error due to sampling, the scale of the model used, as well as the threshold value used to binarize and segment vessels [121]. Furthermore, estimation of vessel width using the STD of Gaussian-based models is also prone to error since a Gaussian model is a continuous curve of infinite extent that does not contain a clear-cut point to indicate the exact location of the vessel edges. Such a method for estimation of width requires an assumption regarding suitable, yet arbitrary, weighting of the STD of the Gaussian model to estimate vessel width [183]. Methods that use a vessel’s center-line for measurement of width are prone to error in case the center-line is not precisely in the middle of the vessel; it is especially challenging to guarantee such a requirement. Furthermore, such methods require accurate detection and subpixel representation of vessel edges. In addition, the methods available in the literature for measurement of vessel width in case of plus disease either manually distinguish between venular and arteriolar branches [102, 103, 123, 127], or do not distinguish between them at all [121]; these methods also obtain the width measurement for only manually selected vessel segments.

Using RISA, a few studies [102, 103, 123, 127] have attempted to measure vessel width in retinal fundus images of premature infants and have correlated their findings to the presence of plus disease. The results of these and two other studies [121, 122] are presented and compared with the results obtained using the methods proposed in this work in Section 5.6.

2.2.2 Measurement of the Openness of the MTA

In order to quantify the openness of the MTA, an angle of insertion of the MTA (or TAA) has been loosely defined as the angle between the STA and the ITA as they diverge from the ONH towards the periphery of the retina [41, 55, 109, 110]. Even though three of the studies cited above [41, 55, 110] have demonstrated statistical significance of the changes in the MTA angles with respect to the stages of the diseases studied (myopia and ROP), they have used different definitions. Only the location of the vertex of the MTA angle has been consistently defined as the center of the ONH. The locations of the other two points used to measure the
TAA have been defined in different manners.

Fledelius and Goldschmidt [55] measured the angle between the STA and the ITA and correlated its decrease to progression of myopia based on follow-up data over a 38-year period. They defined the arcade angle by manually marking cardinal points at the first or the second major arteriole-venule crossings away from the ONH (decided subjectively to represent the direction of the temporal arcade vessels and not just the venule), with the vertex of the angle being at the center of the ONH. The cardinal points were used as landmarks from image to image. Two lines were drawn from the center of the ONH to the marked cardinal points on the ITA and the STA. The angle between the two lines was measured using a transparent protractor.

In a study by the Cryotherapy of Retinopathy of Prematurity Cooperative Group [109], the arcade angle was measured by manually tracking the MTA in 30° sectors; however, the normal range of the arcade angle was not defined.

Wilson et al. [110] defined the angle of insertion of the MTA as follows: the center of the ONH and the fovea are manually marked by two independent observers. A line is drawn through the manually marked centers of the ONH and the fovea; this is the retinal raphe. The image is rotated so that the retinal raphe is horizontal. A line perpendicular to the retinal raphe is drawn from the fovea until it intersects the ITA and the STA. From the points of intersection, two lines are drawn to the center of the ONH. The TAA is defined as the sum of the inferior and the superior arcade angles (IAA and SAA).

In a related follow-up study by Wong et al. [41], semiautomated measurements were made of four different angles of the temporal and the nasal venules and arterioles. The procedures required manual editing of automatically detected vessels; this step required 10 – 15 minutes per image. As compared to the previous related study of Wilson et al. [110], the angles were measured using reference points selected closer to the center of the ONH.

The published methods to measure the angle of insertion of the MTA may not properly
reflect the changes that occur in the structure of the MTA, as they only define the openness of the MTA based on only three points. Furthermore, only the location of the vertex of the arcade angle has been consistently defined as the center of the ONH; the locations of the other two points have been defined in different manners. Even though the structure of the MTA has been used to estimate the ONH and the macula in previously reported works, only Tobin et al. [150] modeled the arcade for parameterization of its openness; however, they used the openness parameter only to draw the parabolic model on the image. It should be noted that identifying the fovea in fundus images of preterm infants is challenging even for experts as shown by Chiang et al. [204].

The results of quantification of the openness of the MTA using the method of Wong et al. [41] and a modeling approach proposed in the present work are presented and compared against one another in Section 6.4.

2.2.3 Measurement of Tortuosity of Vessels

Although there have been several measures of tortuosity proposed in the literature, tortuosity does not have a specific mathematical definition. The proposed methods can typically be divided into three categories: length-to-chord (LTC) measures, curvature-based measures, and angle-based measures. It should be noted that measures of tortuosity are typically obtained using a skeletal representation of the vasculature, which can either be drafted manually using a specially designed GUI or obtained using image analysis algorithms in an automated or semiautomated environment. All of the methods presented in the literature for measurement of tortuosity in the presence of plus disease have used manual segmentation or selection of vessels.

The simplest definition of tortuosity is the LTC measure, which is the ratio of the true (geodesic) length, or arc-length, of a vessel segment to the length of the line connecting the segment’s end points (chord) [102, 122, 205]; see Figure 2.1. The drawback of the LTC measure is that it does not account for the possible changes in the curvature of a segment;
hence, an arched segment and a sinusoidal segment of the same true length and chord length may lead to the same tortuosity values, as shown Figure 2.1, which is undesirable. Some studies have proposed modified definitions of the LTC measure in order to overcome this inherent limitation of the measure.

![Image](image_url)

Figure 2.1: Illustration of the LTC measure as applied to two line segments of equal true length (in black) and chord length (red line) values leading to equal LTC measures; however, the lower line is considered to be tortuous, while the upper line is not.

Wilson et al. [42] proposed a multiscale approach to the LTC measure, in which a given vessel segment was divided into two subsegments using the perpendicular bisector of the chord of the segment (a line that is normal to the chord and divides it into two equal parts); the point of intersection of the perpendicular bisector with the vessel separates it into two subsegments. Each resulting subsegment was divided using the same method until a minimum length was reached as shown in Figure 2.2. Tortuosity was then defined as the level of increase in total chord length after the final subdivision as compared to the initial chord length. It is expected that a tortuous segment would lead to a large increase in the value of the total chord length, leading to a larger multiscale LTC measure.

Hart et al. [206] originally proposed the use of curvature to define a measure of tortuosity using skeletonized retinal vessel segments, including the total curvature and total squared
Figure 2.2: Illustration of the multiscale approach of Wilson et al. [42] to the LTC measure. The original line segment at the top is divided into two subsegments using the perpendicular bisector of the chord. Each resulting subsegment is further divided into two parts until a certain minimum length (of subsegments) is reached. The proposed multiscale LTC measure was defined as the ratio of increase in the total chord length after the final iteration to the initial chord length.

Curvature measures. Dougherty et al. [207] fitted a polynomial spline to the traced center-line of a vessel segment and used the curvature of the spline to compute a measure of tortuosity.

Using curvature, Grisan et al. [208] separated vessel segments into curved and linear parts and then assigned each half of a given linear subsegment to its previous and next curved subsegments. Grisan et al. defined the tortuosity of the entire segment as the sum of the LTC of each subsegment normalized by the true length of the entire segment and weighted based on the number of subsegments (number of sign changes in the curvature). The authors noted that the proposed measure would provide misleading values if a subsegment does not contain any nonlinear parts.

The angle-based tortuosity measures are derived by defining a set of vectors of fixed
length connecting various points on a given vessel segment and using the angles formed at the tip and tail of two consecutively connected vectors to compute a tortuosity measure.

Gelman et al. \cite{123} defined tortuosity as the sum of all angles (SOA) between a set of vectors, as previously explained, normalized by the total length of the segment. However, Gelman et al. did not specify the length of the vectors used to obtain the angles. The authors noted that the proposed methods would not work on a segment without any branching points.

Makkapati and Ravi \cite{209} proposed a modified definition of the SOA measure by defining the angle for a given skeleton pixel using the two angular bisectors of three consecutive vectors normalized by the sum of the lengths of each subsegment. However, Makkapati and Ravi did not specify the length of each vector.

Lisowska et al. \cite{210} used a similar approach as the SOA method; however, instead of defining an angle between successive vectors, the absolute change in the slope of the vectors was obtained and a measure of tortuosity was defined for a vessel segment as the sum of the slope differences.

By defining the angle of a given pixel based on the coordinates of the current and previous pixels, Poletti et al. \cite{211} proposed an angle-based measure of tortuosity. The tortuosity measure was computed as the sum of the squared angle changes, divided by the total length of the vessel segment, where the local change in angle was defined as the difference in the inverse tangent of the angle of the current and previous pixels. It should be noted that such a definition of the angle at a pixel based on its 8-connected neighboring pixels will only provide angle measures with increments that are integral multiples of $45^\circ$.

The tortuosity measures that use the SOA approach are dependent on the length of the vectors used on a vessel segment to define the angles; short vector lengths may result in large angle values, whereas long vector lengths may miss small local variations. Ideal representation of the skeleton (or center-lines) of vessels is a difficult task that is prone to discretization errors as well as inaccuracies in detection and segmentation of vessels; defining
the orientation of a given vessel pixel using the coordinates of such a skeletal representation of vessels is also prone to errors.

Several studies \cite{102,103,121,123,125,127} have measured vessel tortuosity in retinal fundus images of premature infants and correlated their findings to the presence of plus disease in the images. The results of these studies are presented and compared with the results obtained using the methods proposed in this work in Section 7.6.

### 2.3 Detection of Other Retinal Features

As mentioned in Section 1.1, the ONH is an oval-shaped area that is brighter than the surrounding area in retinal fundus images, and is the point of divergence of retinal blood vessels. The macula is a dark region towards which vessels converge \cite{15}.

Many different methods have been proposed for the detection of the ONH, including the use of morphological operators \cite{212,213}, matched filters \cite{214,216}, the shape and color properties of the ONH \cite{217,222}, convergence point of the blood vessels \cite{223,224}, geometric models \cite{142,149}, and a fractal-based model \cite{225}.

The detection of the macular region often proceeds from the detection of the ONH, as it is situated approximately two ONHWs temporal to the ONH center \cite{6} along the retinal raphe \cite{146,150}. The macula may be detected by analyzing the overall vascular structure, because the retinal blood vessels converge towards the macular region, or by considering color and/or contrast properties of the region \cite{77,91,152,218,226,228}.

### 2.4 Remarks

Detection of the retinal vasculature is the initial and the most important step in computer-aided analysis of retinal fundus images. Upon detection of the retinal vasculature, quantitative analysis of its various properties such as thickness, openness of the MTA, and tortuosity may be performed in various manners. Detection of the retinal vasculature facilitates further
detection of other anatomical and/or pathological features as well. Various published methods for vessel detection and quantitative analysis of thickness, openness, and tortuosity in retinal fundus images were reviewed in this chapter. The methods developed in the present work for the same purposes are described in the following chapters.
Chapter 3

Databases of Retinal Fundus Images and the Experimental Setup

The methods developed in the present work need to be tested using established databases of retinal fundus images that provide information with regard to the presence or absence of disease and/or anatomical features, such as retinal blood vessels. The obtained results in terms of vessel detection and segmentation as well as diagnosis of pathology are assessed in a quantitative and objective manner using various metrics. The present chapter provides the details of a few publicly and privately available databases of retinal fundus images, as well as methods for quantitative evaluation of the obtained results.

3.1 The TROPIC Database

The Telemedicine for ROP In Calgary (TROPIC) database is a private collection of retinal fundus images of preterm infants that is used in the present work for diagnosis of plus disease. Written consent was obtained from the parents of the subjects in order to capture and use the images. The tenets of the Declaration of Helsinki were followed in the proposed study. The images of the TROPIC database were captured using the RetCam II camera equipped with a wide-angle ROP lens (130°) and have a size of 480 × 640 pixels. It should be noted that, in the present work, the convention used to state the size of images or indicate coordinate points specifies the row-coordinates (annotated by \(m\)) first, followed by the column-coordinates (annotated by \(n\)). The spatial resolution of the RetCam 130 images is estimated to be 30 \(\mu m\) per pixel [7].

In total, 110 images from 41 preterm patients (16 females, 25 males) were selected from
the database for the present study. In most cases, there are five different images available for each eye of each patient from each visit, representing different retinal fields to provide collectively an almost full photographic documentation of the retina. In each case, the image with the highest visibility of the entire vasculature was chosen. Images were not selected based on overall quality and/or vessel-to-background contrast. Nineteen of the 110 selected images are from patients diagnosed with plus disease (stages 2 and 3 of ROP) and 91 show no signs of plus disease (stages 0, 1, 2, and 3 ROP). At most, two images from the same patient were included for the same stage of ROP (one image from each eye). Multiple images of the same eye from the same patient were included only if the ROP stages were different at the time of imaging. All diagnoses were performed by the specialist (A. L. Ells) at the time of clinical examination using an ophthalmoscope and the patient’s clinical records; the diagnosis was not based solely on the RetCam images of the patient. Patients corresponding to 90 of the 110 images were diagnosed with no ROP or with stages 1 or 2 ROP (30 images per category), and patients corresponding to 20 images were diagnosed with stage 3 ROP.

A training set of 10 images, including 5 without and 5 with signs of plus disease was also formed. All abnormally tortuous vessel segments in the 5 training images with plus disease were manually identified by the retinal specialist. The images of the training set are mutually independent of the test set described in the previous paragraph, even though they are drawn from the same population of patients.

Figure 3.1 displays two images from the TROPIC database. Part (a) of the figure shows an example of an image without signs of plus or ROP. Part (b) of the same figure illustrates an image with signs of Stage 3 ROP as well as plus disease at the time of imaging.

Table 3.1 provides the mean and standard deviation (STD) for the birth weight (BW), gestational age (GA), and chronological age (CA) of the patients.
Figure 3.1: Two images from the TROPIC database where (a) illustrates no signs of plus disease or ROP, and (b) shows signs of ROP as well as plus disease.

Table 3.1: The mean and STD of BW, GA, and CA, in grams (g), weeks, and days respectively, for normal preterm infants as well as patients diagnosed with plus disease. The p-values indicating the statistical significance between the means of the two classes are also provided. ***p < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without Plus, Mean ± STD (n = 91)</th>
<th>With Plus, Mean ± STD (n = 19)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>818.00 ± 210.78</td>
<td>815.89 ± 203.71</td>
<td>&gt; 0.1</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>26.73 ± 1.88</td>
<td>24.95 ± 1.77</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>CA (days)</td>
<td>71.05 ± 23.67</td>
<td>69.84 ± 13.00</td>
<td>&gt; 0.1</td>
</tr>
</tbody>
</table>

3.2 The DRIVE Database

The DRIVE database [230] is one of the most established and commonly used publicly available databases of retinal fundus images. The images in the DRIVE database were captured using a Canon CR5 nonmydriatic camera, which provides an FOV of 45° [230]. Each image in the DRIVE database was acquired at a of size 768 × 584 pixels. However, the images were cropped to the size of 584 × 565 pixels, because the FOV has a diameter of about 540 pixels [230]. The DRIVE images are considered to be low-resolution fundus images of the retina; considering the size of the FOV, the images have an approximate spatial
resolution of 20 $\mu$m per pixel.

Forty images were selected at random to be included in the DRIVE database, including seven showing signs of mild DR such as exudates, hemorrhages, and pigment epithelium changes. Thirty-three images do not show any abnormal signs. The set of 40 images has been divided into a training set and a test set, each containing 20 images. One manually segmented image of the vasculature is available for each image in the training set, whereas the test set has two manually segmented images of the vasculature per image. The observers who manually marked the vasculature were all trained and instructed by an expert ophthalmologist. The observers were asked to mark a pixel as being part of a vessel only if they were at least 70% certain that the given pixel belonged to the vessel [230]. To evaluate the performance of the methods for the detection of retinal vessels, only one of the two sets of manual segmentation provided with the DRIVE test set was used, which shall be referred to as the ground-truth images.

The DRIVE database does not provide separate manual marking of only the MTA. Hence, for the purpose of evaluation of the performance of the MTA openness measures (Chapter 6), the STAs and ITAs in all of the 40 DRIVE images were traced by an expert ophthalmologist and retinal specialist (A. L. Ells, Alberta Children’s Hospital, Calgary), by magnifying the original image by 145% and using the software ImageJ [231]. Only the main venule (the thickest branch of the vessels) was traced within the FOV. At each branching point, the thicker branch was followed. The availability of separate traces of the STA and the ITA facilitates the assessment of the accuracy of the dual-parabolic modeling procedure, as described in Chapter 6. The hand-drawn traces of the STA and the ITA can be combined to obtain a trace of the MTA.

Figure 3.2 shows an image from the DRIVE database accompanied with the associated ground-truth image of the vessels in a binary format. The image illustrated in Figure 3.2 does not show any signs of DR.
Figure 3.2: (a) An image from the DRIVE database showing no signs of DR and (b) its associated, manually labeled, ground-truth image.

Figure 3.3 shows two images from the DRIVE database with the MTA, STA, ITA, contour of the ONH, the center of the ONH, and the fovea labeled.

3.3 The STARE Database

The STARE database \cite{232} is one of the oldest publicly available databases of retinal images. The images of the STARE database are scanned and digitized color fundus photographs \cite{233}, and as a result, the quality of the images is not as good as retinal images that are directly captured in a digital format. The images of the STARE database have a narrow FOV of 35° and a size of 700 × 605 pixels. The spatial resolution of the images is approximately 15 μm/pixel; however, the spatial resolution appears to vary from one image to another and is not constant.

For the purpose of evaluation of the diagnostic performance of methods for detection and modeling of the MTA in a potential clinical application (see Chapter \textit{6} for results), 11 normal cases and 11 cases of PDR were obtained from the STARE database. The STARE
database has a total of 22 cases that are diagnosed with PDR; however, 11 cases were not used because either the MTA was not in the FOV, or the major nasal/temporal branches were not distinguishable. The eleven images of normal cases were selected starting from the lowest image number in the subset of images of the STARE database that is used for the detection of the ONH; cases that either did not clearly show the entire MTA or possessed low contrast within the FOV were not selected.

Previously, the PDR cases were not part of the publicly available subset of images of the STARE database and were provided by Dr. A. Hoover (Clemson University) upon request; all 397 images of the STARE database are now publicly available [232].

Figure 3.4 illustrates two images from the STARE database, where the image in part (a) is considered to be a normal image and the image in part (b) shows signs of PDR.

3.4 Other Databases

In recent years, several new databases of retinal images of diabetic cases have become publicly available. These databases include, MESSIDOR [234], Retinopathy Online Chal-
Figure 3.4: Two images from the STARE database, where (a) shows no abnormal signs and (b) shows signs of PDR, including neovascularization within the disk and elsewhere, as well as signs of NPDR indicated by hemorrhages and exudates. Note that the dark circular spots on the nasal side of the image in part (b) are laser scars from previous treatment.

3.5 Methods for Evaluation of the Results

Analysis and quantification of the results of detection and segmentation of biomedical features as well as CAD of diseases in images is based on medical statistical analysis [238]. Specifically, measures of sensitivity and specificity utilize the fact that a particular biological feature, or a disease in an image, can only have two states, present (positive) or absent (negative), which is referred to as binary classification.

The ground-truth classification for the presence or absence of vessels in a retinal image is provided in terms of a binary image, as annotated by an expert ophthalmologist, where a value of 1 indicates a vessel pixel and a value of 0 represents a background pixel. Similarly,
in the case of disease diagnosis, the ground-truth classification is provided by an expert in the field in terms of negative (absence, indicated by a 0) or positive (presence, indicated by a 1) diagnosis, for each image. Given such a binary classification, a few measures, including sensitivity and specificity, may be defined based on medical statistical analysis in order to quantify the results of a binary-classification system. In order to define such measures, several notations need to be introduced.

The total number of cases that are actually marked as positive or negative in the ground-truth results are referred to as actually positive (AP) and actually negative (AN) cases, respectively. The total number of correctly classified positive and negative cases are termed true-positive (TP) and true-negative (TN) values, respectively. Similarly, the total number of incorrectly classified positive and negative cases are referred to as false-negative (FN) and false-positive (FP) values, respectively. The true-positive fraction (TPF), also referred to as sensitivity, and true-negative fraction (TNF), also known as specificity, are the ratios of the TP and TN values with respect to the AP and AN values, respectively. In a similar manner, the false-positive and false-negative fractions, FPF and FNF, are defined as the ratios of the FP and FN values with respect to the AN and AP values, respectively.

The four ratios defined above, in particular the sensitivity and specificity, provide an understanding of how accurately the positive and negative cases are identified, at the cost of false classification, for a single operating point (threshold value). Given a feature with a certain range of values, the defined ratios facilitate overall analysis of the trade off between sensitivity and specificity of the feature values by varying the threshold at which the binary classification is performed over the entire range of operating points, and obtaining all the associated TPF and FPF values. Such analysis is referred to as receiver operating characteristic (ROC) analysis, and is represented as the plot of TPF versus FPF. The area under the binormal fit to the ROC curve ($A_z$) provides an overall quantitative measure of classification accuracy. The ROC curve, as a whole, provides information in terms of trade off between
sensitivity and specificity.

A single set of sensitivity and specificity values may be used when a final binary decision threshold has already been applied and the final results are in binary classified form. ROC analysis may be used in instances where a specific threshold for binary decision making, to be applied to a feature, is not available and/or the overall response of the feature over the entire operating range is of interest. In such a setup, ROC analysis may also be used to obtain a suitable threshold based on a certain set of training data, which may later be applied to an independent test set. In an ideal case, the results should provide sensitivity = 1 at specificity = 1, or $A_z = 1$.

3.5.1 Evaluation of the Results of Vessel Detection

Considering the evaluation of the results of vessel detection, as the results are presented in terms of gray-level values (see Section 4.1) and not in a binary format, the most suitable method for assessment of the results would be through ROC analysis. As explained in the previous section, ROC analysis sorts the gray-level values from the results of vessel detection and applies a sliding threshold from the smallest value up to the largest, and provides a plot of the varying TPF and FPF results for each threshold value. The area under the ROC curve, $A_z$, provides a quantitative measure for the overall level of classification and the performance of the underlying method for vessel detection. The results of ROC analysis of the outcomes of vessel detection methods are provided in Section 4.1.5.

3.5.2 Evaluation of the Results of Vessel Segmentation

In the case of vessel segmentation, the results are already in a binary format since a threshold has already been applied to the results of vessel detection to obtain an image, where all white pixels represent vessels and all black pixels represent the background. In such a scenario, only a pair of sensitivity and specificity values may be obtained to assess the performance of the selected threshold value or the thresholding algorithm as a whole. Such analysis is
demonstrated in Section 4.2 to compare the performance of several thresholding techniques.

3.5.3 Evaluation of Diagnostic Classification

Analysis of the diagnostic classification results is concerned with the evaluation of the results of a certain extracted feature, which is meant to serve as an indicator of the presence or absence (diagnostic classification) of a disease in a given image. In most cases, the values of the obtained feature are not in a binary format and usually provide a varying range of values. Similar to the evaluation of the results of vessel detection, ROC analysis is the most suitable method for analysis of the results of feature extraction for the purpose of diagnostic-decision making, as applied to the MTA thickness (Section 5.5) and openness measurements (Section 6.4), as well as the total length of tortuous vessels in a given image (Section 7.5). In the present thesis, the ROCKIT software [239] is used to perform ROC analysis for evaluation of the results of diagnostic classification. The $A_z$ value provided by ROCKIT is based on the area under the binormal fit to the ROC curve, which may vary slightly as compared to the area under a discrete version of the ROC curve (AUC).

Given a diagnostic-decision-making criterion, or a threshold, which presents the results of feature extraction as binary classification (similar to vessel segmentation results), only a single set of sensitivity and specificity values may be obtained to evaluate the results. One such criterion is applied in the present work to the results of measurement of the length of tortuous vessel segments and is evaluated using a pair of sensitivity/specificity values, as explained in Section 7.4.

3.5.4 Evaluation of the Confidence Intervals of the Results

Considering the limited number of cases in both classes in the TROPIC database, the bootstrap method [240][241] was implemented for statistical analysis. The bootstrap is a method used to analyze the level of confidence in an estimate of a parameter of a population such as the mean, when there are a small number of data points available. The bootstrapping
method resamples a population (or a class) by randomly selecting a larger number of cases from the given population, with replacement. The random-selection procedure is repeated hundreds or thousands of times and the confidence intervals are obtained based on the results \[241\]. Assuming a standard normal distribution, the symmetric confidence interval for an estimated parameter is obtained as \([m - t_{1-2\alpha/2} \times S_m, m + t_{1-2\alpha/2} \times S_m]\), where \(m\) is the mean of the sample, \(S_m\) is the standard error of the mean (SE) defined as STD of the sample divided by the square root of the number of samples, and \(t_{1-2\alpha/2}\) is the critical value of the standard normal distribution based on \(\alpha = 0.025\). It is crucial to determine the appropriate distribution for the estimated parameter for correct formulation of the confidence interval (symmetric or asymmetric) and the associated critical values. In the present work, the Jarque-Bera test \[242\] for normality of data was used to determine whether a given set of data belongs to a normal distribution or not. The histogram distribution of the data may be analyzed as to determine a suitable distribution.

In the present work, the bootstrap method was employed with the results of the MTA thickness (Section 5.5) and openness measurements (Section 6.4), as well as the total length of tortuous vessels in a given image (Section 7.5). In all instances, both of the classes with and without plus disease were resampled 100 times with replacement and the associated mean parameter of each class and the AUC values were obtained. The resampling procedure was repeated 500 times and the associated symmetric 95\% (\(\alpha = 0.025\)) confidence intervals (\(CI_s\)), calculated by assuming a standard normal distribution for the obtained mean and AUC values, were computed. An in-house ROC analysis program was used to perform the bootstrapping procedure, since ROCKIT does not allow for batch processing of large amounts of data. The in-house ROC analysis program provides the AUC value and not the \(A_z\) value based on the binormal estimation of the ROC curve as in ROCKIT. As a result, there could be small differences between the values obtained using the two programs.
3.6 Methods to Combine Diagnostic Features

3.6.1 Pattern Classification

Logistic Regression

Logistic regression (LR) is a probabilistic model that attempts to predict the likelihood of occurrence of a class (positive or negative in a binary classification), given a feature vector \[\mathbf{f}\]. The LR model predicts whether a given vector of \(n\) features, \(\mathbf{f} = [f_0, f_1, ..., f_n]\), for a single observation (case) is more likely to belong to the negative (0) or positive (1) class based on the calculated probability of occurrence as \[243\]

\[
p_{\text{LLR}}(\mathbf{f}) = \frac{\exp(f_0c_0 + f_1c_1 + ... + f_nc_n)}{1 + \exp(f_0c_0 + f_1c_1 + ... + f_nc_n)}, \tag{3.1}
\]

where \(n\) is the number of features per observation and \(\mathbf{c} = [c_0, c_1, ..., c_n]\) is a vector of coefficients. The coefficient values are determined using the least-squares method; the most likely coefficient values that provide the smallest sum-of-squared distances are selected and the probability of occurrence of the event is computed accordingly \[243\].

Naïve Bayes

The Bayes rule states that the probability of a given feature vector belonging to a specific class can be obtained using the prior probability of occurrences of a class \(p(C)\), a feature vector \(p(\mathbf{f})\), and the joint probability distributions of the features given \(C\) as \[244\]

\[
p_{\text{Bayes}}(C|\mathbf{f}) = \frac{p(C) \cdot p(\mathbf{f}|C)}{p(\mathbf{f})}. \tag{3.2}
\]

The naïve Bayes rule makes the assumption that the probability distributions of the individual features are independent of one another given a specific class; hence \(p(\mathbf{f}|C)\) can be obtained as \(\prod_{i=1}^np(x_i|C)\) using a training set.
Multilayer Perceptrons

Multilayer perceptrons (MLPs) use the concept of linear discriminant functions to provide the optimal solution to an arbitrary problem by implementing linear discriminants in a space where the inputs have been mapped nonlinearly [244]. The function of such a network is roughly based on the properties of biological neurons. In the present work, the MLP classifier used was made up of three layers [244]: an input layer, one hidden layer, and an output layer. A given hidden node in the hidden layer computes a weighted sum of its inputs as

$$\text{net} = \sum_{i=1}^{n} f_i w_i,$$

where $n$ is number of features per observation (case), and $f_i$ and $w_i$ denote the $i^{th}$ feature and weight of a given hidden unit, respectively. The output of a hidden unit is a nonlinear function of its net activation input, such as a tangent sigmoid (tansig) [244]. The output layer node also computes a weighted-sum of its inputs (hidden-layer outputs) and applies a linear or nonlinear activation function, such as tansig, to the results to produce a new discriminatory value based on the input features.

3.6.2 Feature Selection

All pattern classification methods presented in the previous section require training. It would be ideal to perform any training using a dedicated set of data; however, considering the limited number of available cases in this work (110 cases from the TROPIC database), it is required to use the available data set to perform the training as well as testing steps. One practical approach to this situation is the $k$-fold cross-validation method, in which the given dataset is divided into $k$ parts (folds). One fold is kept outside while the rest of the data are used to train the chosen classifier, and the trained classifier is then applied to the given fold [244,245] in the testing or validation step.

The wrapper method is a feature selection technique that employs supervised learning along with classification algorithms, such as those explained above, to select the most discriminatory combination of features based on a given set of data [245]. The wrapper method requires a search criterion based on which the optimal combination (subset) of features is
selected [245]. Two common search methods are the best-first and the exhaustive methods.
The best-first method starts with either an empty or a full set of features and moving in any
direction, attempts to select/remove features sequentially until the best subset of discrim-
inatory features is determined [245]. The exhaustive search method considers all possible
subsets of features, in any order, to determine the subset with the best discrimination of the
data [244].

The results of feature selection by applying the wrapper method, a given classifier, a
specific search method, and \( k \)-fold cross-validation are presented in terms of the number of
times any given feature was selected out of \( k \) tries [245]. In the present work, the features
that were selected more than half the times (\( \geq k/2 \)), using the proposed setup, were used
for pattern classification using the same classifier as with the wrapper method.

3.7 Remarks

In the present chapter, several databases of retinal images, including one with fundus images
of preterm pediatric cases, were described. The three databases of TROPIC, DRIVE, and
STARE are used in the present work for testing as well as evaluation of the final results
in terms of CAD of ROP and PDR. The evaluative methods used for the detection and
segmentation of vessels as well as diagnostic classification were presented in terms of sensi-
tivity/specificity and ROC analysis. The methods described are used in evaluation of the
results obtained in Chapters 4, 5, 6, 7 and 8.
Chapter 4

Methods for Detection, Segmentation, and Modeling of Retinal Vasculature

As mentioned in Section 2.1, detection and segmentation of retinal vessels are the two main steps required in computer-aided analysis of retinal fundus images. The current chapter provides description of several such methods and their application to retinal fundus images of adults and preterm infants. When testing several methods in order to determine their suitability for an application, it is crucial to be able to quantify their performance for the sake of objective comparative analysis. In the case of vessel detection and segmentation methods, it would be ideal to compare the results against hand-drawn vessel-map (ground-truth) images, as shown in Figure 4.1. However, as explained in Chapter 3, there are no publicly available databases of preterm retinal fundus images. Furthermore, the TROPIC database does not include any ground-truth images. As a result, the DRIVE database (of adult retinal images) is used in the present work in order to quantitatively assess and compare each vessel detection and segmentation method studied. The presented methods are also applied to the images of the TROPIC database; however, in this case, only qualitative assessment of the results is possible. The vessel detection method that provides the highest accuracy is then selected for further assessment of the thresholding methods.

4.1 Detection of Retinal Vessels

4.1.1 Gabor Filters

Gabor filters are sinusoidally modulated Gaussian functions that provide optimal localization in both the frequency and space domains. The frequency response of a real Gabor filter
Figure 4.1: (a) Original color image 7 of the DRIVE database. (b) Ground-truth vessel image for the image in part (a), as provided in the database.

oriented at $\theta = -\pi/2$ can be represented as \[ G(u, v) = \frac{1}{2} \left( \exp \left[ -2\pi^2 \{ \sigma_x^2 (u + f_o)^2 + \sigma_y^2 v^2 \} \right] + \exp \left[ -2\pi^2 \{ \sigma_x^2 (u - f_o)^2 + \sigma_y^2 v^2 \} \right] \right). \tag{4.1} \]

In this equation, the frequency of the modulating sinusoid is given by $f_o$, and $\sigma_x$ and $\sigma_y$ are the STD values in the $x$ and $y$ directions in the image domain. For simplicity of design, a variable $\tau$ is used to represent the average thickness of the vessels to be detected. The value of $\sigma_x$ is defined based on $\tau$ as $\sigma_x = \frac{\tau}{2\sqrt{2\ln 2}}$ and $\sigma_y = l\sigma_x$, where $l$ represents the elongation of blood vessels. A bank of $K$ Gabor filters may be obtained by rotating the main Gabor filter response given in Equation 4.1 over the range $[-\pi/2, \pi/2]$ with the step size of $\pi/K$. For a given pixel, the maximum output value over all of the $K$ filters is saved as the Gabor-magnitude response at that particular pixel; the corresponding angle is recorded as the Gabor-angle response.
4.1.2 Vesselness Measures

Vesselness Measure of Frangi et al.

Frangi et al. [174] defined a vesselness measure to detect pixels that exhibit vessel-like characteristics by considering the properties of the eigenvalues of the Hessian matrix, which is defined as the Jacobian matrix of the second-order partial derivatives of a function with respect to its variables [246]. In the case of an image, the function is the map of intensity values, $L(x, y)$, and the variables are the spatial coordinates $(x, y)$. The Hessian matrix, $H$, is symmetrical with real eigenvalues, and is defined as

$$H = \begin{bmatrix}
\frac{\partial^2 L}{\partial x^2} & \frac{\partial^2 L}{\partial x \partial y} \\
\frac{\partial^2 L}{\partial y \partial x} & \frac{\partial^2 L}{\partial y^2}
\end{bmatrix}.$$  \hspace{1cm} (4.2)

A numerical estimate of the Hessian matrix, $H$, is obtained at multiple scales by convolving the image, $L(x, y)$, with the Gaussian kernel $G(x, y; \sigma)$ of different scales, $\sigma$, defined as

$$G(x, y; \sigma) = \frac{1}{2\pi\sigma^2} \exp \left( -\frac{x^2 + y^2}{2\sigma^2} \right).$$  \hspace{1cm} (4.3)

The Gaussian kernel can be used to generate a suitable scale space with a range of $\sigma$ values related to the range of vessel width. Multiscale partial derivatives of the image $L(x, y)$ can be obtained by linear convolution of the image with the scale-normalized derivatives of the Gaussian kernel.

The signs and ratios of the eigenvalues can be used as signatures of a local structure; the larger eigenvalue ($\lambda_2$) corresponds to the maximum principal curvature at a given location, $(x, y)$. A large ratio between the two eigenvalues ($\lambda_2/\lambda_1$) is generally taken to represent a vessel-like structure.

Based on the property of the eigenvalues of the Hessian matrix, Frangi et al. [174] defined
a vesselness measure to highlight pixels belonging to vessel-like structures as

\[
V_F = \begin{cases} 
\exp\left(-\frac{R^2_{\beta}}{2}\right) \left[1 - \exp\left(-\frac{S^2}{2\gamma^2}\right)\right] & \text{if } \lambda_1, \lambda_2 < 0, \\
0 & \text{otherwise,}
\end{cases}
\] (4.4)

where \( R_{\beta} = \frac{\lambda_1}{\lambda_2} \), \( S = \sqrt{\lambda_1^2 + \lambda_2^2} \) is the Frobenius norm of the Hessian matrix, \( \beta = 0.5 \) (as used by Frangi et al. [174]), and \( \gamma \) is equal to one-half of the maximum of all of the Frobenius norms computed for the whole image. The Frobenius norm is expected to be low in background areas where no vessels are present and the eigenvalues are low, because the magnitudes of the derivatives of the intensities are small. On the other hand, in regions with high contrast with respect to the background, the Frobenius norm will be larger, because at least one of the eigenvalues will be large.

Vesselness Measure of Salem et al.

Another vesselness measure, proposed by Salem et al. [175], uses the orientation of the eigenvectors of the Hessian matrix to estimate the orientation of blood vessels. It has been shown that there is less variation in the orientation of the eigenvectors corresponding to the smaller eigenvalues inside vessels as compared to those outside vessels. The eigenvectors corresponding to the smaller eigenvalues are mainly oriented along the blood vessels; hence, the angle \( \theta_1 \), corresponding to the smaller eigenvalue, is used to analyze the orientation of blood vessels.

Detection of blood vessels can be accomplished by assuming that the maximum value of the larger eigenvalue, \( \lambda_2 \), expressed as \( \lambda_{\max} \), over several scales of \( \sigma \), is at the center of a vessel. Salem et al. [175] defined a vesselness measure as

\[
V_S = \frac{\lambda_{\max}}{\theta_{\text{std}} + 1},
\] (4.5)

where \( \theta_{\text{std}} \) is the STD of the orientation of the smaller eigenvalue over all scales used for the pixel under consideration.
4.1.3 Line Operators

Line operators were proposed by Dixon and Taylor [247] and used by Zwiggelaar et al. [196] for the detection of linear structures in mammograms. The main line operator kernel detects horizontal lines. Assume that $N(x, y)$ is the average gray level of $M$ pixels along a horizontal line centered at $(x, y)$. Next, assume that $S(x, y)$ is the average gray level of pixels in a square of width $M$ pixels that is horizontally aligned and centered at $(x, y)$. The main line operator kernel is defined as $L(x, y) = N(x, y) - S(x, y)$. Detecting lines with various orientations is achieved by rotating the main kernel. Let $L_k(x, y)$ be the line operator kernel rotated to the angle $\alpha_k = -\pi/2 + \pi k/K$, $k = 0, 1, ..., K - 1$. Given $W_k(x, y)$ as the result of filtering the image, $I(x, y)$, with $L_k(x, y)$, the orientation of the detected line is obtained as

$$\theta(x, y) = \alpha_{k_{\text{max}}}, \text{ where } k_{\text{max}} = \arg\{\max[W_k(x, y)]\}. \quad (4.6)$$

The magnitude response of the result is obtained as $W_{k_{\text{max}}}(x, y)$. The line operator does not provide a specific parameter for scaling; multiscale analysis is performed by applying the line operator to each level of the Gaussian pyramid decomposition of the original image.

4.1.4 Matched Filters

The method of Chaudhuri et al. [141] was implemented in the present work for the detection of blood vessels. The method assumes that blood vessels have a negative contrast with respect to the background, so the Gaussian template will need to be inverted. The main kernel of the matched filter is expressed as

$$M(x, y) = -\exp\left(-x^2/2\sigma^2\right), \text{ for } -L/2 \leq y \leq L/2, \quad (4.7)$$

where $L$ represents the length of the vessel segment that is assumed to have a constant orientation and $\sigma$ is the STD of the Gaussian. The main kernel of the filter is oriented along the $y$-axis; in order to detect blood vessels at different orientations, the main kernel is
rotated at multiple angles.

4.1.5 Vessel Detection Using the DRIVE Database

The training set of the DRIVE database (see Section 3.2) and the associated ground-truth images were used in the present work to determine the most suitable set of parameters for each of the five vessel detection methods described in the preceding sections. For each method, the related parameters were varied over a certain range of values, vessel detection was performed, and the results were evaluated via ROC analysis (see Section 3.5). The sets of parameters that provided the highest $A_z$ values were selected and applied to the test set of the DRIVE database.

Preprocessing Steps

All vessel detection methods mentioned above are required to be applied to gray-scale images. In the case of adult retinal images, it has been shown that the luminance component, $Y$, of the $YIQ$ color space, and the green-channel image provide high vessel-to-background contrast [154]. The luminance component, computed as

$$Y = 0.299R + 0.587G + 0.114B,$$  \hspace{1cm} (4.8)

where $R$, $G$, and $B$ represent the red, green, and blue color channels in the $RGB$ color space, respectively, was used as the input to the vessel detection method applied to the DRIVE database.

Segments or parts of the edge of the FOV in retinal images may be interpreted as oriented features by the vessel detection methods. To prevent this, each gray-scale image obtained, as explained above, is extended beyond the limits of its FOV by assigning a gray-level value to the pixels that fall outside the FOV, as computed based on the average of a $21 \times 21$ pixel neighborhood that is adjacent to the pixel under consideration. The process is repeated 50 times, extending the gray-scale image by a ribbon of gray pixels of width 50 pixels.
Results of Vessel Detection

Using the training set of the DRIVE database, values of $\tau = 8$ pixels, $l = 2.9$, and $K = 180$ were determined to provide the best single-scale results with Gabor filters [155]. Figure 4.2 shows the magnitude and angle responses of Gabor filters with $\tau = 8$ pixels, $l = 2.9$, and $K = 180$ as obtained for the image in Figure 4.1 (a). It is seen that the magnitude response is high at pixels belonging to vessels and that the angle response values agree well with the orientations of the vessels at the corresponding pixels.

![Figure 4.2](image)

(a) Gabor magnitude and (b) angle responses for a portion of the image in Figure 4.1 (a). The Gabor angle information is shown for every third pixel on the vessels for a portion of the green-channel image.

The range of scales $\sigma = [1, 6]$ with steps of 0.05 was determined to be the most suitable range for the vesselness measures of Frangi et al. and Salem et al. using the training set of the DRIVE database [155], and was used for subsequent analysis. Note that the two vesselness measures implemented in this work perform multiscale analysis by taking the maximum intensity value among all of the available scales of $\sigma$. The implementation of the method of Frangi et al. used in this work was provided by Dirk-Jan Kroon of the University
Figure 4.3 shows the magnitude response images of the results of applying the vesselness measures of Frangi et al. and Salem et al. to the image in Figure 4.1 (a) using the determined parameters.

Figure 4.3: Magnitude response images of the results of filtering the image in Figure 4.1 (a) obtained using (a) vesselness measure of Frangi et al. and (b) vesselness measure of Salem et al. Note that the result of the method of Frangi et al. provides lower intensity values as compared to the method of Salem et al. and the detected vessels may not be clearly visible in the result.

In the case of the line operators, values of $M = 15$ and $K = 180$ were determined to provide the best results for detection of vessels using the training set of the DRIVE database, and were employed for further analysis. Figure 4.4 (a) shows the magnitude response of the line operators as applied to the image in Figure 4.1 (a) using the mentioned values.

Detection of blood vessels using matched filters is performed by taking the maximum filter response of a bank of $K = 180$ filters over the range $[-\pi/2, \pi/2]$ with $L = 15$ and $\sigma = 1$, as determined using the training set of the DRIVE database. Figure 4.4 (b) represents the magnitude response of the matched filters obtained for the image in Figure 4.1 (a).

Comparative analysis of the proposed methods was performed using the set of 20 test
Figure 4.4: Magnitude response images of (a) line operators, obtained using $M = 15$ and $K = 180$, and (b) matched filters, obtained using $L = 15$, $\sigma = 1$, and $K = 180$, for the image in Figure 4.1 (a).

images of the DRIVE database \[155\]. The ground-truth images of the vasculature were used as reference to perform ROC analysis. The results were evaluated in terms of the area under the ROC curve ($A_z$) (see Section 3.5 for more details), which are provided in Table 4.1.

Based on the results presented in Table 4.1, single-scale Gabor filters were selected for further use in the present work for detection of vessels in preterm retinal fundus images of the TROPIC database. A comparative analysis of Gabor filters, line operators, and steerable filters by Ayres and Rangayyan \[248\] also led to the conclusion that Gabor filters are the most suitable filters for the detection of oriented patterns. Cruz-Aceves et al. \[250\] tested the same methods for detection of coronary arteries in angiograms and concluded that the best results were provided by multiscale Gabor filters.

4.1.6 Vessel Detection Using the TROPIC Database

Ideally, it would be of interest to determine a set of suitable parameters for the Gabor filters using a training set, as performed using the DRIVE database and described in the previous
Table 4.1: Comparison of the efficiency of detection of blood vessels in retinal fundus images obtained using different methods, as implemented in this work for the test set (20 images) of the DRIVE database [230].

<table>
<thead>
<tr>
<th>Detection method</th>
<th>$A_z$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vesselness measure of Salem et al.</td>
<td>0.892</td>
</tr>
<tr>
<td>Vesselness measure of Frangi et al.</td>
<td>0.896</td>
</tr>
<tr>
<td>Line operators</td>
<td>0.905</td>
</tr>
<tr>
<td>Matched filters</td>
<td>0.928</td>
</tr>
<tr>
<td>Single-scale Gabor filters</td>
<td>0.950</td>
</tr>
</tbody>
</table>

section. However, as mentioned in Section 3.1, the TROPIC database does not include a training set with ground-truth vessel images. As a result, for each application, the Gabor parameters were determined empirically by visual assessment of the results. The preprocessing steps required before the application of Gabor filters to the TROPIC images are the same as the steps performed for the DRIVE database images, as explained in Section 4.1.5. However, instead of using the luminance component of the image, the $G$-channel image is used, since it has been observed to provide similar or better performance in the case of preterm retinal images [154]. Figure 4.5 illustrates a color retinal fundus image from the TROPIC database and the results of application of Gabor filters. The Gabor parameters used are $\tau = 6$ pixels, $l = 1$, and $K = 45$. The number of filters used ($K$) is reduced since the number of branching vessels in preterm retinal images is less than that in adult retinal images.

For the sake of comparison, the other four vessel detection methods studied were also applied to the image in Figure 4.5 (b). The results of vessel detection using the vesselness measures, the line operators, and matched filters are presented in Figure 4.6. The two vesselness measures (parts (a) and (b)) generally provide low response values, similar to the results of application to the DRIVE database. The magnitude response of the line operators provides relatively high response values for the background, which is undesirable. The result of matched filters is visually comparable to that of Gabor filters as shown in Figure 4.5 (c).
Figure 4.5: (a) Image 2302 of the TROPIC database. (b) G-channel image, (c) Gabor magnitude, and (d) angle responses for the image in part (b). The Gabor angle information is shown for every third pixel on the vessels for a portion of the G-channel image.
Both matched and Gabor filters provide relatively high response values for choroidal vessels. However, Gabor magnitude-response images generally possess higher response values for vessels as compared to the matched filters. It should be noted that quantitative comparison between the results of various vessel detection methods presented in this chapter, as applied to the TROPIC database, is not possible due to the lack of ground-truth images in the TROPIC database.

Figure 4.6: Magnitude responses of (a) vesselness measure of Frangi et al., (b) vesselness measure of Salem et al., (c) line operators, and (d) matched filters obtained using the image in Figure 4.5 (b).

For the remainder of the present work, single-scale Gabor filters are employed for vessel detection for the three applications of thickness, openness, and tortuosity measurements,
as discussed in Chapters 5, 6, and 7. The selected parameters of Gabor filters for various applications are determined specifically with regard to the particular requirements of each application and may be different from one to another.

4.2 Segmentation of Retinal Vessels

All of the vessel detection methods discussed in Section 4.1 produce gray-scale images of the vessels which can be thought of as vessel-strength images. Any further analysis of the retinal vasculature would require the vessel-strength image to be transformed to an image that indicates what is (unity-valued) or is not an object of interest (zero-valued) through binary representation of the image. Binarization of a gray-scale image is achieved through thresholding methods, where the values above and below a given threshold are set to one and zero, respectively. The binarized vasculature image may require to be processed further in order to obtain the required measurements or information, which is usually achieved through morphological image processing methods. The following sections present the details of several vessel segmentation methods, as well as morphological image processing methods for postprocessing of the results.

4.2.1 Thresholding Methods

Considering the ground-truth data provided for the 20 training images of the DRIVE database, on the average, only 13% of the FOV of a retinal image is covered by vessel pixels. This fact is further observed through the histogram of the gray-scale intensity values of the result of vessel detection, which is unimodal and skewed towards zero, as shown in Figure 4.7, there is no clear separation between the pixels belonging to blood vessels from the background pixels. Such unimodality is also observed in the histograms of the magnitude-response images obtained using the TROPIC database, as illustrated in Figure 4.8. It should be noted that, in most instances, there exists a large spike specifically at zero intensity; such a spike at
only a single intensity value cannot be taken as a peak. Based on these observations, it can be concluded that thresholding the gray-scale output images of vessel detection methods with high accuracy is a rather difficult task, especially since most automated thresholding methods assume a bimodal histogram or a certain level of separation between the foreground and background pixel values. For these reasons, several automated thresholding methods, including Otsu’s method [251], a moment-preserving thresholding method [252], the Ridler and Calvard thresholding method [253], and an entropy-based thresholding method [254] are explored in this work.

Otsu’s Method
In this method [251], the probability density function (PDF) of the gray-level values is obtained using the histogram of the gray-scale image. Next, an initial threshold is assumed and two classes are defined based on the threshold value: foreground (object of interest) and background classes. The associated statistics of each class, namely, mean and variance, are then computed, after which a new threshold is obtained that optimizes a discriminant criterion. The method works by defining a threshold value which provides the largest separation between the two classes [251].

Ridler and Calvard’s Method
In the method of Ridler and Calvard [253], two classes of pixels, foreground and background, are assumed based on the histogram of the gray-level values. The initial means of the background and foreground classes are set to 0 and to the mean of all nonzero intensity values, respectively. Next, a threshold is defined as the average of the means of the two classes. The threshold value is then iteratively computed by selecting the next gray-level value in the histogram as the class divider and obtaining the means of the classes. The process is repeated until the value of the threshold does not vary between two successive iterations [253].
Figure 4.7: Histograms of the normalized intensity values associated with (a) Figure 4.2 (Gabor filters), (b) Figure 4.3 (a) (vesselness, Frangi et al.), (c) Figure 4.3 (b) (vesselness, Salem et al.), (d) Figure 4.4 (a) (line operators), and (e) Figure 4.4 (b) (matched filters).
Figure 4.8: Histograms of the normalized intensity values associated with (a) Figure 4.5 (c) (Gabor filters), (b) Figure 4.6 (a) (vesselness, Frangi et al.), (c) Figure 4.6 (b) (vesselness, Salem et al.), (d) Figure 4.6 (c) (line operators), and (e) Figure 4.6 (d) (matched filters).
Moment-preserving Method

The moment-preserving thresholding method \cite{252} finds a suitable threshold by finding the gray-level value that allows the first three moments of the gray-scale image to remain the same in the resulting binary image. An advantage of the moment-preserving method is that it can provide multiple thresholds and does not require a given image to possess a bimodal histogram.

Entropy-based Method

Similar to the methods of Otsu and Ridler and Calvard, the entropy-based thresholding method works by initially assuming two PDFs for the foreground and background pixels. A threshold is then selected that maximizes the sum of the entropies of the two PDFs \cite{254}.

Figure 4.9 illustrates the results of thresholding the image in Figure 4.2 (a) using the four mentioned methods. In all instances, except for the entropy-based methods [part (d)], the results of segmentation are similar; most major branches are segmented, whereas the minor branches as well as single-pixel-thick vessels are missing. However, it has been shown that none of the tested thresholding methods provides adequate sensitivity values when compared against the ground-truth images in the test set of the DRIVE database \cite{155}.

The same four thresholding methods were also applied to the TROPIC images for comparison to the results obtained using the DRIVE database. Figure 4.10 illustrates the results of thresholding the image in Figure 4.5 (c). In comparison to the results of thresholding of the DRIVE image shown in Figure 4.9, it can be concluded that the entropy-based method consistently under segments the image, resulting in very few vessels being segmented.

For both adult and preterm retinal images, the method of Ridler and Calvard seems to provide a suitable trade-off between FPF and TPF values (see Section 3.5). However, as shown in Figure 4.11 (c), the same thresholding method fails to segment any vessel when tested with an image that differs from the image in Figure 4.5 (a) in terms of pigmentation and vessel-to-background contrast. In comparison, the magnitude response of the same
Figure 4.9: Results of binarization of the image in Figure 4.2 (a) obtained using (a) Otsu’s method with $t = 0.1333$, (b) Ridler and Calvard’s method with $t = 0.1522$, (c) the moment-preserving method with $t = 0.1726$, and (d) the entropy-based method with $t = 0.4196$. In all instances, the threshold values are fractions of the normalized magnitude response.
Figure 4.10: Results of binarization of the image in Figure 4.5 (c) obtained using (a) Otsu’s method with $t = 0.0588$, (b) Ridler and Calvard’s method with $t = 0.0821$, (c) the moment-preserving method with $t = 0.1216$, and (d) the entropy-based method with $t = 0.2667$. In all instances, the threshold values are fractions of the normalized magnitude response.
image when thresholded using a slider via the GUI by the user [Figure 4.11 (d)] maximizes detection of the retinal vessels while minimizing the artifacts due to choroidal vessels. Based on the results of this study, it can be concluded that accurate and precise segmentation of retinal vessels for both adult and pediatrics cases requires more sophisticated and complex thresholding techniques that adaptively adjust to local variations in each given image.

Figure 4.11: (a) Image 0801 of the TROPIC database. (b) Gabor magnitude-response image of part (a). Results of binarization of the image in part (b) using (c) the Ridler and Calvard method with \( t = 0.4448 \) and (d) a manually selected threshold value = 0.0250. The Ridler and Calvard method fails to segment any vessel. It should be noted that the image in part (b) provides relatively low intensity values and only appears to be completely black.
4.2.2 Morphological Image Processing Methods

Morphological image processing methods \[255\] are mainly based on set theory \[256\], which considers the outcome of the interaction of sets (of numbers) with one another. In image processing, the sets are the object(s) of interest as well the object used for manipulation of the former, referred to as a structuring element (SE). The geometrical factors defining an SE are crucial and are determined based on the relevant information that is required to be extracted from the image.

Although morphological image processing techniques were originally developed for binary images, in which unity-valued objects are presented against a zero-valued background (the same as for the SEs), the methods have been extended to applications with gray-scale images as well \[257\]. In the present work, morphological image processing methods are only applied to binary images; hence, the following review of the relevant methods covers only binary morphological image processing techniques.

Binary Erosion and Dilation

The two most basic morphological operators are erosion and dilation. As the names imply, erosion will shrink and dilation will enlarge an object.

Binary erosion is based on the intersection of the SE, \( E \), and the object of interest, \( X \), in the image as \[257\][258]

\[
\varepsilon_E(X) = \bigcap_{e \in E} X_{-e},
\]  

(4.9)

where \( e \) indicates a shift of \( E \) and the negative sign indicates reflection about the origin. The result of erosion can be thought of as the set of pixels where, if \( E \) were to be centered at, it would fit entirely within the object \( X \). In practical implementation, the erosion of a pixel, \( x \), is obtained as the minimum value of \( X \) within a window defined by the SE, \( E \), centered
at \( x \), as

\[
\varepsilon_E[I(x)] = \min_{e \in E} I(x + e),
\]  

(4.10)

where \( I \) is the given binary image.

Binary dilation is essentially the complement of binary erosion; binary dilation of an image is the same as complementing the result of erosion of the complement of the image using the same SE [257]. Binary dilation is based on the union of the SE, \( E \), and the object of interest, \( X \), in the image [257, 258]. In mathematical terms,

\[
\delta_E(X) = \bigcup_{e \in E} X_{-e},
\]  

(4.11)

where \( e \) indicates a shift of \( E \) and the negative sign indicates reflection about the origin. The result of dilation can be thought of as the set of pixels where, if \( E \) were to be centered at, it would touch the object \( X \). In practice, the dilation of a pixel, \( x \), in the given image, \( I \), is determined as the maximum value of \( X \) within a window defined by the SE, \( E \), centered at \( x \), as

\[
\delta_E[I(x)] = \max_{e \in E} I(x + e).
\]  

(4.12)

The level or amount of erosion and/or dilation in an image is determined by the shape and size of the SE. Figure 4.12 shows the results of applying binary erosion and dilation to the image in Figure 4.10 (b) using different SEs. It can be observed that the level of change is associated with the shape and size of the SE being used.

In the present work, binary erosion and dilation are used in the preprocessing steps before the application of Gabor filters for generation of a mask image representing the FOV, as well as in extending the gray-scale image beyond the limits of the FOV, in order to avoid the detection of the edges of the FOV by Gabor filters (see Section 4.1.5 for more details).
Figure 4.12: Results of eroding and dilating the image in Figure 4.10 (b) using two different SEs: a disk of radius 1 pixel and a line of length 3 pixels oriented at $\pi/4$. Erosion using (a) the disk and (b) the line SEs. Dilation using (c) the disk and (d) the line SEs.
Skeletonization

The process of skeletonization refers to a procedure that extracts single-pixel-thick piece-wise curvilinear segments, $S$, related to a binary object, $O$, which are spatially situated about the medial region of $O$ [257][259]. There are several skeletonization algorithms available in the literature that provide a skeleton that is situated medially about the object; however, depending on the type of algorithm used, minor details and attributes of the resulting skeleton may vary. Medial-axis transformation [260], thinning algorithm [261], and curvature-skeletonization algorithm [259] are three such methods.

In general, topology-preserving skeletonization algorithms work by first distinguishing a set of contour pixels (neighbors of background pixels), $C$, of an object, $O$, and proceed to remove a given contour pixel, $c$, only if its removal from the pattern satisfies a connectivity condition. Such an approach guarantees that there will be no holes in the final result, $S$, i.e., the object does not fall apart in the process of skeletonization. In order to explain better the concept of connectedness as used in skeletonization algorithms, a few terms must be explained first.

A pixel, $p$, has eight immediate neighbors, as shown in Figure 4.13. The pixel $p$ is said to be 8-adjacent to a neighbor pixel if $N(p) \geq 1$ for $1 \leq k \leq 8$ ($p$ has at least one neighbor that belongs to $O$), where $N(p)$ represents the summation of the values of the immediate neighboring pixels. Similarly, $p$ is 4-adjacent to a neighbor pixel if $N(p) \geq 1$ for $k = 1, 3, 5, \text{and} 7$. $O$ is said to be 8- or 4-connected if it cannot be partitioned into two subsets that are 8- or 4-connected, respectively. Using 4-connectivity is suitable for applications that contain objects with sharp transitions, whereas 8-connectivity is appropriate for more general patterns [262]. Generally, an $m$-component is a subset of pixels of $O$ that are $m$-connected and are not $m$-adjacent to any other pixel [262]. A pixel, $p$, is $m$-deletable only if its removal does not change the number of $m$-components that make up $O$ and does not create a hole in the object. It has been shown that, if the removal of a pixel does not change the topology of
a 3 × 3 window centered on the pixels under consideration, the global topology of the object is also preserved \[263\]. The crossing \[264, 265\] and the connectivity numbers \[266\] are two tests that can determine whether disconnectedness will result by the removal of a pixel, \( p \), within a 3 × 3 window. Using 8-connectedness, the crossing and the connectivity numbers are defined as

\[
X_8(p) = \sum_{k=1}^{4} h_k, \text{ where } h_k = \begin{cases} 
1, & \text{if } n_{2k-1} = 0 \text{ and } (n_{2k} = 1 \text{ or } n_{2k+1} = 1) \\
0, & \text{otherwise},
\end{cases}
\]

(4.13)

and

\[
C_8(p) = \sum_{k=1}^{4} (\bar{n}_{2k-1} - \bar{n}_{2k-1} \times \bar{n}_{2k} \times \bar{n}_{2k+1}),
\]

(4.14)

respectively, where \( \bar{n} = 1 - n \) and \( n_9 = n_1 \). Equations \[4.13\] and \[4.14\] both count the number of 8-components of white pixels in \( N(p) \) in two different ways. A crude skeletonization algorithm, using Equation \[4.14\] may work as follows: determine the contour pixels (\( C \)) of \( O \) and proceed to remove a contour pixel only if \( X_8 == 1 \); determine \( C \) again and repeat until \( O \) and \( C \) are equal.

\[
\begin{array}{ccc}
  n_2 & n_3 & n_4 \\
  n_1 & p & n_5 \\
  n_8 & n_7 & n_6 \\
\end{array}
\]

Figure 4.13: Illustration of the adjacent neighbors of a given pixel in an image. \( N(p) = \{n_k \mid 1 \leq k \leq 8\} \) determines 8-adjacency and \( \tilde{N}(p) = \{n_k \mid k = 1, 3, 5, 7\} \) gives 4-adjacency, where \( n_k \) is the neighboring pixel value; a unity-valued pixel belongs to the object and a zero-valued pixel resides in the background.

An iterative implementation of a skeletonization algorithm, such as the one explained
above, is inefficient and computationally resource intensive. It has been shown that, based on the pattern of object pixels in a $3 \times 3$ window centered on the pixel under consideration, $p$, a look-up table (LUT) could be used to indicate whether $p$ could be deleted or not. Such an implementation will be significantly faster than an iterative algorithm. In the present work, MATLAB’s implementation of the curvature-skeletonization algorithm, which uses LUTs to perform the skeletonization procedure, is used.

Figure 4.14 shows the result of applying the curvature-skeletonization algorithm to the image in part (b) of Figure 4.10. It can be observed that the skeleton of an object can provide useful information for further analysis and detection of oblong and branching patterns.

![Figure 4.14: The result of skeletonization of the image in Figure 4.10 (b) using the curvature-skeletonization algorithm.](image)

In the present work, the curvature-skeletonization algorithm is used to obtain the structure of vessels using 8-connectivity to be utilized in thickness measurement, quantification of MTA openness, and assessment of vascular tortuosity. The details of application of the preprocessing steps that use the skeletonization procedure are provided in Sections 5.1, 6.2, and 7.1.

Area Open Procedure

The morphological procedure of area open utilizes the concept of $m$-connectedness to locate sets of white pixels in a binary image and proceeds to remove those $m$-connected sets of
pixels that are smaller than a specified size, which is expressed in terms of a certain number of pixels \[268\]. The choice of the value for \(m\) is 4 or 8 in the case of binary images.

Figure 4.15 shows the effect of applying the area open operation using 8- and 4-connectivity to the image in part (b) of Figure 4.10. It is evident that small regions related to choroidal segments have been removed in all instances. Furthermore, it can be observed that the 4-connectivity criterion is a more restrictive; given the same area (number of subset of connected pixels), 4-connectivity removes fewer segments in comparison to 8-connectivity. It is clear that the larger the area that is used to check for connectivity, the larger the objects that are removed from the image.

In the present work, the morphological operation of area open is used in two separate steps to remove unwanted segments of white pixels left behind after the binarization process, as explained in Sections 5.1, 6.2, and 7.1.

4.3 Modeling of the MTA

4.3.1 The Generalized Hough Transform

The Hough transform was originally proposed by Hough \[269\] for the detection of straight lines in images and has since been extended and modified for detection of curves of various orders and even arbitrary shapes in the form of the generalized Hough transform (GHT) \[270\]–\[272\]. The main advantage of the GHT is being able to perform well in noisy images with irrelevant or even missing data \[270\],\[271\]. Various forms of the GHT have been employed in different biomedical image processing applications \[217\],\[273\],\[277\].

The GHT turns a global image domain problem of shape detection into a global parameter domain (Hough space) problem of peak detection. Every nonzero point in the image domain leads to a vote on different combinations of parameters that could have led to its presence, if it were part of the shape to be detected. An accumulator matrix is used to store and count the votes. The final count for each accumulator cell indicates the likelihood of the
Figure 4.15: The result of applying the area open procedure to the image in Figure 4.10 (b) using 4-connectivity to find subsets of pixels that have area less than (a) 50 and (b) 100 pixels. Results of application of area open using 8-connectivity to find subsets of pixels that have area less than (c) 50 and (d) 100 pixels.
corresponding parameter values of the accumulator cell having created the given pattern in the image domain. The size of the accumulator matrix is determined by the number of parameters and their range of values.

4.3.2 The GHT for Parabolic Modeling

The GHT can be used to detect parametric curves such as circles and parabolas [270,272,273,278–280] in images. As mentioned in Section 1.1, the MTA and the MNA take parabolic, or semiparabolic paths if considering their superior and inferior parts separately, as they branch into the retina from the center of the ONH. Such arch-like structure allows for easy detection and modeling of the MTA using the GHT for detection of parabolas to quantify the openness of the MTA.

The general formula defining a parabola with its directrix parallel to the vertical axis and its symmetrical axis parallel to the horizontal axis is $(m - m_v)^2 = 4a(n - n_v)$, where $(m_v, n_v)$ are the coordinates of the vertex of the parabola and the quantity $4a$ is known as the latus rectum [281]. The absolute value of the parameter $a$ controls the openness of the parabola and its sign indicates the direction of the opening of the parabola; for a negative $a$ value, the parabola opens to the left. The parameters $(m, n, a)$ define the Hough space, where $(m, n)$ are the row and column indices of the original image, respectively. The size of the parameter $a$ is theoretically unbounded; however, in many applications, a relevant range of the parameter $a$ may be obtained empirically or based on physical limitations. A single point in the Hough space defines a parabola in the image domain; for every nonzero pixel in the image domain, there exists a parabola in the Hough space for each value of $a$ that opens in the direction opposite to that of the parabola in the image domain.

In the procedure implemented in the present work [153,154,278], for each nonzero pixel in the image domain $(m, n)$, the parameter $a$ is computed for each $(m_v, n_v)$ in the Hough space, and the corresponding accumulator cell, $(m, n)$, is incremented by the Gabor magnitude response of the same pixel, only if the value of $a$ is within a specified range and the pixel
falls within an ellipse centered at the center of the ONH with axes lengths equal to the average ONHW and ONHH (see Section 1.1). The point in the Hough space with the highest value is taken to present the parameters of the best-fitting parabolic model. The coordinates of the detected vertex, along with the corresponding $a$ value, define the parameters of the obtained parabolic model.

4.3.3 Parabolic Modeling of the MTA

The GHT algorithm requires a binary skeletal representation of the image to be processed. In the present work, the Gabor filters (with a large $\tau$ value) were employed to detect the vasculature and the results were binarized using a sliding threshold via a GUI to obtain a binary image of the vasculature, which was then skeletonized and cleaned using the area open procedure, and finally passed on to the GHT algorithm. By dividing the input image into its superior and inferior parts and processing each image separately, it is possible to perform semiparabolic modeling of the STA and ITA.

Figure 4.16 shows the results of single and dual-parabolic modeling of the MTA, STA, and ITA. In this example, the Gabor magnitude-response image was obtained using $\tau = 10$ pixel, $l = 2$, and $K = 30$ filters and the result was thresholded at $t = 0.175$ of the normalized intensity value. Segments containing 150 pixels or less were removed using the area open procedure. It is clear that the dual-parabolic modeling procedure provides a better fit to the STA; the fit to the MTA is the same as the one to the ITA.

In the present work, the GHT for detection of parabolas and semiparabolas is employed for quantitative analysis of the openness of the MTA, STA, and ITA to perform CAD of plus disease, as explained in Chapter 6.
Figure 4.16: (a) The result of applying Gabor filter to the image in Figure 4.5 (b) with a large $\tau$ value to emphasize the MTA. (b) Result of thresholding the image in part (a) at $t = 0.0175$ of the normalized intensity value. Results of (c) single-parabolic modeling of MTA (249, 249, 42) and (d) dual-parabolic modeling of the STA (236, 248, 32) and ITA (249, 249, 42), respectively, using the image in part (b).
4.4 Remarks

The present chapter provided detailed description of the main image processing methods used in this work for preprocessing and postprocessing of images as well as for detection and segmentation of vessels. Various vessel detection and segmentation methods were discussed and quantitative measures were used to select the best performing methods using the DRIVE database. Results of application of the selected methods to images from the TROPIC database were also demonstrated in this chapter. Detailed descriptions of the methods used for measurement of the width and openness of the MTA as well as the tortuosity of vessels are provided in Chapters 5, 6, and 7 respectively.
The process of measurement of the thickness of the MTA consists of several different stages. The current chapter presents methods for detection, segmentation, and extraction of the MTA skeleton as well as detection and interpolation of the edges of the MTA, and ultimately, computation of the width of the MTA at all available pixels along its skeleton in retinal fundus images of preterm infants. The methods include Gabor filters and morphological image processing to extract the MTA skeleton, Canny’s method for selection of vessel-edge candidates, least-squares fitting to interpolate the vessel edges, and geometrical procedures to measure vessel width. The diagnostic performance of the average MTA width measure alone in distinguishing between cases with and without plus disease is analyzed in terms of sensitivity, specificity, $A_z$, and $p$-value. The obtained results are compared with results of similar studies in the literature.

5.1 Extraction of the Skeleton of the MTA

The procedure for extraction of the MTA skeleton consists of several stages:

1. preprocessing,
2. selecting the seed labels,
3. extracting the MTA skeleton segments, and
4. postprocessing.

The MTA extraction algorithm was originally developed based on adult retinal images from the DRIVE database and was validated by obtaining the mean-distance-to-the-closest-point (MDCP) measure from the tracked MTA to the hand-drawn traces of the MTA as
delineated by A. L. Ells [282]. The validation results indicated a low average MDCP error of about 2.3 pixels in 40 images of the DRIVE database [282], indicating that the methods are reliable for detection and extraction of MTA.

The MTA extraction algorithm requires the approximate location of the center of the ONH; for this purpose, the ONH centers in all images were manually marked by the user (F. Oloumi) via a GUI [283]. Note that this is the only manual input in the entire procedure used for vessel width measurement and may be replaced by an automated procedure [224].

5.1.1 Preprocessing

Figure 5.1 shows a flowchart representation of the preprocessing steps for extraction of the skeleton of the MTA.

In order to detect the MTA, Gabor filters, as explained in Section 4.1.6, were employed. A large thickness value ($\tau = 10$ pixels) and elongation factor $l = 1.2$, determined empirically, were used to emphasize the magnitude response of the MTA. Using the responses from a bank of 45 Gabor filters spaced evenly over the angular range of $[-\pi/2, \pi/2]$, the Gabor-magnitude response at each pixel was defined as the largest response of the filters, and the Gabor-angle response was defined as the orientation of the corresponding Gabor function.

Given the fact that vascular changes have been observed to occur close to the ONH (posteriorly) in the presence of ROP [40], an average ONHW of 1.05 mm in infants [7], and the spatial resolution of the TROPIC images, the skeleton extraction procedure and the subsequent analysis of each image were limited to the horizontal range from $0.25 \times$ ONHW nasal to $2 \times$ ONHW in the temporal direction with respect to the center of the ONH.

The algorithm is designed to detect the STA and ITA separately. The part of the image above the center of the ONH was taken as the superior part (STA), and the part below it was taken as the inferior part (ITA) and processed separately.

Each superior and inferior Gabor-magnitude-response image was normalized and gamma correction (with $\gamma = 1.5$) [273] was applied to increase the high-intensity values (related to
Figure 5.1: Flowchart of the preprocessing steps required for extraction of the skeleton of the MTA.
the MTA) and decrease the low-intensity values (related to choroidal vessels).

The moment-preserving thresholding method \[252\] (see Section 4.2.1) was used to binarize the Gabor-magnitude-response images of the STA and ITA separately. The resulting binary image was then skeletonized using the curvature-skeleton algorithm, as explained in Section 4.2.2.

All vessels in the retina originate from the center of the ONH; hence, it is difficult to distinguish between the various branches of vessels within the ONH. Therefore, a circular region centered at the center of the ONH, with diameter equal to the average ONHW, was removed from the skeleton of the detected vessels.

In order to remove the pixels in the skeleton that represent the boundary of the ONH, the difference between the radial angle (originating from the center of the ONH) and the Gabor angle for skeleton pixels within an annular region (the area between two circles with diameters = 1× and 2× ONHW) was obtained. If the angle difference was more than 60°, the associated pixel was removed \[155\].

The horizontally restricted skeleton images were then pruned to remove short branches. Unconnected segments that are not part of the MTA were removed by using the morphological process of area open only if there were at least 10 different segments available in the image. The minimum number of connected pixels to be removed was automatically determined based on the mean and STD of the number of pixels in all available segments in the skeleton image, as mean + 0.5×STD.

Based on the orientation of the MTA at each skeleton pixel, as indicated by the Gaborangle response, pixels that do not belong to the STA and ITA were removed separately. When considering the STA/ITA of the right/left eye, respectively, pixels with orientation in the range \[0, \pi/2\] were removed; similarly, when considering the STA/ITA of the left/right eye, pixels with orientation in the range \[-\pi/2, 0\] were removed.
5.1.2 Selecting the Seed Labels

Figure 5.2 illustrates a flowchart representation of the seed-label-selection algorithm for extraction of the skeleton of the MTA.

The branching points of the skeleton were determined using morphological image processing and by considering the number of neighboring pixels in an area of size $3 \times 3$ pixels. The segments resulting from breaking the skeleton were each labeled separately by numbering them. The average Gabor-magnitude response of each labeled segment of the skeleton was then calculated to obtain the seed label. The annular search area used to find seed label candidates was adaptively increased to ensure that there are at least two labels present for comparison for each of the STA and ITA parts; the procedure makes an exception to this criterion if only a single label is available in the image.

5.1.3 Selecting the MTA Skeleton Segments

Figure 5.3 shows a flowchart representation of the part of the algorithm for extraction of segments of the skeleton of the MTA.
Start

CurrentLabel = SeedLabel
ArcadeLabels = CurrentLabel

Does CurrentLabel exist in BranchingPoints_Matrix? Yes

Find neighboring labels of CurrentLabel in BranchingPoints_Matrix.

Select the neighbor label with the highest average Gabor-magnitude response (maxGaborMag) as the next label of the arcade and remove the current branching point from the BranchingPoints_Matrix.
CurrentLabel = Labels(maxGaborMag),
ArcadeLabels = [ ArcadeLabels; CurrentLabel ]

Yes

Is length(ArcadeLabels) > 30 pixels & maxColumnIndex(ArcadeLabels) > ColumnSize/2?

ArcadeLabels = SecondSeedLabel

Is # of ArcadeLabels > 1?

No

Yes

Remove the last selected label from ArcadeLabels.
CurrentLabel = ArcadeLabels(last)

End

Is length(ArcadeLabels) > 30 pixels & maxColumnIndex(ArcadeLabels) > ColumnSize/2?

No

Yes

Is length(SecondSeedLabel) > 30 pixels & maxColumnIndex(SecondSeedLabel) > ColumnSize/2?

No

Yes

Is length(ArcadeLabels) > length(SecondSeedLabel)?

No

Yes

Figure 5.3: Flowchart representation of the algorithm for extraction of the MTA skeleton.
When selecting the label with the highest average Gabor-magnitude response as the next potential segment of the arcade being analyzed, the algorithm ensures that the distance between the ending point of the current label and the starting point of the next potential label is no more than 10 pixels. The algorithm checks the total length of the selected segments and how far they extend collectively, with respect to the horizontal size of the image, as the exit criteria; specific details are provided in the flowchart in Figure 5.3.

5.1.4 Postprocessing

The postprocessing steps after the selection of the segments of the skeleton of the MTA are stated below.

a) Construct a binary image of the extracted arcade consisting of the selected labels in the previous stage.

b) Combine the extracted STA and ITA to obtain a binary image of the MTA (see Figure 5.3).

It is possible that the extracted MTA skeleton consists of disconnected segments because of the removal of the branching points in the preprocessing stage. This design factor is advantageous to the measurement of width as explained in Section 5.7.

5.2 Segmentation of the MTA Skeleton into Linear Parts

The selected skeleton segments are analyzed in terms of their associated Gabor-angle information to determine whether each selected segment is approximately linear or not. If a segment is not linear, breaking points are determined to separate the given segment into a set of linear subsegments.
5.2.1 Sequencing the Skeleton Pixels

The first step in obtaining linear subsegments of each selected skeleton segment is to identify
the pixels in the correct sequential order as follows:

a) Locate the first of the two end points of the current segment.

b) Start from the selected end point and sequentially move along the skeleton segment one
   pixel at a time in a $3 \times 3$-pixel neighborhood.

c) Store the sequential position of each traversed pixel in the segment as well as its associated
   Gabor-angle response in vectorial format.

d) Repeat steps b-c) until the other end point is reached.

5.2.2 Statistical Analysis of the Gabor-Angle Sequence

In order to detect possible changes in the orientation of the extracted skeleton segments, the
statistical measure of median absolute deviation (MAD) [284] was obtained in a window of
length 7 pixels applied to the sequenced Gabor-angle response obtained in the previous step.
Given a set of data points, $M$, $MAD = \text{median}[|M - \text{median}(M)|]$, where $|$ indicates the
absolute value. The analysis was only performed on segments with a minimum length of 7
pixels. The steps involved in obtaining the MAD measure are as follows:

a) Obtain the MAD measure of the previously obtained Gabor-angle sequence.

b) Set all nonzero values in the MAD measure equal to unity.

All nonzero values in the MAD measure are set to be equal to avoid detection of local
changes and emphasize the global variations in the orientation of the segment under analysis.
Note that, given the angle increment of $\pi/45$, due to the number of Gabor filters used (45),
the MAD measure provides values of 0 or multiples of $\pi/45$. 

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The obtained MAD measure needs to be analyzed further to detect any possible significant variations in the orientation of the segment being evaluated and subsequently any possible cutting points. The analysis is performed as follows:

a) Obtain the forward and backward derivatives ($d_f$ and $d_b$) of the MAD measure.

b) Set values of $d_f > 0$ and $d_b < 0$ to 0.

c) Set $d_{combined} = d_f + d_b$.

d) Analyze $d_{combined}$ based on possible patterns in the derivative values.

e) Determine the breaking points if any expected patterns are detected.

f) Break apart the segment only if any breaking point is determined.

g) Assume the entire segment to be linear if no breaking point is determined.

The forward and backward derivatives are combined to take advantage of the fact that a positive backward derivative value indicates the exact point of a positive change in the MAD measure; similarly, a negative forward derivative value indicates the exact point of a negative change in the MAD measure. Given a variation in the orientation of a segment, there are three possibilities and patterns to detect: a) A change that starts and stops within a sequence, indicated by a leading positive and a trailing negative derivative. b) A change that starts from the first pixel in a sequence and ends within it, indicated by only a negative derivative (no leading positive value). c) A change that starts within a sequence, but does not end, indicated by only a positive derivative (no trailing negative value). Any one of these conditions indicates a possible cutting point on the segment under analysis. Any single index obtained from cases b) and c) was taken as a cutting point. The median point between the two indices obtained in case a) was taken as a cutting point as well.

By the end of this step, each extracted MTA skeleton segment is either broken into piecewise linear subsegments or is considered to be a linear segment by itself.
5.3 Interpolation of Vessel Edges

In this work, Canny’s edge detection method is used to detect initial edge-pixel candidates in the inverted green-channel of the original image. Canny’s method detects edges by looking for local maxima in the gradient of the given gray-scale image. The gradient is obtained using the derivative of a Gaussian filter. The Canny method uses two threshold values to detect strong and weak edges. Pixels belonging to weak edges are included only if they are connected to strong edge pixels. As a result, the outcome of Canny’s thresholding step is less prone to error due to noise as compared to other edge detection methods. However, given the low spatial resolution of the TROPIC images, the results of Canny’s method provide vessel edges that are jagged and suffer from sampling errors. Considering the average width of vessels in pediatric cases of about 90 µm [121], the low spatial resolution of the TROPIC images, and small variations between normal and abnormal MTA width, it is crucial to detect and represent the vessel edges as accurately as possible and consequently obtain precise width measurements.

In order to represent vessel edges accurately, for every linear segment or subsegment of the extracted MTA, two separate sets of nearest edge pixels (in Canny’s output) to each skeleton pixel on both sides were automatically selected. Two first-order polynomials were fitted to the two sets of edge-pixel candidates in order to obtain accurate, continuous-form representations of the two edges as $Y_{e_{1,2}} = m_{e_{1,2}}X_{e_{1,2}} + C_{e_{1,2}}$, where the subscripts $e_{1,2}$ indicate edge lines 1 and 2, respectively, and the variables $m_{e_{1,2}}$ and $C_{e_{1,2}}$ their corresponding slopes and $y$-intercepts. Such a continuous-form representation of vessel edges allows for subpixel measurement of MTA width.

5.4 Measurement of MTA Width

The width of a vessel is defined as the distance along the normal to the skeleton between the two adjacent edges. Based on the Gabor-angle response, i.e., vessel orientation, the slope of
the normal, \( n_i \), at a given skeleton pixel, \( i \), is obtained as \( m_{n_i} = -1 / \tan \phi(i) \), where \( \phi(i) \) is the Gabor-angle response at the given skeleton pixel. The normal line is then obtained using the coordinates of the given skeleton pixel, \((x_i, y_i)\), and its corresponding slope, as \( Y_{n_i} = m_{n_i}X_{n_i} + C_{n_i} \), where \( C_{n_i} = y_i - (m_{n_i}x_i) \) is the y-intercept.

Given the continuous-form representation of the two edges (also in slope-and-y-intercept form) and the normal lines along a linear vessel segment/subsegment, it is possible to obtain the width at each skeleton pixel precisely as the Euclidean distance between the two exact points of intersection between the normal line and each edge line. At a given skeleton pixel, \( i \), the coordinates of the two points of intersection are defined as \( x_{i1,2} = (C_{n_i} - C_{e_{1,2}}) / (m_{e_{1,2}} - m_{n_i}) \) and \( y_{i1,2} = m_{e_{1,2}}x_{i1,2} + C_{e_{1,2}} \). The vessel width at the given pixel is computed as \( w_i = \sqrt{(x_{i1} - x_{i2})^2 + (y_{i1} - y_{i2})^2} \).

### 5.5 Results

Figures 5.4 and 5.5 demonstrate the application of the proposed methods for measurement of the width of the MTA to a normal case from the TROPIC database. The procedure selects the skeletons of the STA and ITA separately as shown in parts (d) to (g) of Figure 5.4. Even though both the extracted STA and ITA look linear at first glance, it can be seen from the results in part (i) that the originally extracted arcades are, indeed, made of up of several linear segments and are not linear by themselves. The linear skeleton segments of the MTA [part (i)] and the vessel-edge image [part (j)], are used to obtain the interpolated edges and ultimately the width measurements of the STA and ITA as illustrated in Figure 5.5.

Figures 5.6 and 5.7 illustrate the application of the methods to a case diagnosed with plus disease. Visual comparison of the width lines illustrates that the MTA is thicker as compared to the normal case in Figure 5.4; this observation is confirmed by the average MTA width measured for the two images (101.2 \( \mu m \) for the normal case compared to 146.8 \( \mu m \) for the plus case).
Figure 5.4: (a) The original color image 2405 of the TROPIC database that shows no signs of plus disease. (b) Gray-scale, inverted green-channel image used to obtain (c) the Gabor-magnitude-response image. (d) and (e) Horizontally restricted and gamma-corrected Gabor-magnitude-response images of the superior and inferior parts, respectively. Skeleton images of the (f) superior and (g) inferior parts obtained after binarization of the images in parts (d) and (e), respectively, and removal of the ONH region as well as the boundary of the ONH. (h) The extracted MTA skeleton obtained using the proposed algorithm. (i) Results of obtaining linear subsegments of the extracted MTA skeleton shown in part (h). (j) Horizontally restricted result of edge detection using Canny’s method as applied to the image in part (b). (k) Illustration of the calculated vessel widths for the entire MTA. The average calculated MTA width for this image is 101.2 \( \mu m \) (3.37 pixels). The images in parts (f) to (i) have been dilated using a disk of radius one pixel for better illustration. See also Figure 5.5.
Figure 5.5: Illustration of the calculated vessel widths for (a) STA (enlarged) and (b) ITA (enlarged), respectively, of the image in part k of Figure 5.4. The length of each normal line (in blue) represents the vessel width at that exact location.

Figure 5.8 (a) illustrates the steps used in statistical analysis of the Gabor-angle response to segment the extracted skeleton into linear parts as indicated in Section 5.2.2. The analysis is shown only for an extracted STA segment [part (b) of the same figure] corresponding to the image in Figure 5.4 (h). The resulting subsegments shown in part (c) are all considered to be linear. Note that the first/last two pixels in each resulting linear subsection are excluded from width measurement and are not shown in part (c) of the figure.

Table 5.1 presents the statistics of MTA width measurements obtained for cases with and without plus disease in the TROPIC database. The average width of the MTA in the images that show signs of plus disease is about 15 µm higher than the average width of the MTA in the normal cases. The median of the width measurements shows a larger difference between the two classes (about 22 µm); this is likely due to the fact that the median is less sensitive to the presence of outliers as compared to the mean. The differences between the mean and median MTA width measurements for all 91 normal images as compared to the mean and median measurements for the 19 images with plus disease were found to be statistically extremely significant with $p < 0.001$ in both cases, using the Wilcoxon rank-sum
Figure 5.6: (a) The original color image 4402 of the TROPIC database that shows signs of plus disease. (b) Gray-scale, inverted green-channel image used to obtain (c) the Gabor-magnitude-response image. (d) and (e) Horizontally restricted and gamma-corrected Gabor-magnitude-response images of the superior and inferior parts, respectively. Skeleton images of the (f) superior and (g) inferior parts obtained after binarization of the images in parts (d) and (e), respectively, and removal of the ONH region as well as the boundary of the ONH. (h) The extracted MTA skeleton obtained using the proposed algorithm. (i) Results of obtaining linear subsegments of the extracted MTA skeleton shown in part (h). (j) Horizontally restricted result of edge detection using Canny’s method as applied to the image in part (b). (k) Illustration of the calculated vessel widths for the entire MTA. The average calculated MTA width for this image is 146.8 µm (4.89 pixels). The images in parts (f) to (i) have been dilated using a disk of radius one pixel for better illustration. See also Figure 5.7.
Figure 5.7: Illustration of the calculated vessel widths for (a) STA (enlarged) and (b) ITA (enlarged), respectively, of the image in part (k) of Figure 5.6.
	est [285][286]. A total of 13,878 width measurements were obtained over the 110 images, leading to 126 measurements per image on the average.

Table 5.1: Mean, SE, median, minimum (min.), and maximum (max.) of MTA width values measured for cases with and without plus disease using the proposed methods. All values are in $\mu m$.

<table>
<thead>
<tr>
<th>Class</th>
<th>MTA Width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SE)</td>
</tr>
<tr>
<td>Without Plus (n = 91)</td>
<td>110.6 (1.94)</td>
</tr>
<tr>
<td>With Plus (n = 19)</td>
<td>125.0 (3.97)</td>
</tr>
</tbody>
</table>

The MTA extraction algorithm failed to detect all or most of a half arcade (STA or ITA, but not both) in a total of seven images out of the 110 analyzed. This is mainly due to poor quality images and/or lack of contrast between vessels and the background. See Section 5.7 for more discussion on the quality of the TROPI C images.

The $p$-values indicating the statistical significance between the mean MTA width measurements for various stages of ROP, not including any case with plus disease, were obtained to test the hypothesis that there is a range of MTA width changes that occurs prior to plus
Figure 5.8: (a) The Gabor-angle response along a sequenced skeleton, its associated MAD measure obtained over a 7-pixel-long window, and the analysis of the MAD measure in terms of its derivatives are plotted against the sequence index. The resulting cutting points are shown in the last row of the same plot. (b) The extracted STA segment on which the statistical analysis of part (a) is performed. (c) The result of segmenting the skeleton in part (b) into linear subsegments based on the cutting points indicated in part (a). Note that the first/last two pixels of each resulting subsegment are dropped and not used for any further measurements.
disease [40]. The results are presented in Table 5.2. The differences between the mean MTA width measurements of normal (stage 0, \( n = 30 \)), stage 1 (\( n = 30 \)), and stage 2 (\( n = 23 \)) ROP cases as compared to stage 3 (\( n = 8 \)) ROP were all found to be statistically highly significant (\( p < 0.01 \)).

Table 5.2: \( p \)-values indicating the statistical significance of the differences of mean MTA width values measured between images of various stages of ROP (S0 to S3). All cases with plus disease (stages 2 and 3) were excluded from this analysis. Cases without ROP are labeled as S0. \( ** p < 0.01 \).

<table>
<thead>
<tr>
<th></th>
<th>S1 (( n = 30 ))</th>
<th>S2 (( n = 23 ))</th>
<th>S3 (( n = 8 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0 (( n = 30 ))</td>
<td>0.603</td>
<td>0.397</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>S1 (( n = 30 ))</td>
<td>−</td>
<td>0.641</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>S2 (( n = 23 ))</td>
<td>−</td>
<td>−</td>
<td>&lt; 0.01**</td>
</tr>
</tbody>
</table>

The ROC curve provides the overall sensitivity and specificity of the diagnostic feature used, as mentioned in Section 3.5. The most suitable operating point is often taken as the point on the ROC curve that is closest to the point (0, 1). Figure 5.9 shows the binormal approximation of the ROC curve obtained using the mean MTA width measure, via the ROCKIT software [239], to distinguish between cases without and with plus disease. The red error bars indicate the confidence interval at a given point on the ROC curve. The mean and median width measurements obtained using the proposed methods both provided \( A_z = 0.75 \).

Table 5.3 presents the results of applying the bootstrapping approach as explained in Section 3.5.4. The results indicate similar average AUC and mean values as compared to the result in Table 5.1 and Figure 5.9 with a narrow 95% confidence band. The results of bootstrapping indicate that there is confidence in the estimated values for the average MTA width for cases with and without plus disease, as well as in discrimination between the two classes using ROC analysis. As mentioned in Section 3.5.3, the ROC analysis procedure used for the bootstrapping approach provides the AUC values as opposed to the \( A_z \) values provided by ROCKIT; hence, there is a small difference between the \( A_z \) value related to
Figure 5.9: ROC curve (blue line) presenting the diagnostic accuracy of the mean MTA width measure obtained using the proposed methods to distinguish between cases without and with plus disease with $A_z = 0.75$. The red error bars indicate the confidence interval at a given point on the ROC curve. Two possible operating points could be obtained based on the ROC curve; one point is selected using the shortest distance to the point $(0, 1)$ and another point is selected to show the trade off between having a higher sensitivity and a lower specificity. The optimal point is the point on the ROC curve that is closest to the point $(0, 1)$ with sensitivity $= 0.73$ and specificity $= 0.66$. The second operating point provides a higher sensitivity value of 0.90 at the expense of a lower specificity of 0.45. The trade off between sensitivity and specificity must be determined based on the clinical application.

Figure 5.9 and the AUC values in Table 5.3. The numbers of trials out of 500 for which $p$-values indicated statistically significant differences between the cases with and without plus disease are also indicated in Table 5.3.

5.6 Comparative Analysis

Table 5.4 presents comparative analysis of the results of studies in the literature that have performed diagnosis of plus disease using vessel width measurements in retinal images of
Table 5.3: Values of the mean of the area under the discrete ROC curve (AUC) and their 95% symmetric confidence interval $CIs$ obtained in the discrimination of 100 cases with plus disease against 100 cases without plus disease, all randomly selected with replacement and repeated 500 times. The confidence intervals were obtained by assuming a standard normal distribution for the $A_z$ values based on the results of Jarque-Bera test for normality of a distribution [242]. In the last column, $M^{***}$ means that, out of the 500 trials, the results indicated statistically extremely significant differences in the $M$ trials with $p < 0.001$; $N^{**}$ means that the differences were highly statistically significant in $N$ trials with $0.001 \leq p < 0.01$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC, Mean, $[CIs]$</th>
<th>Without Plus, Mean (SE)</th>
<th>With Plus, Mean (SE)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width</td>
<td>0.744, [0.741, 0.747]</td>
<td>110.46 (0.086)</td>
<td>125.07 (0.077)</td>
<td>491***, 8**</td>
</tr>
</tbody>
</table>

preterm infants. It should be noted that the sensitivity and specificity values of other studies provided in Table 5.4 have been calculated using criteria and assumptions other than the common practice as defined in Section 3.5. The details of the results of each study are described in the following paragraphs.
Table 5.4: Comparative analysis between the present work and similar state-of-the-art studies available in the literature. If a particular parameter or result was not specified in a study, it is denoted by NA (not available). Manual editing refers to manual correction of the results by the user. Manual input refers to one or more manually selected parameters and/or inputs. The $p$-values indicate the statistical significance of the difference between the mean vessel width of the cases without plus disease as compared to the cases with plus disease. Sen. and Spe. stand for sensitivity and specificity, respectively. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Manual Editing and/or Input</th>
<th>MTA and/or MNA Analyzed</th>
<th># of cases without/with plus disease</th>
<th>$A_z$</th>
<th>$p$-value</th>
<th>Sen.</th>
<th>Spe.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heneghan et al. [121]</td>
<td>Both</td>
<td>Both</td>
<td>12/11</td>
<td>NA</td>
<td>&lt; 0.01**</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Wallace et al. [122]</td>
<td>Both</td>
<td>Both</td>
<td>11/5</td>
<td>NA</td>
<td>NA</td>
<td>1.00</td>
<td>0.55</td>
</tr>
<tr>
<td>Gelman et al. [123]</td>
<td>Both</td>
<td>MTA</td>
<td>20/12</td>
<td>0.76</td>
<td>&lt; 0.01**</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gelman et al. [102]</td>
<td>Both</td>
<td>Both</td>
<td>21/13</td>
<td>0.82</td>
<td>NA</td>
<td>0.76</td>
<td>0.76</td>
</tr>
<tr>
<td>Koreen et al. [103]</td>
<td>Both</td>
<td>Both</td>
<td>14/6</td>
<td>0.79</td>
<td>&lt; 0.05*$</td>
<td>0.50</td>
<td>0.86</td>
</tr>
<tr>
<td>Thyparampil et al. [127]</td>
<td>Both</td>
<td>Both</td>
<td>61/13</td>
<td>0.79</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Present work</td>
<td>Input</td>
<td>MTA</td>
<td>91/19</td>
<td>0.75</td>
<td>&lt; 0.001***</td>
<td>0.73</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Heneghan et al. [121] found the difference between the total average vessel width of cases without ROP as compared to cases that progressed to threshold ROP and required treatment to be statistically highly significant. Since distinguishing between venules and arterioles is a difficult task, Heneghan et al. combined the measurements from both arterioles and venules to obtain the average thickness values. The associated analysis was limited to an annular region between two circles of radii one and two ONHW centered at the center of the ONH. The methods required manual selection of two appropriate threshold values to be used for hysteresis thresholding, as well as manual selection of the vessel segments to be analyzed. The user was also required to correct manually the path indicating the vessel segment to be analyzed. The study did not indicate values of sensitivity and specificity using only the width measurements.

The study conducted by Wallace et al. [122] did not provide mean, STD, and $p$-values for the obtained measurements. The sensitivity and specificity values provided were based on a simple diagnostic decision-making criterion. Wallace et al. obtained measures of width for the standard image, and if an image had equal or greater width measures in at least two quadrants, the patient was diagnosed with plus disease. Their methods required manual correction and selection of the major vessel to be analyzed by the user. Wallace et al. did not provide an $A_z$ value.

The studies conducted by Gelman et al. [102,123], Koreen et al. [103], and Thyparampil et al. [127] used the software package RISA (see Section 2.2.1). In all of the four studies, the center of the ONH was marked manually and the images were cropped to a circle centered at the center of the ONH with radius $= 2 \times \text{ONHW}$ in a preprocessing step. In studies where sensitivity and specificity are indicated in Table 5.4, the values were determined by finding the intersection point between the sensitivity and specificity curves, plotted separately as functions of the ratio of correctly identified vessels against the actual width measures. Note that such a measure does not classify the image, but classifies each single vessel. A drawback
of RISA is that it requires the vessel segment to be analyzed to have at least one branching point. RISA requires manual correction of the detected branching points, as well as manual input regarding which vessels are to be analyzed and whether they are arterioles or venules. RISA provides a single estimated mean value for each selected segment of a vessel and not a width measure at each pixel on the vessel segment. Such computation of width does not allow for analysis of the associated statistics of the width of a vessel segment over its entire length. In all of the four studies, median values of mean width measures were used to compare the results in terms of statistical significance between the two classes.

5.7 Discussion

Retinal images of preterm infants vary substantially in terms of pigmentation, contrast, and quality. Many of the studies that have dealt with measurement of vessel dilation in ROP have selected only images with high quality and contrast, and hence have limited the number of available cases used in their analysis. This work is based on one of the largest sets of preterm retinal fundus images in the literature for such a study, and is not limited to only images with high quality and contrast. Figures 5.10 and 5.11 show examples of images from the TROPIC database illustrating a wide range of pigmentation, vessel-to-background contrast, and overall quality (sharpness). The proposed methods are robust and work well in the presence of large variations in the characteristics of the images used, as demonstrated by the results, which show a statistically highly significant difference in the width of the MTA between the two classes and good accuracy in the detection of plus disease. However, as can be seen in parts (a) to (d) of Figure 5.11, image artifacts and low quality can cause the proposed algorithm either to miss parts of an arcade, or not detect vessel edges properly. Although such inaccuracies are not common or severe in the present work, methods need to be developed to address such challenges because, in real-world applications, it may not be possible to obtain only high-quality and sharp pediatric retinal fundus images. It would be
of interest to test the proposed methods with more images, including more cases with plus disease, as well as against combined diagnosis of the same set of images by multiple experts. It should be noted that the MTA width measurements obtained for all of the images shown in Figures 5.10 and 5.11 were included in the analysis performed in the present work.

![Image](image-url)

**Figure 5.10:** (a) An image with a high vessel-to-background contrast, which leads to accurate width measurement as shown in (b). (c) An image possessing low vessel-to-background contrast for which (d) the proposed methods were able to extract and measure accurately the width of the STA.

The thresholding step has an effect on the skeleton selection step. The wide range of pigmentation and contrast that exists in preterm retinal images could cause the thresholding
Figure 5.11: (a) An image showing a dark patch on the superior side near the ONH, which leads to (b) the thresholding algorithm failing to extract the entire STA. (c) An image with poor quality and contrast for which, (d) despite the low quality, the proposed methods were able to extract the ITA; however, due to low vessel-to-background contrast and the presence of an artifact near the ONH, Canny’s method incorrectly detected one of the edges, which led to inaccurate width measures close to the ONH.
step not to provide the best possible results as shown in part (d) of Figure 5.11. Analysis of various automatic thresholding methods and their combinations may lead to more suitable methods for this purpose [155].

In case there are local variations in the illumination or brightness of an image, by dividing the image into its superior and inferior parts and normalizing each part separately as done in the present work, it can be expected that both the STA and ITA will have the highest intensity values in their respective Gabor-magnitude-response images. Furthermore, in case the average intensity values of the two half-arcades are not close to each other, they will not affect each other when choosing an appropriate threshold.

The $A_z$ values obtained in various studies as listed in Table 5.4 are within a relatively small range (0.75–0.82). It is questionable whether such small differences (maximum = 0.07) are statistically significant. The methods proposed in this work perform as well as the state-of-the-art methods available in the literature without the need for user correction of the results and discrimination in selection of images to be analyzed based on quality, which all of the other mentioned methods required.

Except for the step requiring the location of the center of the ONH, the proposed methods are automated. In the future, the method of Rangayyan et al. [224], which performs phase portrait analysis of the Gabor-angle information, could be adapted for pediatric retinal fundus images to detect automatically the center of the ONH, which could make the entire procedure of vessel width measurement fully automated.

The design factor to segment the extracted MTA into linear subsegments is especially advantageous in case of vessel edge interpolation. When interpolating data points, it is of importance to know the order of the required fit. MTA skeleton segments can vary from being linear to highly tortuous. Given a skeleton segment with varying orientation, or lack there of, and consequently the same mirrored orientation in the corresponding vessel edges, it is not appropriate to use a single order for interpolation of all vessel edges, as done in
previous studies [199]. By breaking a given skeleton segment into linear parts, it is expected that the edges of the resulting vessel subsegments are also linear; hence, the order of the fit used to interpolate the two vessel edges can always be set to unity.

The methods that use the center-line of a vessel to determine its width require the center-line to be precisely in the middle of the vessel, which is arguably improbable when using automated image processing techniques, and challenging even when manually drafting the center-line. The use of the skeleton of the vessel being analyzed, which does not need to be positioned at the exact center of the vessel, obviates the difficulties associated with the detection and use of the center-line. The use of the Gabor-angle response to determine the slope of the normal, and consequently compute vessel width measures, is advantageous since the Gabor-angle response provides the dominant orientation of a vessel at a given point, which does not need to be at the center of a vessel.

The design factor of the proposed algorithm that removes parts of the MTA at branching points may, at first, seem disadvantageous as it results in slightly fewer points on the MTA being available for width measurement; however, measuring the width of a vessel at branching points could lead to errors since such measures may not represent the width of a single branch but instead larger regions that include parts belonging to both branches of the vessel. The proposed design factor helps to reduce such errors in measurement of the width of the MTA.

The average width measurements provided by Heneghan et al. [121] were obtained over all branches of venular and arteriolar vessels on the nasal side as well as the temporal side. The present study provides average width measurements over only the thickest branch (no child branches) of the temporal-venular vessel, which is expected to be, on the average, thicker than the average width of both venules and arterioles combined; this point is confirmed by the larger measurements provided in Table 5.1 as compared to the results of Heneghan et al.

The difference between the median widths of cases with and without plus disease was found to be less than one pixel in the present work, which is comparable to the median
differences found in the work of Gelman et al. [102, 123] and the mean differences provided by Heneghan et al. [121].

The range of changes in the width of retinal vessels that is observed in later stages of ROP prior to the presence of plus disease is designated as preplus [40]. Based on this observation, it can be expected that an increase in vessel width should be observed as the ROP stage progresses prior to the diagnosis of plus disease. Indeed, as indicated in Table 5.2, the differences in vessel width between stages 0 and 3 ROP, stages 1 and 3 ROP, and stages 2 and 3 ROP were found to be statistically highly significant, when excluding cases with plus disease. It would be of interest to test this observation further and strengthen the statistical analysis using more cases that show no signs of plus disease.

Most of the other available studies in the literature have quantified the changes in the width of both the MTA and the MNA in the presence of plus disease. It would be of interest to verify if the width of the MNA on its own shows significant changes in the presence of plus disease using the proposed methods.

Since the retina and its vasculature are not fully developed in preterm infants, it would be of interest to verify if there are any correlations between the gestational age and birth weight of the infants and the MTA width.

5.8 Remarks

The current chapter presented methods for the detection, extraction, and measurement of the width of the MTA in retinal fundus images of preterm infants. The results indicate that there is a statistically highly significant difference between the MTA width of cases without plus disease as compared to those with plus disease. The obtained width measure shows good accuracy in the diagnosis of plus disease with $A_z = 0.75$. The results show potential in CAD and clinical management of plus disease and ROP.
Chapter 6

Measurement of the Openness of the MTA

As mentioned in Section 1.3.2, a change in the openness of the MTA has been cited as a sign of ROP, PDR, and myopia. As shown in previous related studies [45, 287, 288], parabolic modeling of the MTA can be useful in analyzing changes to the openness of the MTA for the diagnosis of PDR [45], plus disease [288], and ROP [287].

Fledelius and Goldschmidt [55] reported a decrease of more than 4° in the arcade angle in 25% (6 of 24) of the cases with high and stable myopia, and in 60% (12 of 20) of the cases with high and progressive myopia. The change in the arcade angle of the progressive myopia group as compared to the stable myopia group was shown to be statistically highly significant ($p < 0.01$). For the high and progressive myopia group, the change in the arcade angle was shown to be correlated with the degree and increase of myopia.

Wilson et al. [110] reported a high degree of interocular symmetry with a mean total arcade angle of 82° for both eyes. They indicated that interocular asymmetry of above 14° to 20° between the two eyes of a patient should be treated with suspicion [110]. A significant level of acuteness in the IAA of the left eye was associated between stage 0 and 1, stage 1 and 2, and stage 1 and 3 of ROP (higher numbers indicate higher severity of ROP) [110].

Wong et al. [41] reported that the nasal arcade angles show no statistically significant differences between normal cases and ROP of various stages. The angles of the temporal venules and arterioles were found to have statistically significant differences between normal cases and stage 3 ROP [41]. However, when all stages of ROP were combined, only the angle of the temporal arterioles indicated statistically significant difference as compared to the normal cases [41].

The aim of the methods described in the present chapter is to assess the diagnostic power
of the openness of the MTA in discrimination between cases with and without plus disease. The openness of the MTA is quantified via parabolic modeling of the MTA as well as by measuring the TAA, using the method of Wong et al. [41], for comparative analysis.

6.1 Procedure for Measurement of the Arcade Angle

The present work employs the principal concepts of the method of Wong et al. [41] for the measurement of the TAA via a GUI [45] for comparative analysis. The semiautomated procedure for measurement of the TAA starts by prompting the user to mark the center of the ONH, after which a circle with a radius that is specified by the user is drawn on the image [45]. The procedure then prompts the user to mark the point of intersection of the circle with the superior venule; the same is repeated for the inferior venule. The TAA is measured as the angle between the three manually marked points, where the center of the ONH is the vertex of the angle [45]. In the present work, circles of radii $r = 60$ and 120 pixels were used to measure the TAA. The values for the radii were selected based on the values provided in the work of Wong et al. [41]. The method of Wilson et al. [110] (see Section 2.2.2) was found to be less accurate [283] as compared to the method of Wong et al. and was not employed in the present work.

6.2 Detection and Modeling of the MTA

In the present work, Gabor filters are used for detection of the MTA [164] using a large value for the parameter $\tau$ (see Section 4.1.1). The GHT [278] for the detection of parabolic forms is used with the result of the filtering step to perform single- and dual-parabolic modeling of the MTA, STA, and ITA [278], as described in Section 4.3. The proposed methods are implemented via the GUI, which facilitates user input for selection of the required parameters for detection and modeling of the MTA [45].

The gray-scale magnitude-response image of the Gabor filters is binarized using a sliding
threshold via the GUI and then the binarized image is skeletonized to obtain a single-pixel-thick representation of the vessels. For each image, a suitable threshold is selected to obtain a binary image containing only the MTA. The user can specify the maximum number of connected pixels to be removed to eliminate small vessel segments that may remain after the thresholding step.

The user is required to indicate if the current image is an image of the left eye or the right eye for the GHT modeling procedure. The user is then prompted to mark the approximate location of the ONH in a separate window. Given the average width of the ONH (ONHW) of about 1.05 mm in preterm infants [7] and the spatial resolution of the RetCam images, the vessel skeleton map used as input to the GHT modeling procedure is horizontally restricted from 0.25×ONHW in the nasal direction to 2×ONHW in the temporal direction with respect to the ONH center to enable the modeling procedure to fit a parabola to the MTA close to the posterior pole.

6.3 Evaluation of the Diagnostic Performance

The $p$-value, indicating the statistical significance of the differences between the values of the openness parameters of the parabolic models ($a_{MTA}$, $a_{STA}$, and $a_{ITA}$, see Section 4.3) as well as the TAA for cases without plus disease as compared to the values of the same for the cases with plus disease were obtained via the Wilcoxon rank-sum test in MATLAB [285,286]. To assess the diagnostic performance of the parameters derived, ROC analysis was performed using ROCKIT [239] (see Section 3.5.3). The values of the area under the ROC curve, $A_z$, and the associated asymmetric 95% confidence intervals ($CI_a$) were obtained from ROCKIT.

6.4 Results

Figures 6.1 and 6.2 show the results of single- and dual-parabolic modeling as well as the measurement of the TAA using circles of radii $r = 60$ and 120 pixels for two images from the
TROPIC database; one image contains no signs of plus disease (Figure 6.1) and the other shows signs of plus disease (Figure 6.2). The ITA portion of the dual-parabolic model is providing an accurate fit close to the ONH, whereas the MTA model in part (b) is providing an average fit to the ITA. The TAA obtained using the circle of radius \( r = 120 \) pixels is providing a measure close to the macular region, whereas the TAA obtained using the circle of radius \( r = 60 \) pixels is providing a measure close to the posterior pole. The parameters of Gabor filters used for detection of vessels were \( \tau = 10 \) pixels, \( l = 2 \), and \( K = 30 \).

Table 6.1 shows the results of statistical analysis of the single- and dual-parabolic model parameters as well as the TAA measures. The results obtained using 91 cases without plus disease as compared to 19 cases with plus disease indicate statistically highly significant differences for the TAA measures obtained using both circles of radii \( r = 120 \) and 60 pixels as well as the openness obtained using single-parabolic modeling (\( |a_{MTA}| \)). The openness parameters obtained using dual-parabolic modeling show statistically significant differences between cases with and without plus disease. The areas under the ROC curves, \( A_z \), for the TAA with \( r = 60 \) pixels and the \( |a_{STA}| \) parameter are \( \geq 0.70 \), indicating satisfactory performance in classification or diagnosis.

Table 6.1: Values of the area under the ROC curve (\( A_z \)), their 95\% (\( \alpha = 0.025 \)) asymmetric confidence interval (\( CI_a \)), and \( p \)-values obtained in the discrimination of 19 cases with plus disease versus 91 cases without plus disease using the TAA with radii of \( r = 60 \) and 120 pixels, the parameter of the single-parabolic model (\( |a_{MTA}| \)), and the parameters of the dual-parabolic models (\( |a_{STA}| \) and \( |a_{ITA}| \)). The mean and the SE of each parameter for cases with and without plus disease are also provided. (\(^* p < 0.05 \) and \(^{**} p < 0.01 \).)

<table>
<thead>
<tr>
<th>Parameter ( r )</th>
<th>( A_z ), ( CI_a )</th>
<th>Without Plus, ( n = 91 )</th>
<th>With Plus, ( n = 19 )</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( TAA_{r=60} )</td>
<td>0.73, [0.589, 0.844]</td>
<td>132.14 (1.55)</td>
<td>119.19 (3.86)</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>( TAA_{r=120} )</td>
<td>0.69, [0.560, 0.805]</td>
<td>115.25 (1.53)</td>
<td>104.88 (3.12)</td>
<td>&lt; 0.01**</td>
</tr>
<tr>
<td>(</td>
<td>a_{MTA}</td>
<td>)</td>
<td>0.67, [0.513, 0.801]</td>
<td>42.31 (2.55)</td>
</tr>
<tr>
<td>(</td>
<td>a_{STA}</td>
<td>)</td>
<td>0.70, [0.560, 0.808]</td>
<td>50.44 (5.51)</td>
</tr>
<tr>
<td>(</td>
<td>a_{ITA}</td>
<td>)</td>
<td>0.66, [0.511, 0.790]</td>
<td>56.51 (5.65)</td>
</tr>
</tbody>
</table>
Figure 6.1: (a) Image 1701 of the TROPIC database, which does not show any signs of plus disease. (b) Single-parabolic model with $a_{MTA} = 60$. (c) Dual-parabolic model with $a_{STA} = 66$ and $a_{ITA} = 40$. TAA measured using circles of radii (d) $r = 120$ pixels with TAA = 128.14° and (e) $r = 60$ pixels with TAA = 141.99°. The vertical lines in yellow show the extent of the arcade used in the modeling procedure.
Figure 6.2: (a) Image 3602 of the TROPIC database, of a patient diagnosed with plus disease. (b) Single-parabolic model with $a_{MTA} = 14$. (c) Dual-parabolic model with $a_{STA} = 12$ and $a_{ITA} = 13$; both models are providing fits close to the posterior pole. TAA measured using circles of radii (d) $r = 120$ pixels with $TAA = 94.38^\circ$ and (e) $r = 60$ pixels with $TAA = 100.28^\circ$. The vertical lines in yellow show the extent of the arcade used in the modeling procedure.
Table 6.2 presents the results of applying the bootstrapping approach as explained in Section 3.5.4. The results indicate similar average AUC and mean values as compared to the results in Table 6.1. However, the 95% confidence bands are fairly narrow, indicating that there is a high level of confidence in the estimated values for the average openness for cases with and without plus disease, as well as in discrimination between the two classes using ROC analysis. The average STA and ITA openness values for each class (91 vs. 19 cases) have relatively large SEs, as stated in Table 6.1. However, based on much smaller SEs obtained using the results of bootstrapping, it may be concluded that the original set of values obtained (Table 6.1) can be considered to be representative of the population distribution that they belong to. As mentioned in Section 3.5.3, the ROC analysis procedure used for the bootstrapping approach provides the AUC values as opposed to the $A_z$ values provided by ROCKIT; hence, there is a small difference between the $A_z$ values in Table 6.1 and the AUC values in Table 6.2. The numbers of trials out of 500 for which $p$-values indicated statistically significant differences between the cases with and without plus disease are also indicated in Table 6.2.

Analysis of the openness measures related to images from the same eye of the same patient that progressed to plus disease, and in some instances to a higher stage of ROP, on average over six patients and eight imaging instances, showed decreases of 6.5, 4.3, 8.4, 4.4°, and 5.3° in the $|a_{MTA}|$, $|a_{STA}|$, $|a_{ITA}|$, TAA, and TAA parameters, respectively.

6.5 Discussion

This is the first study to quantify the effects of plus disease on the openness of the MTA, STA, and ITA, using semiautomated methods to perform single- and dual-parabolic modeling as well as measurement of the TAA for comparative analysis. In the present study, the diagnostic performance (in terms of $A_z$ or AUC, as shown in Tables 6.1 and 6.2) of the parameters of the single- and dual-parabolic models is comparable to that provided by the
Table 6.2: Values of the mean of the area under the discrete ROC curve (AUC) and their 95% symmetric confidence interval $CI_s$ obtained in the discrimination of 100 cases with plus disease against 100 cases without plus disease, all randomly selected with replacement and repeated 500 times. The confidence intervals were obtained by assuming a standard normal distribution for the $A_z$ values based on the results of Jarque–Bera test for normality of a distribution [242]. In the last column, $M^{***}$ means that, out of the 500 trials, the results indicated statistically extremely significant differences in the $M$ trials with $p < 0.001$; $N^{**}$ means that the differences were statistically highly significant in $N$ trials with $0.001 \leq p < 0.01$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC, Mean, $[CI_s]$</th>
<th>Without Plus, Mean (SE)</th>
<th>With Plus, Mean (SE)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAA$_{f=60}$</td>
<td>0.700, [0.697,0.703]</td>
<td>132.10 (0.068)</td>
<td>119.09 (0.074)</td>
<td>490^{*<strong>}, 9^{</strong>}</td>
</tr>
<tr>
<td>TAA$_{f=120}$</td>
<td>0.685, [0.682,0.688]</td>
<td>115.27 (0.066)</td>
<td>104.80 (0.058)</td>
<td>475^{*<strong>}, 23^{</strong>}</td>
</tr>
<tr>
<td>$</td>
<td>a_{MTA}</td>
<td>$</td>
<td>0.685, [0.682,0.688]</td>
<td>42.45 (0.109)</td>
</tr>
<tr>
<td>$</td>
<td>a_{STA}</td>
<td>$</td>
<td>0.660, [0.657,0.663]</td>
<td>50.63 (0.239)</td>
</tr>
<tr>
<td>$</td>
<td>a_{ITA}</td>
<td>$</td>
<td>0.643, [0.639,0.647]</td>
<td>56.56 (0.234)</td>
</tr>
</tbody>
</table>

TAA measures obtained based on the method of Wong et al. [41] via the GUI. However, the TAA measures provide better statistical performance as compared to the parameters of the parabolic models. All of the studied measures show a similar trend: there is a decrease in the openness of the MTA in the presence of plus disease. The decreasing trend in the openness of the MTA is also observed over time in patients who progressed to plus disease and/or higher stages of ROP.

Ells and MacKeen [289] illustrated that the changes that occur in the MTA may be dynamic as they alter the posterior architecture of the MTA over time. Based on this observation, it may be possible that the TAA measures proposed by Wilson et al. [110] and Wong et al. [41] may not accurately reflect such changes that occur over the entire posterior architecture of the MTA, as the TAA measures define the openness of the MTA based on only three points on the arcades. Furthermore, the TAA is sensitive to the exact position of the center of the ONH. The parabolic modeling procedure is dependent only on an approximate location of the ONH instead of the specific location of the center of the ONH [278].
The radius of the circle used for the measurement of the TAA in the work of Wong et al. [41] is not clearly defined or justified. This parameter needs to be formally related to a physiological measure, such as the average ONHW.

If the angle of the retinal raphe (the line passing through the center of the ONH and the fovea) is large with respect to the horizontal axis of the image, it could lead to a much larger openness parameter for one of the dual-parabolic models than the other. The retinal raphe angle may be corrected either manually or by using methods to detect the center of the ONH and the fovea. However, as shown by Chiang et al. [204], retinal fundus images of preterm infants typically lack a clear depiction of the fovea, which makes such analysis difficult.

The two parameters of the dual-parabolic models may be combined using pattern classification techniques to incorporate the independent information from both the STA and ITA models into classification methods. It may also be possible to estimate the retinal raphe as the principal axis [290] of the skeleton of the MTA and correct for the rotation that might exist in the image.

The large variations that could exist in the openness parameter of the parabolic models, as explained in Section 6.4, could be the reason for the high SE observed, which could also be the reason for obtaining large $p$-values for the results of parabolic modeling as shown in Table 6.1. However, given the narrow confidence intervals obtained using the results of bootstrapping (Table 6.2), it may be argued that high confidence could be placed on the estimated mean parameters for the AUC and the class statistics.

Upon close inspection, it becomes clear that, first, the STA and the ITA are asymmetric, and second, more accurate modeling of each arcade may be possible by applying models based on higher-order curves as compared to using parabolic (second-order) curves. A high-order curve fitting method may provide more accurate results in terms of modeling and parameterization of the MTA, at the cost of an increased number of parameters.
It has been observed that changes in the tortuosity and thickness of the vessels that occur in the presence of plus disease are dynamic and that analysis of such changes from one visit to another could lead to improved diagnosis and analysis of plus disease \[127\]. Indeed, a decrease in the openness of the MTA was observed in the present study in images of the same eyes of the same patients who progressed to plus disease. Further longitudinal analysis of this observation with more cases would be of interest.

Given that the results of the TAA with \( r = 60 \) pixels are providing the best discriminatory performance in the present study and that this angle is measured closer to the ONH as compared to the parabolic modeling procedure, it could be beneficial to restrict the GHT modeling procedure to fit a parabolic model closer to the ONH, i.e., more posteriorly than in the proposed method. It is possible that the changes that occur in the MTA are locally contained within the immediate area around the ONH, such as a circle with radius \( \approx 40 \) pixels centered at the center of the ONH, as opposed to 70 pixels \((2 \times \text{ONHW})\) as used for parabolic modeling in the present work.

Using the results of MTA tracking as explained and demonstrated in Chapter 5, the same single- and dual-parabolic modeling procedures were applied to the 110 tracked MTAs and ROC analysis was performed. The results, as assessed visually, showed close fits to the MTA. However, the results indicated poorer discrimination between cases with and without plus disease. This result may be further indication that the changes that occur in the MTA and are signified as a decrease in the angle of insertion of the MTA in the presence of ROP may be more complex and locally concentrated as previously observed \[40\].

Many of the previously conducted studies that have performed diagnosis of plus disease have used relatively small databases of images including even smaller numbers of cases with plus disease \[101,103,122,123\] than the present study. The number of cases (both with and without plus disease) used in the present study, although limited, are larger in comparison to the cited studies with a maximum of 92 cases and a minimum of 16. Inclusion of more
cases with plus disease will help to strengthen the related statistical analysis.

The methods used in the present study are semiautomated as are other published studies with a similar application [57]. The parameters of the methods used for detection and modeling of the MTA will need to be set automatically or derived adaptively based on the characteristics of each individual image.

The proposed methods for quantification of the openness of the MTA were also applied to a subset of images of the STARE database for the purpose of diagnosis of PDR [45]. The results indicated statistically highly significant differences between the openness of the MTA of the normal cases as compared to cases with PDR and provided $A_z = 0.87$ in diagnosis of PDR [45].

6.6 Remarks

In this chapter, procedures were presented for quantification of the openness of the MTA using parabolic modeling and an angular measure. It was demonstrated, for the first time, that the openness of the MTA decreases in the presence of plus disease. It should be noted that the changes to the MTA may be more posteriorly defined than the range used in the present work, which has previously been expected and observed by other researchers.
Chapter 7

Measurement of Vascular Tortuosity

In this chapter, a measure of tortuosity based on the orientation at each pixel of a vessel segment obtained during the vessel detection stage using Gabor filters is presented. The methods include the use of Gabor filters for detection of vessels as well as obtaining the dominant orientation of vessels at each pixel, and morphological image processing methods to segment vessels and obtain their associated skeletal representation. Furthermore, statistical analysis of the variations in the orientation of vessels is used to detect linear segments and the measure of angle-variation-based tortuosity (AVT) is developed to determine abnormally tortuous vessels. Finally, clinically relevant diagnostic-decision-making procedures are designed to assess the diagnostic power of vascular tortuosity alone in diagnosis of plus disease. The results are assessed in terms of sensitivity, specificity, $A_z$, and $p$-value. The obtained results are compared with the results of similar studies in the literature.

7.1 Skeletal Representation of Vasculature

The first step in quantitative computer-aided analysis of retinal vasculature is to obtain a skeletal representation of the vessels. In the present work, oriented-feature detectors (Gabor filters) were used initially to detect the vasculature and represent the same in terms of vessel-strength (Gabor-magnitude response) and vessel-orientation (Gabor-angle response) images \[164\]. The vessel-strength image is a gray-scale representation of the vessel detection measure and was used to obtain a vascular skeleton. The vessel-orientation image, which provides the dominant vessel orientation at each pixel, was used to compute a measure of tortuosity for segments of the vascular skeleton.

The vessel-strength image was first normalized to contain values in the range $[0, 1]$ and
then binarized using a threshold slider operated by the user (F. Oloumi) via a GUI to segment vessels from the background. Next, 8-connected segments shorter than a certain maximum length as specified by the user, expected to be caused by choroidal vessels, were removed using the morphological area-open procedure. Finally, the segmented binary image of vessels was skeletonized using morphological image-processing methods.

The approximate center of the ONH marked by the user (F. Oloumi) was used to remove an elliptical area, centered at the center of the ONH, from the skeleton image. The height and width of the ellipse were set based on the average and STD of ONHW and ONH height (ONHH) in preterm infants [7] as 44 and 60 pixels (mean plus two times the STD in each case), respectively. Next, spurs of up to 5 pixels in length were removed from the skeleton image. The branching points on the skeleton were then automatically identified and an area of $3 \times 3$ pixels, centered at each branching point, was removed from the skeleton image to separate vessel segments for further analysis.

### 7.2 Detection of Linear Vessel Segments

Each disconnected vessel segment obtained in the previous step was automatically identified and numbered for further analysis. The vessel-orientation information at each pixel for each identified segment was analyzed to separate it into linear and nonlinear subsegments. Such analysis requires the correct sequential order of pixels from one end point to the other of each segment, which was automatically obtained.

To detect linear portions of the extracted and sequenced skeleton segments, the statistical measure of MAD [284] (see Section 5.2.2) was obtained in a window of length 7 pixels applied to the sequenced vessel-orientation information, $\phi$. The MAD measure was normalized by setting all nonzero values equal to 1. A pixel on a given segment is marked as being a linear part of a subsegment only if all normalized MAD measures in a 7-pixel-long window centered at the current pixel are zero; a skeleton image of only linear subsegments was obtained in
this manner. In addition, a skeleton image of nonlinear vessel subsegments was obtained by subtracting the image of the linear subsegments from the original skeleton image.

### 7.3 Quantification of Vascular Tortuosity

A local tortuosity index (LTI) [291] based on vessel orientation was obtained at every pixel as

\[
\text{LTI}(p) = \frac{1}{2} \left\{ \left| \sin[\phi(p) - \phi(p-1)] \right| + \left| \sin[\phi(p) - \phi(p+1)] \right| \right\},
\]

(7.1)

where \( p \), \( p - 1 \), and \( p + 1 \) are the current, previous, and next pixels, respectively, along a sequenced nonlinear vessel subsegment. This formulation ensures that the LTI has a theoretical maximum value of 1 and a minimum of 0. The AVT for a given vessel segment is defined as the average of the LTI values along the segment as

\[
\text{AVT} = \frac{1}{P} \sum_{p=1}^{P} \text{LTI}(p),
\]

(7.2)

where \( P \) is the total number of pixels in a given segment. The AVT is normalized, so that it provides a maximum value of 1 and a minimum of 0 for each vessel segment. By definition, \( \text{AVT} = 0 \) for a straight line.

Figure 7.1 illustrates a test image after detection of its linear parts, derivation of the AVT measure, and color coding the segments based on their level of tortuosity. Values of \( \tau = 5 \) pixels, \( l = 1 \), and \( K = 180 \) were used to apply Gabor filters to the test image. The green-coded segments are either considered to be straight via the MAD measure or have a value of \( \text{AVT} < 0.045 \), and the red-coded segments possess \( \text{AVT} \geq 0.045 \). The advantage of application of the MAD measure to detect linear segments prior to measurement of the AVT is well demonstrated in this figure; entire segments are not simply identified in terms of their increasing frequencies, but linear parts of the segments are first removed before the
application of the AVT to the nonlinear parts of the segments and consequently color coding them.

Figure 7.1: A test image of size 480 × 640 pixels representing skeletons of vessels using sinusoids and a straight line. Assuming a pixel size of 30 µm, the frequency of the sinusoids from top to bottom (excluding the straight line) are: 1.32, 1.32, 7.94, 5.29, 4.5, 10.75, 15.87, and 41.67 cycles/mm. The skeletons have been dilated using a disk of radius one pixel for better illustration.

The AVT thresholds illustrated in Figure 7.1 need to be set according to the application. In case of the TROPIC database, the training set of images was used to obtain a suitable threshold for the AVT measure by comparing the AVT measures for the abnormally tortuous and normal vessels, as marked by the retinal specialist. Based on this analysis, a threshold of $t = 0.07$ was determined in order to discriminate between abnormally tortuous and normal vessel segments. Each nonlinear vessel segment with $\text{AVT} > t$ was automatically marked as being abnormally tortuous and as normal if $\text{AVT} \leq t$.

All 110 test images were randomly presented to the user (F. Oloumi) via the GUI, one
at a time, without revealing the associated diagnosis. The two binarization parameters (see Section 7.1) were selected with the objective of maximizing the number of segmented retinal vessels obtained while minimizing the artifacts due to choroidal vessels. On the average, it took about 45 seconds to analyze each image.

7.4 Diagnostic Decision Making

Clinical diagnosis of plus disease requires the presence of sufficient increase in tortuosity and thickness, as compared to the standard photograph, in at least two quadrants of the image. Since tortuosity is not formally defined, numerical representations of its quantitative measurement, such as AVT, may not be directly meaningful to an ophthalmologist. Furthermore, providing one average or maximum measure of tortuosity for an entire retinal fundus image, or even for each quadrant of an image, may be misleading if the tortuous segments are not sufficiently long, i.e., two highly tortuous but short segments in two different quadrants should not lead to a positive diagnosis. A diagnostic-decision-making procedure that follows the clinical definition of plus diagnosis concerning vessel tortuosity as well as a minimum-length criterion is proposed in this work.

The total length of tortuous vessels in each quadrant was computed by dividing the skeleton image of the automatically labeled abnormally tortuous vessels into four quadrants with reference to the center of the ONH. All vessel segments in each quadrant were then identified and their associated chain-code representations were obtained. The length of each segment was computed as the number of even codes plus $\sqrt{2}$ multiplied by the number of odd codes. Such an approach ensures that the diagonal distance between two pixels is taken as $\sqrt{2}$ pixels. The true length of a segment was obtained based on the total length of a segment (in pixels) and the spatial resolution of the TROPIC images (30 $\mu m$/pixel). The proposed analysis is performed in three regions; the posterior, the periphery, and the entire FOV of the image. The posterior section of the image is defined as a circle centered at the
center of the ONH with a radius = 2× ONHH and the peripheral section is taken as the entire FOV minus the posterior area.

A suitable minimum-tortuous-vessel-length threshold for diagnosis of plus disease was obtained by analyzing the length of the abnormally tortuous vessels, over the entire FOV, in the training set, as marked by the retinal specialist. A diagnosis of plus disease is made if an image contains at least 2.5 mm of abnormally tortuous vessels in each of at least two quadrants, or at least 5 mm of abnormally tortuous vessels in any one quadrant. Note that no separate minimum-tortuous-vessel-length thresholds were obtained for the posterior and the periphery of the retina, as the abnormally tortuous vessels were marked over the entire FOV in the training set. The total length of abnormally tortuous vessels in a given image, in the posterior, the periphery, and the entire FOV, was also considered as a diagnostic feature; a sliding threshold method (see Section 7.4.1) was used to determine the overall diagnostic accuracy of this feature.

7.4.1 Evaluation of the Diagnostic Performance

Using the minimum-tortuous-vessel-length criterion, a single set of sensitivity and specificity values was obtained to characterize the diagnostic accuracy of the proposed methods at a single operating point over the entire FOV. To assess the overall diagnostic performance of the results at multiple operating points, ROC analysis was performed on the total length of abnormally tortuous vessels in the posterior, the periphery, and the entire FOV of the image using the ROCKIT software [239]. The values of the area under the ROC curve, $A_z$, as well as their SE and 95% CI were obtained from ROCKIT.

7.5 Results

Figure 7.2 illustrates the results of applying the proposed methods to an image without any signs of plus disease from the TROPIC database. No segment was found to be abnormally
tortuous in all four quadrants based on the predetermined AVT threshold.

Figure 7.2: (a) Image 4002 of the TROPIC database showing no signs of plus disease. (b) The vessel orientation for every third pixel on the vessels for a portion of the image in part (a) is shown by the blue needles. (c) The vessel-strength image obtained using the inverted green channel of the image in part (a). (d) The skeleton image after thresholding the image in part (c) at 0.14 of the normalized intensity value and removal of 8-connected segments having fewer than 100 pixels each. (e) The skeleton image after removal of the ONH area and the branching points. (f) The skeleton image of the linear vessel subsegments obtained using the MAD measure. (g) Skeleton image of the nonlinear vessel subsegments obtained by subtracting the image in part (f) from the image in part (e). (h) Color-coded skeleton image distinguishing the abnormally tortuous vessel segments, if any, in red and the normal vessel segments in green, using the AVT threshold of 0.07; in this case, no segment was found to be abnormally tortuous. Images in parts (d)–(h) have been morphologically dilated using a disk of radius one pixel for better visual representation in the figure.

Figure 7.3 illustrates the same steps for an image of a patient diagnosed with plus disease. The sum of the lengths of abnormally tortuous vessels in the first to fourth quadrants of the image were computed to be 11.75, 4.20, 1.99, and 1.42 mm, respectively.

Figure 7.4(a) illustrates the steps used in statistical analysis of the vessel-orientation information to break apart an extracted vessel skeleton segment into its linear and nonlinear parts. The analysis is shown only for an extracted segment of the STA [part (b) of the
Figure 7.3: (a) Image 2903 of the TROPIC database that shows signs of plus disease. (b)–(c) The same as the caption in Figure 7.2(b)–(c). (d) The skeleton image after thresholding the vessel-strength image at 0.045 of the normalized intensity value and removal of 8-connected segments having fewer than 100 pixels each. (e)–(g) The same as the caption in Figure 7.2(e)–(g). (h) Color-coded skeleton image distinguishing the abnormally tortuous vessel segments in red, and the normal vessel segments in green, using the AVT threshold of 0.07.

The same figure corresponding to the image in Figure 7.3. The resulting subsegments shown in part (c) are considered to be linear and those shown in part (d) are considered to be nonlinear. Using the AVT threshold of 0.07, only the rightmost nonlinear subsegment (the longest of the three subsegments) in Figure 7.4(d) was found to be abnormally tortuous, with AVT = 0.15; this subsegment is shown in red in Figure 7.3(h) along with other abnormally tortuous subsegments.

Table 7.1 shows the results of statistical and diagnostic analysis of the total abnormally tortuous vessel length using 91 cases without plus disease and 19 cases with plus disease over the posterior, the periphery, and the entire FOV of the image. The area under the ROC curve indicates excellent performance in classification and diagnosis of plus disease, with $A_z = 0.98$ over the entire FOV. The same measure provides lower values of $A_z = 0.89$
Figure 7.4: (a) The vessel orientation along a sequenced skeleton, its associated normalized MAD measure obtained over a 7-pixel-long window, and the detected linear samples are plotted against the sequence index. (b) The extracted STA segment [in quadrant 1 of the image in Figure 7.3(e)] on which the statistical analysis of part (a) is performed. (c) The result of segmenting the skeleton in part (b) into linear and (d) nonlinear subsegments. The AVT measures computed for the three nonlinear subsegments in part (d) of the figure are 0.05, 0.04, and 0.15, from left to right, respectively.
and 0.95 over the posterior and the periphery, respectively (see Figure 7.3).

Table 7.1: Computed total average abnormally tortuous-vessel lengths and their associated SEs in the posterior, the periphery, and the entire FOV. The $p$-values obtained using the Wilcoxon rank-sum test \cite{285,286}, area under the ROC curve ($A_z$), SE, and 95% asymmetric confidence interval ($CI_a$) in the discrimination of 19 cases with plus disease versus 91 cases without plus disease are provided. \textit{***}$p < 0.001.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without Plus, Mean (SE) $\quad n = 91$</th>
<th>With Plus, Mean (SE) $\quad n = 19$</th>
<th>$p$-value</th>
<th>$A_z$ (SE)</th>
<th>$CI_a$ $\alpha = 0.025$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior Tortuous-Vessel Length (\textit{mm})</td>
<td>0.53 (0.08)</td>
<td>4.62 (0.61)</td>
<td>$&lt; 0.001$***</td>
<td>0.89 (0.05)</td>
<td>[0.712, 0.978]</td>
</tr>
<tr>
<td>Peripheral Tortuous-Vessel Length (\textit{mm})</td>
<td>0.95 (0.14)</td>
<td>11.91 (2.26)</td>
<td>$&lt; 0.001$***</td>
<td>0.95 (0.04)</td>
<td>[0.859, 0.987]</td>
</tr>
<tr>
<td>Total Tortuous-Vessel Length (\textit{mm})</td>
<td>1.48 (0.17)</td>
<td>16.53 (2.42)</td>
<td>$&lt; 0.001$***</td>
<td>0.98 (0.02)</td>
<td>[0.910, 0.997]</td>
</tr>
</tbody>
</table>

Using the minimum-tortuous-vessel-length threshold derived in the present work (see Section 7.4), sensitivity = 0.89 and specificity = 0.99 were obtained. The results indicate statistically extremely significant differences ($p$-value $< 0.001$) between the means of the total abnormally tortuous vessel lengths for cases with and without plus disease over the entire FOV. Note that the same minimum-tortuous-vessel-length threshold may not be applicable to the results obtained over the posterior and the periphery of the retina.

It is possible to select the most suitable threshold for a minimum-tortuous-vessel-length criterion for the entire image using the point on the ROC curve that provides the best trade off between sensitivity and specificity values. As shown in Figure 7.5, it is clear that, based on the results in the present work, analysis of the total length of abnormally tortuous vessels over the entire FOV provides higher diagnostic accuracy and narrower confidence bounds.
as compared to analysis of only the posterior or the periphery of the retinal fundus images. A potentially optimal operating point is the point on the ROC curve, associated with the entire FOV, that is closest to the point \((0, 1)\), with sensitivity = 0.91 and specificity = 0.92 in the present case. The ROC curves associated with the posterior and the periphery provide potential optimal operating points with sensitivity = 0.80 and 0.86, as well as specificity = 0.90 and 0.89, respectively. The trade off between sensitivity and specificity must be determined based on the clinical application.

Table 7.2 presents the results of applying the bootstrapping approach as explained in Section 3.5.4. The results indicate similar average AUC and mean values as compared to the results in Table 7.1. However, the 95% confidence bands are narrow, indicating that there is a high level of confidence in the estimated values for the average tortuous-vessel lengths for cases with and without plus disease, as well as in discrimination between the two classes using ROC analysis. Based on the results of bootstrapping, it may be concluded that the original set of values obtained (Table 7.1) can be considered to be representative of the population distribution that they belong to. As mentioned in Section 6.3, the ROC analysis procedure used for the bootstrapping approach provides the AUC values as opposed to the \(A_z\) values provided by ROCKIT; hence, there is a small difference (of the order of 0.01) between the \(A_z\) values in Table 7.1 and the AUC values in Table 7.2.

Analysis of the total tortuous-vessel length related to images from the same eye of the same patient that progressed to plus disease and a higher stage of ROP, on average over the three patients and four imaging instances available in the TROPIC database, showed an increase of 10.82 \(\text{mm}\) in the total length of abnormally tortuous vessels detected.

7.6 Comparative Analysis

Only eight studies found in the literature [102,103,121–123,125–127] have performed CAD of plus disease based on quantification of vessel tortuosity; however, the approaches to analysis,
Figure 7.5: (a) ROC curves presenting the diagnostic accuracy of the total length of tortuous vessels obtained using the proposed methods to distinguish between cases without and with plus disease over the posterior ($A_z = 0.90$), the periphery ($A_z = 0.95$), and the entire FOV ($A_z = 0.98$). Parts (b), (c), and (d) of the Figure illustrate each ROC curve separately with added error bars, or confidence bounds, (red lines) at different points on the curve obtained using the total tortuous-vessel lengths in the posterior, periphery, and the entire FOV, respectively.
Table 7.2: Values of the mean of the AUC, the mean of each class, and their associated 95% ($\alpha = 0.025$) symmetric confidence intervals $CI_s$, obtained by randomly selecting 150 cases with and without plus disease, repeated 500 times for total tortuous-vessel lengths in the posterior, the periphery, and the entire FOV. The confidence intervals were obtained by assuming a normal distribution for the AUC and class-mean values. In the last column, $M^{***}$ means that, out of the 500 trials, the results indicated statistically extremely significant differences in $M$ trials with $p < 0.001$; $N^{**}$ means that the differences were statistically highly significant in $N$ trials with $0.001 \leq p < 0.01$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AUC, Mean, $[CI_s]$</th>
<th>Without Plus, Mean (SE)</th>
<th>With Plus, Mean (SE)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posterior Tortuous-Vessel Length (mm)</td>
<td>0.905, [0.904, 0.907]</td>
<td>0.533 (0.003)</td>
<td>4.623 (0.012)</td>
<td>500***, 0**</td>
</tr>
<tr>
<td>Peripheral Tortuous-Vessel Length (mm)</td>
<td>0.942, [0.941, 0.943]</td>
<td>0.950 (0.006)</td>
<td>11.822 (0.044)</td>
<td>500***, 0**</td>
</tr>
<tr>
<td>Total Tortuous-Vessel Length (mm)</td>
<td>0.970, [0.970, 0.971]</td>
<td>1.487 (0.008)</td>
<td>16.485 (0.050)</td>
<td>500***, 0**</td>
</tr>
</tbody>
</table>

Diagnosis, and validation of the results have not been consistent or standardized across all of the studies. All of the mentioned studies have limited the area of analysis to the posterior of the retina, defined as a circle centered at the center of the ONH with radius $= 2 \times$ ONHW. All of the studies have used manual selection and correction of the vessel segments to be analyzed. Table 7.3 presents comparative analysis of the results of the mentioned studies. The main differences between these studies are in the approach to diagnosis and validation of the results.
Table 7.3: Comparative analysis between the present study and similar state-of-the-art studies available in the literature. If a particular parameter or result was not specified in a study, it is denoted by NP (not provided). Sen. and Spe. stand for sensitivity and specificity, respectively. All studies have used manual markings of the center of the ONH and used threshold values provided by the user for binarization. * indicates et al. "Diagnosis performed on each selected arteriole, not the image." Diagnosis performed on each selected venule, not the image. "Diagnosis performed based on the maximum tortuosity value of selected vessels for each quadrant, not the image." "Value obtained at the point of intersection of the sensitivity and specificity plots of diagnosis of each vessel, not using ROC analysis." "Diagnosis performed using tortuosity and thickness measures combined." "Requires two parameters to be set by the user.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Vessel Detection</th>
<th>Selection of Vessels to Analyze</th>
<th>Analysis of Venules &amp; Arterioles</th>
<th># of Cases</th>
<th>$A_z$</th>
<th>Sen. &amp; Spe. (Threshold)</th>
<th>Sen. &amp; Spe. (ROC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelman* [123]</td>
<td>Manual</td>
<td>Manual</td>
<td>Separate</td>
<td>20 Without Plus, 12 With Plus</td>
<td>0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Gelman* [102]</td>
<td>Manual</td>
<td>Manual</td>
<td>Separate</td>
<td>21 Without Plus, 13 With Plus</td>
<td>0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NP</td>
<td>0.76 &amp; 0.76&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Koreen* [103]</td>
<td>Manual</td>
<td>Manual</td>
<td>Separate</td>
<td>14 Without Plus, 6 With Plus</td>
<td>0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NP</td>
<td>1.00 &amp; 0.85&lt;sup&gt;a,d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Thyparampil* [127]</td>
<td>Manual</td>
<td>Manual</td>
<td>Separate</td>
<td>61 Without Plus, 13 With Plus</td>
<td>0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Zhao* [125]</td>
<td>Manual</td>
<td>Manual</td>
<td>Combined</td>
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<td>0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>Kiely* [126]</td>
<td>Manual</td>
<td>Manual</td>
<td>Combined</td>
<td>92</td>
<td>0.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.00 &amp; 0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.91 &amp; 0.86&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Wallace* [122]</td>
<td>Manual</td>
<td>Manual</td>
<td>Combined</td>
<td>11 Without Plus, 5 With Plus</td>
<td>NP</td>
<td>0.82 &amp; 0.80</td>
<td>NP</td>
</tr>
<tr>
<td>Heneghan* [121]</td>
<td>Semiautomatic&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Manual</td>
<td>Combined</td>
<td>12 Without Plus, 11 With Plus</td>
<td>NP</td>
<td>NP</td>
<td>0.82 &amp; 0.75&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Present work</td>
<td>Semiautomatic&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Automatic</td>
<td>Combined</td>
<td>91 Without Plus, 19 With Plus</td>
<td>0.98</td>
<td>0.89 &amp; 0.99</td>
<td>0.91 &amp; 0.92</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diagnosis performed on each selected arteriole, not the image.
<sup>b</sup>Diagnosis performed on each selected venule, not the image.
<sup>c</sup>Diagnosis performed based on the maximum tortuosity value of selected vessels for each quadrant, not the image.
<sup>d</sup>Value obtained at the point of intersection of the sensitivity and specificity plots of diagnosis of each vessel, not using ROC analysis.
<sup>e</sup>Diagnosis performed using tortuosity and thickness measures combined.
<sup>f</sup>Requires two parameters to be set by the user.
The studies conducted by Gelman et al. [102,123], Koreen et al. [103], and Thyparampil et al. [127] all used the software package RISA [187]. RISA provides two different measures of tortuosity and analyzes venules and arterioles separately. Only the best diagnostic results, as provided by these works, are reported in Table 7.3. Two studies [123, 127] only provided $A_z$ values, whereas the other two studies listed above [102,103] provided $A_z$ as well as sensitivity and specificity values. However, the sensitivity and specificity values were determined by finding the intersection point between the sensitivity and specificity curves, plotted separately as functions of the ratio of the number of correctly identified vessels to the actual tortuosity measures. RISA requires the vessel segment under analysis to have at least one branching point. Manual correction of the detected branching points and manual input regarding whether the selected vessels are arterioles or venules, which were measured separately, were also required. Although the studies of Gelman et al. [102] and Koreen et al. [103] provided results for various combinations of venular and arteriolar tortuosity and dilation measures, none of the four mentioned studies provided diagnostic results for combination of only the arteriolar and venular tortuosity measures. The values in Table 7.3 as provided by Thyparampil et al. [127] were obtained using venular tortuosity, whereas the other three studies [102,103,123] obtained higher diagnostic accuracy using arteriolar tortuosity.

Studies by Zhao et al. [125] and Kiely et al. [126] used the ROPTool software, which combines the tortuosity measures obtained from both venules and arterioles. However, the maximum tortuosity value in each quadrant was taken as the overall measure and diagnosis of plus disease was performed on each quadrant of the image. The separate counts of the number of images with and without plus disease were not provided in either study. Zhao et al. provided only an $A_z$ value; the threshold obtained based on this analysis was used in the work of Kiely et al. to obtain measures of sensitivity and specificity, as well as an overall $A_z$ value.

Heneghan et al. [121] obtained the average thickness and tortuosity values for an image.
by combining measurements from both arterioles and venules. Sensitivity and specificity values in the diagnosis of threshold disease were then obtained using a combination of the two measures. The study did not provide $A_z$ values or diagnostic results using only the tortuosity measurements.

The study conducted by Wallace et al. [122] is the only study that is almost directly comparable to the present work. Wallace et al. used measures of tortuosity obtained from the standard photograph (venules and arterioles combined) and if an image had equal or greater tortuosity measures in at least two quadrants, the image was diagnosed as a case with plus disease. The study did not provide $A_z$ values.

7.7 Discussion

The proposed diagnostic-decision-making criteria combine the clinical definition of plus disease with respect to tortuosity [40] with practical understanding of the characteristics of a sufficiently tortuous vessel. The results indicate high performance in the diagnosis of plus disease using a single feature with sensitivity $= 0.89$ (17/19) and specificity $= 0.99$ (90/91). Furthermore, considering the entire length of tortuous vessels in a given image, an excellent overall diagnostic accuracy is achieved with $A_z = 0.98$. The proposed methods provide the highest $A_z$ and specificity rates reported in the literature using tortuosity. However, a direct comparison between the results of the present study and those listed in Table 7.3 may not be appropriate since the diagnosis and evaluation have been performed differently in the studies.

The clinical diagnostic definition of plus disease provides a diagnosis for the entire image (or eye or patient) based on at least two quadrants having vessels with abnormal tortuosity. However, diagnosis of each single vessel or each quadrant (based on a single maximum value) may not qualify as diagnosis of plus disease, as plus diagnosis is performed on a per-eye (image) or per-patient basis.
All studies that have performed diagnosis of plus disease via quantification of vascular tortuosity have included only the desired vessels for further analysis via manual marking of vessel segments, or manual selection and/or correction of parts of automatically detected vessels. Such manual marking of images may not be feasible in a clinical or teleophthalmological setting. The methods proposed in the present work are capable of distinguishing tortuous vessels in a given image without any manual selection and/or correction.

Studies that have performed CAD of plus disease using tortuosity have provided a single measure of tortuosity for each selected vessel segment [102, 103, 123, 127], the entire image [121], or for each quadrant of the image [122, 125, 126]. However, as previously mentioned, because tortuosity is not formally defined, numerical representations of its quantitative measurement (per vessel, quadrant, or image) may not be meaningful to an ophthalmologist. Use of the AVT measure first to identify abnormally tortuous vessel segments and then to obtain the total length of all of such segments should be more practical from a CAD point-of-view, and more meaningful to an ophthalmologist.

Separate analysis of arterioles and venules may not be necessary for the diagnosis of plus disease. It has been observed that the distinction between arterioles and venules becomes difficult in the presence of zone I disease [129]; even experts cannot distinguish between arterioles and venules in retinal images of preterm infants about 20% of the time [130]. Furthermore, based on the findings in the present work, it is questionable whether peripheral-venular tortuosity is less important than posterior-arteriolar tortuosity (see Figure 7.3 as an example).

As previously mentioned, even though the standard photograph of plus disease has been used for clinical diagnosis, the image is believed to be atypical because it shows more vascular dilation and less tortuosity as compared to most cases with plus disease [57]. Secondly, the image possesses a narrow FOV, and does not reveal possible tortuous vessels in the periphery of the retina. All studies mentioned in Section 7.6 limited the area of analysis to the posterior
of the retina. However, peripheral-vessel tortuosity has been shown to be more correlated to the presence of plus disease as compared to posterior-vessel tortuosity \cite{105}. Indeed, as presented in Table 7.1, considering the total length of abnormally tortuous vessels over the entire FOV, as compared to the posterior and the periphery of the retina, provides higher diagnostic accuracy. Given such limitations, it is beneficial to obtain any necessary thresholding parameters using an independent training set of images. The methods proposed in the present work are capable of detecting all tortuous vessels regardless of location.

Based on the results provided in Table 3.1, the patient attributes of BW and CA do not show any statistically significant differences in the mean for the cases without plus disease as compared to the plus cases, whereas the difference in the mean GA for the two classes is statistically highly significant. Furthermore, considering the Pearson correlation coefficient, a statistically highly significant correlation ($p < 0.01$) was found between GA and the total length of tortuous vessels. These results could indicate that plus disease may not be a developmental process and may be more probable in under-developed retinas, i.e., the lower the GA, the higher the probability of occurrence of plus disease. No correlation was found between the total length of tortuous vessels and the patient attributes of BW and CA.

The proposed methods are automated, except for the vessel binarization step, the initial removal of small segments, and marking of the center of the ONH (the same is true for all studies listed in Table 7.3). No single automated thresholding method provides consistent results for binarization of all images due to the variable nature of the retinal images of preterm infants, including varying pigmentation, blurring, and low vessel-to-background contrast. Combination of the results of multiple thresholding methods may lead to better binarization results \cite{155,293}. The method of Rangayyan et al. \cite{224} could be adapted in the future to detect automatically the center of the ONH.

Retinal images of preterm infants vary substantially in terms of pigmentation, contrast, and quality. All of the studies mentioned above have selected only images with high vessel-
to-background contrast and quality in order to observe more precisely the variations in the vasculature. In real-world applications (clinical or teleophthalmological), it may not be possible to obtain only high-quality and sharp pediatric retinal fundus images. In the present work, the largest database of retinal images of preterm infants in the literature (see Table 7.3) has been used, which is not limited to images with high quality and contrast. Despite some limitations in the thresholding step, the proposed methods are robust to be able to diagnose plus disease with high accuracy regardless of the quality of the image.

Whereas the clinical diagnosis at the time of imaging has been taken as the reference diagnosis in the present study, given the interexpert variability in the diagnosis of plus disease, the inclusion of more cases with and without plus disease as well as multiple diagnoses of the same cases provided by several experts, could help to strengthen the results and analysis.

It has been observed that changes in vascular tortuosity that occur in the presence of plus disease are dynamic [127]. Indeed, as mentioned in Section 7.5, using the proposed methods, an average increase of about 11 mm in the total length of abnormally tortuous vessels was detected in the eyes of three patients that progressed to plus disease. Further longitudinal analysis of this aspect with more cases is of interest.

The present work focused on evaluation of the diagnostic power of retinal vascular tortuosity alone. Combining the results of measurement of tortuosity with measurements of vessel thickness [294, 295] as well as the openness of the MTA [44] using pattern classification methods could lead to better and robust discrimination in CAD of plus disease, and ultimately, timely treatment of ROP.

In comparison to previous related studies on vascular tortuosity due to plus disease [291, 296], the use of the MAD measure to detect and remove linear parts of vessel segments significantly improved the accuracy of the obtained tortuosity measure. The use of a training set of images to obtain thresholds for the AVT measure and to define the minimum-tortuous-
vessel-length criterion is advantageous and does not bias the final findings since the training
and test sets are mutually independent. Furthermore, the minimum-tortuous-vessel-length
threshold obtained from ROC analysis in this study can be used as a threshold for plus
diagnosis and applied to any dataset of preterm retinal fundus images.

Although the clinical diagnosis of plus disease requires the presence of abnormally tor-
tuous and dilated vessels in at least two quadrants in the posterior of the retina, based on
the results of the present work, it may be argued that considering the total length of all
abnormally tortuous vessels in the entire image, as compared to only the posterior or the pe-
riphery, may be a more accurate and quantitatively comprehensible indicator of the presence
of plus disease that could lead to higher diagnostic accuracy. Further analysis and testing of
this observation, using the minimum tortuous-vessel length threshold obtained in this study
with the TROPIC database, with independent databases of preterm retinal fundus images
and including multiple expert diagnoses per image, would be of interest.

7.8 Remarks

The methods presented in this chapter are capable of distinguishing abnormally tortuous
vessels, present high accuracy in the diagnosis of plus disease, and may be used for CAD in
a clinical or teleophthalmological setting. Future research could include combining measures
of tortuosity and thickness of retinal vessels with the openness of the MTA, which may yield
better and more robust results in the diagnosis of plus disease.
Chapter 8

Clinical Applications

8.1 A GUI for Feature Extraction and Analysis

To facilitate the application of CAD of plus disease using the proposed methods and features in a clinical setting, a GUI was developed and tested in consultation with a pediatric ophthalmologist and retinal specialist (A.L. Ells). The GUI adheres to the main principles of GUI development, such as human factors, knowledge of the user’s requirements and expectations, ease of use, intuitiveness, error handling capabilities, proper documentation, and visual aesthetics [283].

A few GUIs have been developed and used for the analysis of retinal images in different contexts in the literature. Zhang et al. [297] used a GUI for manual segmentation, analysis, and grading of glaucoma. Fiorin and Ruggeri [199] used a GUI for manual segmentation and analysis of the thickness and tortuosity of blood vessels in ROP images. Tsai et al. [298] used a GUI for fusion of retinal images of the same patient into a composite mosaic. Venkatatalakshmi et al. [299] developed a GUI for detection and analysis of exudates for diagnosis of DR. Lalonde et al. [148] designed a GUI for detection of the ONH and the macula, image quality assessment, lesion detection, and image registration and fusion.

The GUI in the present work was designed using MATLAB’s GUI development environment (GUIDE) and contains modules for detection, segmentation, and analysis of retinal vascular architecture based on the methods proposed in this work. All of the underlying code to run the various modules of the GUI was written in the form of MATLAB functions. The GUI is deployable as a stand-alone installation package.

Upon opening the main GUI window, the user is presented with a message box stating the various functionalities and capabilities of the GUI as well as general tips on how to get
started. The message box opens at every startup unless the user chooses not to receive it anymore. The GUI is capable of opening all commonly used image types (jpg, tif, png, bmp, etc.) using a typical “Open File” window (Figure 8.1). The selected image is then displayed within the internal display area of the GUI, as shown in Figure 8.2. The internal display area of the GUI is always updated with the processed image after completion of a selected procedure. Opening a new image enables several functionalities, including vessel detection using Gabor filters (see Section 4.1.1), measurement of the thickness of the MTA (see Chapter 5), and measurement of the TAA (see Section 6.1).

Figure 8.1: The main GUI showing the “Open File” window.
When a functionality requires the location of the center of the ONH, if the information is not available (not previously saved), the GUI opens the G-channel image in a new window, prompts the user to mark the center of the ONH, and saves the information in terms of $(x, y)$ coordinates to a text file.

![Image of the main GUI showing an opened image.]

**Figure 8.2:** The main GUI showing an opened image.

The TAA measurement module (Figure 8.3) launches through a button click and provides the user with the option of choosing between the methods of Wilson et al. [110] and Wong et al. [41]. The user has the option of choosing the full-color or the G-channel image; by default, the GUI provides the G-channel image. See Section 6.1 for details on TAA measurement procedures.

Tracking and measurement of the thickness of the MTA is available through a button click.
and performed automatically, as explained in Chapter 5 and only requires the approximate location of the center of the ONH as input from the user.

Figure 8.3: The arcade angle measurement window is launched through the main GUI by a button click.

The vessel-detection module is part of the main GUI window (see Figure 8.4) and allows the user to set the parameters of Gabor filters as well the option to choose the $G$-channel or the luminance component as the gray-scale input image. Default values for each Gabor parameter are also provided. See Section 4.1 for details on Gabor filters.

Figure 8.4: The vessel-detection module providing default values as well as user control over each parameter of Gabor filters.

Following the application of Gabor filters, the vessel-segmentation module becomes avail-
able to the user through the main GUI window (Figure 8.5). The Gabor magnitude-response image is normalized before binarization is applied. The user has the option of using a sliding threshold, entering a specific threshold value in the range \([0, 1]\), or using one of the automated thresholding methods explained in Section 4.2. The module also provides the option of removing 8-connected segments using the area open procedure (see Section 4.2.2) by specifying the maximum number of connected pixels in a segment to be removed. See Section 4.2 for details on thresholding methods and morphological image processing techniques.

Figure 8.5: The vessel-segmentation module becomes available only after the application of Gabor filters and obtaining a gray-scale image of vessels.

Segmentation of the detected vessels through the binarization module leads to the arcade modeling and tortuosity measurement functionalities becoming available through buttons located on the main GUI window. The arcade modeling window, as shown in Figure 8.6, allows the user to perform single- or dual-parabolic modeling of the MTA or the STA/ITA, respectively (see Chapter 6 for details on parabolic modeling). Tortuosity measurement, as explained in Chapter 7, is performed through a button click using the obtained binary image. Both the arcade modeling and the tortuosity measurement procedures require the approximate location of the ONH, obtained as previously explained.

Figure 8.7 shows the four buttons that enable the quantification of the three diagnostic features of MTA width, MTA openness (in terms of TAA and the openness of the parabolic model), and tortuous-vessel length. The TAA-measurement and MTA-width-measurement buttons become available as soon as an image is opened by the user. The arcade-modeling
Figure 8.6: The parabolic-modeling window allows the user to perform single- and dual-parabolic modeling using the previously obtained binary image of vessels.

and the tortuous-vessel-length-measurement buttons become available after the detected vessels have been segmented using the binarization module.

Figure 8.7: The buttons that allow for quantification of the three diagnostic factors of MTA openness, MTA thickness, and abnormally-tortuous vessel length used for CAD of plus disease in the present work.

Figure 8.8 illustrates the internal display of the GUI as it is updated with the results of single- and dual-parabolic modeling as well as detection of abnormally-tortuous vessels. The GUI also displays the relevant measured and/or obtained quantitative results in a message box; the user is able to save the obtained results in a textual format through the “Save” tab.
Figure 8.8: The embedded internal display of the GUI showing the results of (a) single-parabolic modeling fit, (b) dual-parabolic modeling fits, and (c) color-coded results of detection of abnormally tortuous vessel segments.
The GUI also provides other functionalities through the menu bar, as shown in Figure 8.9, and is capable of displaying various gray-scale forms of the input image as well as all of the processed images. The GUI provides the option of saving all of the obtained results and the values of the various parameters used in a text-file format. The principal axis and zone I functions allow for estimation of the retinal raphe (the line going through the centers of the ONH and fovea), in automated and manual manners, respectively. The ‘Help’ tab provides information regarding all of the available methodologies and functionalities of the GUI and how to use them.

![AnalysisOfRetinalVasculation](image)

Figure 8.9: The GUI menu bar provides functionalities such as viewing of various versions of the opened image and the ability to save the computed results.

Table 8.1 presents the execution run times for various parts of the proposed methods in this work on a Lenovo Thinkpad T510, equipped with an Intel Core i7 (Hyper-threaded-dual-core) 2.67-GHz processor, 8 GB of DDR3 RAM, running 64-bit Windows 10 Professional, and using 64-bit MATLAB R2013a software. The MATLAB codes developed may be translated to a low-level programming language such as C or C++ for faster and more efficient implementation.

8.2 Clinical Application of Feature Selection and Pattern Classification

As mentioned in Section 3.6, the wrapper method was used with three classifiers and two search methods to determine whether combinations of the different clinical features obtained in the present work based on the openness, thickness, and tortuous-vessel length, could lead to better diagnostic classification and higher discrimination between cases with and without
Table 8.1: Table of execution run times, in seconds, for various procedures as proposed in the present work. The time given for the measurement of MTA thickness includes the preprocessing as well as the tracking steps. The times given for the parabolic modeling procedures include the preprocessing of the binary image. The time provided for the tortuosity measurement includes the preprocessing as well as the MAD measure analysis steps.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Execution time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gabor Filters – Preprocessing</td>
<td>4.2</td>
</tr>
<tr>
<td>Gabor Filters – {τ, l, K} = {7, 1, 45}</td>
<td>2.2</td>
</tr>
<tr>
<td>Measurement of MTA Thickness</td>
<td>1.8</td>
</tr>
<tr>
<td>Measurement of MTA Openness – Single Modeling</td>
<td>2.6</td>
</tr>
<tr>
<td>Measurement of MTA Openness – Dual Modeling</td>
<td>4.3</td>
</tr>
<tr>
<td>Measurement of Vessel Tortuosity</td>
<td>1.0</td>
</tr>
</tbody>
</table>

plus disease, as compared to the use of each single feature separately. The WEKA software package [300] was used to perform all feature selection and pattern classification tasks. The following nine features were included in the feature selection procedure: \(|a_{MTA}|, |a_{STA}|, |a_{ITA}|, TAA_{r=60}, TAA_{r=120},\) average MTA width, posterior tortuous-vessel length, peripheral tortuous-vessel length, and total tortuous-vessel length.

The classifiers used were an LR model, a naïve-Bayes model, and a three-layer MLP (with one hidden layer). The best-first search method was set to start with an empty set of features and search in both directions to include/exclude features to obtain the set of features with the highest level of classification based on the cross-fold-validation scheme. The exhaustive search method traverses the entire search space and examines all possible combinations of features to determine the feature sets with the highest accuracy of discrimination, also based on the cross-fold-validation method. In all instances, 11 folds (10 images each) were used for cross-validation. See Section 3.6 for details of the pattern analysis methods used.

Table 8.2 presents the results of feature selection using the wrapper method and cross-fold validation. A feature was taken as a valid choice for pattern classification only if it was selected in at least 50% of the folds during the 11-fold cross-validation process. In
all instances, the tortuous-vessel length over the entire FOV was selected in 100% of the folds; only the naïve-Bayes classifier selected other features as well, including the openness parameter of the single-parabolic model and the two TAA measures.

Pattern classification was performed using the results of feature selection via the naïve-Bayes model using the two search methods. However, in both instances, the results of pattern classification did not yield higher \( A_z \) values as compared to using the total tortuous-vessel length alone (\( A_z = 0.98 \)). The pattern classifier based on the naïve-Bayes model achieved the same sensitivity or specificity as compared to classification using the minimum-vessel-length criterion with total tortuous-vessel length (see Section 7.5), at the expense of lower specificity or sensitivity values, respectively.

Various other classifiers were tested for feature selection, but the results are not provided here as they were consistently the same. All methods always included the total tortuous-vessel length with at least 90% selection rate during cross-fold validation. None of the methods that selected additional feature(s) along with total tortuous-vessel length led to improved diagnostic results.

The results indicate that the total tortuous-vessel length over the entire FOV alone can lead to the best CAD performance in the detection of plus disease with the database used (TROPIC). The MTA thickness measure derived from images of higher quality and resolution may lead to better results. Larger databases with a large range of image characteristics and the need for robust performance may call for the use of multiple diagnostic features.

8.3 Remarks

The proposed GUI can facilitate practical application, in a clinical setting, of the methods developed in the present work. The layout and design of the GUI ensure ease of use and provide various useful functionalities in addition to CAD of plus disease. The feature-selection and pattern-classification experiments performed in the present work indicate that the total
Table 8.2: Results of feature selection using various pattern classification methods employed via the wrapper method and cross-fold validation. In all instances, 11 folds were used during validation and a feature was included if it was selected in at least 50% of the folds.

<table>
<thead>
<tr>
<th>Classifier</th>
<th>Selected Feature(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Best-First</td>
</tr>
<tr>
<td>LR</td>
<td>{Tortuosity_total}</td>
</tr>
<tr>
<td>Naïve Bayes</td>
<td>{Tortuosity_total,</td>
</tr>
<tr>
<td>MLP</td>
<td>{Tortuosity_total}</td>
</tr>
</tbody>
</table>

tortuous-vessel length in the entire image is the single most discriminatory feature among all of the diagnostic features extracted and evaluated.
Chapter 9

Concluding Remarks

This chapter provides details on various contributions made by the author, through the present Ph.D. work, to the field of computer-aided analysis and diagnosis of retinal fundus images. Summarizing discussion and conclusion regarding each proposed method, the associated quantitative diagnostic feature, as well as feature selection and pattern classification are also provided. Possible areas of expansion and future work are identified.

9.1 Discussion and Remarks

The results of the study on vessel detection indicate that single-scale Gabor filters are adequate for detection of retinal vasculature in fundus images of preterm infants. However, since the retina is still developing in preterm infants and is fairly thin and transparent at the time of imaging, the choroidal vessels may also possess relatively high vessel-to-background contrast and Gabor-magnitude responses. Gabor filters are advantageous over the other vessel detection methods studied (see Section 4.1) as they provide the vessel-strength (magnitude-response) and vessel-orientation (angle-response) results in one step.

In the case of detection and tracking of the MTA, since a large thickness ($\tau$) value is used, the magnitude-response image will provide relatively high intensity values for the MTA as opposed to other vessels. Such a representation, along with the use of gamma correction and the area open procedure, allow thresholding techniques such as the moment-preserving method to automatically segment the MTA. However, obtaining a binary image in which the entire vasculature is segmented while the effects of choroidal vessels are removed (in retinal fundus images of preterm infants) in an automatic manner may require more comprehensive locally adaptive preprocessing and postprocessing methods as well as thresholding methods...
that account for the nearly unimodal histogram of the intensity values of the results of vessel-detection methods (see Section 4).

The combined application of tracking the MTA, use of the MAD measure for analysis of variations in vessel orientation, and geometrical methods enables measurement of the width of the MTA to subpixel accuracy. The difference between the mean width measurements for cases with and without ROP is about one-half of a pixel (as also shown by other researchers) and was found to be statistically highly significant. Blurring and image artifacts due to movement may introduce errors in measurement of the width even when using the proposed methods. A washout effect may be introduced when averaging the width measure over all available segments in an image or over the entire length of a segment. To avoid such effects, it may be of interest to use a training set where abnormally dilated vessel segments are marked by an expert, to determine a threshold to detect abnormally dilated vessel segments, and to use the total length of such segments for diagnosis. Analysis of variation of the width of segments of the MTA over its entire length based on statistical measures such as coefficient of variation, skewness, and kurtosis may provide useful diagnostic information regarding the presence of plus disease. Such statistical measures may be used along with MTA openness and abnormally tortuous vessel length as inputs to machine learning algorithms. Longitudinal analysis and diagnosis may also be performed using the proposed methods.

As mentioned in Chapter 1, an increase in the openness of the MTA has been observed as a sign of ROP [40]. The results of the analysis presented in the present work may point to a realization that the restructuring of the MTA, referred to as the change in the openness of the MTA, may be more complex than defined clinically at this time. The parabolic fits to the tracked MTAs provide low MDCP errors when overlaid on the original image. However, as shown in Chapter 6, the related results of such analysis did not improve the diagnostic performance. This result may be related to an observation that the restructuring of the MTA originates close to the boundary of the ONH and could be contained in an annular
region up to one ONHW from the center of the ONH, within which the simple arch of the STA/ITA starts curving at a higher rate leading to a third- or fourth-order curve. Shortening the horizontally restricted image and use of higher-order curve fitting may provide improved results.

The AVT measure, computed via segmentation of vessels with the MAD measure based on the Gabor-angle information, is capable of detection and quantification of tortuous-vessel segments. The use of a training set is advantageous in finding a suitable threshold to indicate abnormal tortuosity. Obtaining the total (or per quadrant) length of abnormally tortuous vessels eliminates the use of arbitrary and unitless values of tortuosity; tortuous vessel length in mm is easily comprehensible. Furthermore, by breaking apart all available vessels segments and by using the length of the abnormally tortuous vessels, it is possible to avoid any washout effect that may result from averaging the measure of tortuosity over the entire length of a segment, including its child branches, or averaging over all vessel segments in the entire image. Using the derivatives of the sequenced MAD measure, it should be possible to determine $180^\circ$ changes in the orientation (a twist) and to use the total number of such changes in vessel orientation as a weight to modify the AVT measure.

Considering the results of feature-selection analysis (see Section 8.2), it may be concluded that, based on the proposed methods and materials used, the measure of total tortuous-vessel length alone, which provides the highest diagnostic accuracy in CAD of plus disease based on the $A_z$, sensitivity, and specificity values, may be the single most important diagnostic factor in the diagnosis of plus disease; no combination of features led to better diagnostic accuracy.

The methods developed in this work can provide for quantitative assessment, objective evaluation, and diagnosis of plus disease in retinal fundus image of preterm infants in a clinical setting.
9.2 Potential Future Work

In addition to the suggestions presented at the end of Chapters 5, 6, and 7, future work could include incorporation of the method of Rangayyan et al. [224] to detect the center of the ONH automatically via phase portrait analysis of Gabor-angle responses. More research work could be considered on preprocessing steps that take into consideration the varying nature of the images of preterm infants and provide an enhanced gray-scale image to be used at the vessel-detection stage. The magnitude-response (vessel-strength) image could be improved by postprocessing steps for normalization to provide an image with uniform contrast for all vessels before the application of vessel segmentation methods. Thresholding methods that consider the nearly unimodal nature of the histogram of vessel-strength images are required for more accurate and automated segmentation of the entire vasculature. The reproducibility of the proposed features in relation to the various parameters used in the present work needs to be studied. Given the wide FOV of the RetCam images, it would of interest to determine whether any geometrical distortion may have affected the appearance of vessels in the periphery of the image and whether such distortion may affect the features proposed in this work. Further training, testing, and validation of the methods proposed and the GUI could lead to a practical approach for CAD of plus disease and ROP.

9.3 Contributions to the Field

The material contributions of the author in the present Ph.D. work are in the form of MATLAB software (code) for segmentation, tracking, and quantification of the thickness of the MTA (Chapter 5); detection of linear parts of vessel subsegments via analysis of vessel orientation (Sections 5.2 and 7.2); definition and computation of the AVT measure for quantification of tortuosity (Section 7.3); detection and measurement of the length of abnormally tortuous vessels (Section 7.4); and the GUI housing and presenting all of the methods for clinical application (Section 8.1).
One of the major conceptual contributions of the present Ph.D. work is the novel quantitative and objective method of accounting for sufficient abnormal tortuosity related to plus disease in terms of the total length of abnormally tortuous vessels as opposed to an arbitrary measure of tortuosity per vessel segment or per image as in related works by other researchers (Section 7.4).

9.4 Contributions to the Literature

9.4.1 Journal Publications

List of journal publications related to and developed during the Ph.D. program:


List of journal publications not directly related to, but developed during the Ph.D. program:


List of journal publications developed prior to the Ph.D. program:


9.4.2 Papers in Conference Proceedings

List of papers in conference proceedings related to and developed during the Ph.D. program:


List of papers in conference proceedings not directly related to, but developed during the Ph.D. program:


List of papers in conference proceedings developed prior to the Ph.D. program:


9.4.3 Books, Book Chapters, and Theses


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