CHAPTER 1
INTRODUCTION

In medical research, the diagnosis of ocular diseases is an important area for early and in-time treatment of people at high-risk. Retinal vasculature map is one unique feature of fundus image which is used for diagnosis of these ocular diseases. Various algorithms have been developed for computation of retinal vessel tree features from medical images, particularly using fundus retinal images. Vessel segmentation algorithm should be robust in nature so that we can extract the vessels accurately even in the presence of pathologies in the retinal image. With the use of automatic vasculature segmentation techniques, diagnostic performance has been improved a lot. Although promising techniques have been proposed, the extraction of accurate vasculature from the fundus image with the different type of pathologies is still the main issue. Superior image segmentation algorithms are needed for understanding and management of these circumstances in a better way. Segmentation of vessels in an accurate manner is a tedious job because of the following reasons:

- Fewer variations in the contrast between vasculature and surrounding tissue
- Presence of noise in the retinal fundus image
- Variation in the vessel width, branching angle, shape, and brightness.
- Presence of lesions, haemorrhage, exudates, and other pathologies.

Segmentation of vessels is possible using manual segmentation methods, semi-automatic methods, and fully automatic methods. Blood vessels segmentation using the manual method and the semi-automatic method is a very complex and time-consuming task because high skills and training are required in both these methods. Moreover, these segmentation techniques are susceptible to errors. With the use of fully automatic segmentation techniques, the problem of manual segmentation and semi-automatic segmentation can be overcome. These automatic techniques are helpful in the diagnosis of ophthalmic disorders in an efficient manner.
1.1. Motivation

Vascular diseases critically affect an individual, thereby presenting a health problem for the society which is challenging in nature. The task of managing and understanding all challenges in a better way motivates the requirement for superior imaging techniques. Retinal abnormalities can be examined and analyzed manually or semi-automatically using computer-assisted methods. The manual method used for analysis of retinal photographs is time-consuming, expensive, and subjective to each grader expertise. Semi-automatic methods require intervention from experts and consume a significant amount of time. The development of a fully-automated system for retinal image analysis would allow the assessment to be done on a huge set of retinal fundus images for disease screening on a large population. To develop such a system, highly accurate methods are needed to extract reliable results at each stage of the whole process. This research aims at studying novel and accurate algorithms for the automated extraction of the retinal vasculature map and its abnormalities from color retinal fundus images which are further used for prediction of various diseases.

1.2. Medical and technical background

Medical imaging includes algorithms for collecting quantitative information about the different parts of the body. Different properties of the tissues can be visualized by employing various modalities. Most widely used visual examination methods are stated as follows:

- Visible spectrum based photography
- Multi-spectral imaging: Various wavelengths that are invisible to the human eye are used for improving the spectral resolution. Information about the composition of the object to be photographed can be easily obtained by these imaging techniques.
- Imaging based on ultrasound
- Tomographic approaches
- MRI
Although all these imaging techniques are used only for the collection of quantitative information about various tissues and organs of the body, still the analysis of the image is possible only by image processing. All the images produced after visual examination methods cannot be used directly for analysis. So, different pre-processing, processing and post-processing techniques are employed for an understanding of these images in a better way. A very vast field of processing includes segmentation and characterization of various anatomical structures of images. The main motive of segmentation technique is the extraction of the various anatomical structures like an optic disk, blood vessels, and various organs, etc. and the main motive of the characterization technique is to offer a set of measures which are utilized to distinguish pathological and healthy structures. In the proposed study, the main work is focused on segmenting the vascular structure from the fundus image and characterizing the feature points of the retinal image.

### 1.3. The human eye: Rich source of information

The human eye is the most vital part of the human body. Our eyes are so tiny, but their action is very much important as they provide us with the power of vision. Light is passed onto the lens with the help of pupil and iris. Lens refracts the light onto the retina for the formation of the image. The image is transformed into electrical energy with the help of rods and cones. This electrical energy is initially sent to the optic disk on the retina and further, the energy is transferred to the brain with the help of optic nerve. Fig. 1.1 shows the anatomy of the eye. Detailed information about each part of the eye is mentioned in the coming sections.

- **Iris**

Iris is a visible and colored part of the eye lies in front of lens which helps in controlling the amount of light to be entered into eyes by adjusting the size of pupil i.e. in dark conditions, pupil expands so that more light can enter into eyes and in bright light, pupil contracts so that amount of light to be entered into the eyes is less. So, the narrowing and widening of the pupil are controlled with the help of iris.
- **Pupil**
  Pupil lies in the center of the iris, helps in forming the image of the object by allowing the light to enter into our eyes. The light entered through pupil is further directed towards the lens which further sends the light onto the retina.

- **Cornea**
  It is of circular shape which is transparent in nature. It is used to refract the light enters into the eye onto the lens, which is further used to focus the light onto the retina.

- **Lens**
  In a normal camera, the image of an object is produced with the help of a lens inside the camera. Similarly, our eyes also have a lens which is a transparent part located behind the pupil. The purpose of this lens is to bend or refract the incoming light onto the retina.

- **Sclera**
  It is the white part of the eye, which is used to provide external protection to the eye.

- **Choroid**
  The choroid is present in between the sclera and retina. Excess light is absorbed with the help of pigment present in choroid to prevent blurring of vision.
• **Ciliary body**  
  It is used to make a connection between choroid and iris which is the part of the eye.

• **Retina**  
  It is a layer which is sensitive to light and is present at the back surface of the eye. Approximately 65% of the interior surface is covered only by the retina. The working of a retina in the eye is similar to working of a film in the camera.

• **Rod cells**  
  These are light-sensitive cells which are approximately 125 million in number present in the retina of the eye. In the dim light, vision is possible due to the presence of only rod cells.

• **Cone cells**  
  These are light-sensitive cells which are approximately 6-7 million in number present in the retina of the eye. In bright light, vision is possible due to the presence of only cone cells. Different cons are sensitive to different wavelengths of color. Based on that, there are three types of cons- red, green and blue. Other colors can be produced after the combination of these primary colors. Other than this, macula, fovea, optic disc, optic nerve are the important parts of an eye which are also discussed in further sections.

### 1.4. Retinal image capture

Retinal image is captured with the help of specialized camera known as fundus camera. The image captured using fundus camera contains all features of retina; this image is known as fundus image. Details of fundus camera and fundus image are given in coming sections.

1.4.1. **Fundus camera**

A fundus camera as shown by Fig. 1.2 is just like a microscope on which camera is attached for photography purpose. It is manufactured to take pictures of the interior anatomical structures of the eye like an optic disc, blood vessels, macula, fovea, optic nerve, and posterior pole. A retinal fundus camera which is non-mydriatic in nature is used to capture details of interior parts of the fundus image.
Initially, the image is captured with the help of a camera which needs dilation of pupil 30-minutes before the photography. But with the use of today’s cameras which are non-mydriatic in nature, fundus photography can be performed in less time due to the elimination of the requirement for bright lights and dilating drops.

A non-mydriatic fundus camera has many features which are discussed below:

- Does not require pupil dilation in the majority of cases and is painless for patients.
- Provides identification and monitoring of abnormalities present in the eye like glaucoma, high blood pressure, and diabetic retinopathy, etc.
- Offers images that visually add to ophthalmologist notes, and creates a fuller picture of eye health that can be shared with other physicians easily in the collaborative management of patient health.
- Easy to operate because little training is required in the operation of a camera.
- Offers a wide angle of the targeted retina.
- It has different filters to choose from for the purposes of image enhancement.

1.4.2. Fundus image

The fundus of the eye as shown by Fig. 1.3(a) is the surface which is present opposite to the lens. This surface includes the optic disc, blood vessels, macula, fovea, and posterior pole. The fundus of the eye is examined using fundus photography. Medical symptoms [3-5] that can be identified from eye's observation fundus include cotton wool spots, haemorrhages, and exudates, various blood vessel abnormalities like tortuosity, neovascularization, pulsation, and pigmentation.

1.5. Features of the retinal image

There are two types of features which are observed by the retinal fundus image i.e. normal features and abnormal features of the retinal fundus image. Normal features are those which include the interior of the human eye and are necessary for vision. Abnormal features are those which are present in the diseased eye and needs to be diagnosed on time.
Different features of the retinal fundus image as shown by Fig. 1.3 (b) are described below.

Fig.1.2: Fundus camera [2]

Fig.1.3: (a) Normal camera image and fundus camera image of the eye [6], and
(b) Different features of retinal fundus images [7]
1.5.1. Normal features of the retina

Normal features of retina required for vision are optic disc, blood vessels, optic nerve, macula lutea, fovea, and peripheral retina as shown by Fig. 1.3(b).

(i) Optic disc

The optic disc is just like a bright hole located at the back area of the eye. All major retinal blood vessels enter through this optic disk. The optic nerve which is used to send signals to the brain also starts from the optic disc.

(ii) Blood vessels

Retinal vasculature is a complex network, which consists of hollow pipes of various dimensions. This tree-like structure is useful to find the other normal features of the retina such as macula or fovea or optic disk or for the automatic identification of pathological elements like haemorrhage, microaneurysms, exudates or lesions. Blood vessels are classified into two main types i.e. arteries and veins. Arteries are brighter in color and they are used to transport blood which is high in oxygen level from the lungs and heart to the eye and the veins are darker in color and they are used to transport blood which is low in oxygen level from the eye back to the lungs and heart.

(iii) Optic nerve

The optic nerve is just like a wire of nerve fibers which is used to pass entire visual information towards the brain. With the help of optic nerve, all electrical signals are carried to the brain from the retina for further processing.

(iv) Macula lutea

It is a highly sensitive area which is responsible for central vision. Macula appearance is like yellow spot which lies in the center of the retina and fovea lies in the center of the macula. The requirement of macula is to perform all activities that require fine, sharp vision like reading, recognizing faces, etc.

(v) Fovea

The fovea which is avascular in nature is located in the center of macula. The diameter of fovea is approximately 1.5mm. Fovea contains the large concentration of photoreceptors, mainly cone cells, which are responsible for color vision with high
resolution. Whenever our eye watches any object, the image which is produced on the fovea is the image which is accurately registered and processed by the brain.

(vi) **Peripheral retina**
The peripheral retina is the portion of the eye on which rods and cones are present. Peripheral vision is possible only with the help of peripheral retina.

**1.5.2. Abnormal features of the retina**
In diabetic retinopathy progression of different blockages and damage to different vessels of the retina can cause the development of areas of retinal ischemia. Due to damage of different retinal blood vessels, fluid and blood may leak on the retina which produces features like haemorrhages, exudates, cotton wool spots, and new vessels, etc. as shown by Fig. 1.4. The appearance and significance of some of the main features of diabetic retinopathy are discussed in detail.

(i) **Microaneurysms**
The structure of microaneurysms is just like a balloon which arises on the surface of capillaries walls as shown by Fig. 1.4(a). Microaneurysms appear like round, small and dark isolated red dots which are unattached to any blood vessel. Whenever there is a retinal breakdown or arterial blockage in the eye, these microaneurysms get produced.

(ii) **Haemorrhages**
Haemorrhages which are of variable size and shape are produced when the blood leaks from the vessels due to the breakdown of the capillary walls as shown by Fig. 1.4(b). Causes of haemorrhage are arterial vein blockage and hypertension. Some retinal haemorrhage on retina also leads to major impairment of the vision.

(iii) **Exudates**
Exudates are situated in the posterior pole of the retinal fundus image. These appear as dark yellowish spots as shown by Fig. 1.4(c) and usually produced when fat or lipid leaks from aneurysms or abnormal retinal blood vessel. Among diabetic patients, exudates are the most common reason for impairment of vision.
(iv) Cotton wool spots
Cotton wool spots appear on the retina as fluffy white patches as shown by Fig. 1.4(d). Due to damage of various nerve fibers and due to deposition of axoplasmic material within the nerve fiber, cotton wool spots appear.

(v) New vessels
Due to the revascularization of the oxygen-deprived tissue, growth of new vessels gets triggered. Retinal detachment [13] can also occur due to the development of fibrous tissue around new vessels. Generation of new vessels as shown by Fig. 1.4(e) may lead to severe vision loss which can be reduced using laser treatment. The characterization of new vessels is difficult due to the following reasons:

- Unregulated growth of vessels
- New vessels are very faint, so it’s difficult to distinguish it from the background.

1.6. Challenges in retinal image processing
The retina of patient is scanned by the oculists with the help of high-resolution fundus camera. Various retinal diseases can be detected using the retinal vasculature map. Mostly, the contrast of the retinal vasculature is low with respect to its background. So, the use of accurate image segmentation algorithm is required for segmentation of vasculature map from the fundus image, since it further leads to an accurate diagnosis. Different challenges faced by segmentation of retinal vasculature are stated as follows.

- **Variation in pixel width**

Retinal vasculature map is a complex network of various blood vessels having different pixel width. The range of intensity of color for a vessel in the fundus image may vary from one pixel up to more than five pixels, as shown by Fig. 1.5(a). So, identification of technique which is highly flexible in nature is a challenging task for detection of vasculature map of the fundus image.
Fig. 1.4: Retinal fundus images with (a) Microaneurysms [8], (b) Haemorrhages [9], (c) Exudates [10], (d) Cotton wool spots [11], (e) New vessels [12], and (f) Retinal detachment [13]
➢ **Variation in contrast**

Retinal blood vessels either tiny [15-16] or large have the same color intensity as that of its background color as shown by Fig. 1.5(b). So, identification and extraction of vasculature map due to fewer contrast variations is a highly challenging task.

![Variation in the pixel width of various retinal vessels, and Vessel type distribution on retina surface](image)

**Fig.1.5:** (a) Variation in the pixel width of various retinal vessels, and (b) Vessel type distribution on retina surface [14]
Presence of pathologies

Identification of vascular map near pathologies like exudates, haemorrhage leads to false detection of vessel pixels. So, segmentation of vasculature map from the retinal fundus image having pathologies is also one of the most challenging tasks.

1.7. Retinal image analysis applications

The retinal vasculature map is the unique property that can be readily photographed and can be visualized non-invasively. The images which contain the extracted microvascular map can be further used for analysis of digital images. There are a lot of areas in which the retinal vasculature map can be used. Some of them are mentioned below:

- For detection and early diagnosis of pathologies

Information about the location of the retinal vasculature and assessment of its morphological attributes like length, diameter, width, tortuosity and branching angle is important for diagnosis, screening, and treatment of various disorders such as stroke, cardiovascular diseases, hypertension, diabetes, and arteriosclerosis.

So, the quantitative measurements of the topography of the retinal vasculature map [17-18] can be used as a tool for research for better understanding the relationship between the microvasculature map and various cardiovascular diseases.

- For authentication purpose

For each individual person, our retinal microvascular system [19-20] is unique in nature and there is no evolvement of vasculature map in complete life span of an individual person. So, this microvascular system termed as retinal “fingerprint” can be further utilized to check the authenticity.

1.8. Databases used in retinal image processing

Retinal databases are those which contain normal as well as pathological retinal fundus images. Various public databases have been used for quantitative comparison
of the performance of various algorithms developed by different authors. A list of
different databases used for retinal fundus images is mentioned below:

- DRIVE (Digital Retinal Images for Vessel Extraction) [21]
- STARE (Structural Analysis of Retina) [22]
- ARIA (Automated Retinal Image Analysis) online [23]
- Messidor [24,25]
- REVIEW (Retinal Vessel Image set for Estimation of Widths) [26]
- ROC (Retinopathy Online Challenge) microaneurysm set [27]
- VICAVR [28]
- CHASE_DB1 (Child Heart and Health Study in England) [29]
- DIAbietes RETina Data Base 0 (DIARETDB0) dataset [30]
- DIAbietes RETina Data Base 1 (DIARETDB1) dataset [31]
- DIAbietes RETina Data Base v2.1 (DIARETDBv2.1) dataset [32]
- High Resolution Fundus (HRF) [33].

Table 1.1 represents the information about each database in detail.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Source</th>
<th>Number of Images</th>
<th>Resolution</th>
<th>Field of View (FOV)</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRIVE</td>
<td>[21]</td>
<td>40</td>
<td>768x584 pixels</td>
<td>45°</td>
<td>Ground truth images are available for both test and training dataset images.</td>
</tr>
<tr>
<td>STARE</td>
<td>[22]</td>
<td>397</td>
<td>700x605 pixels</td>
<td>___</td>
<td>For 40 images, manual blood vessel segmentation is available. For 10 images, artery/vein labeled images are available. For 80 images, ground truth of optic disc is available.</td>
</tr>
<tr>
<td>ARIA</td>
<td>[23]</td>
<td>143</td>
<td>768x576 pixels</td>
<td>50°</td>
<td>This database contains both normal and abnormal fundus</td>
</tr>
</tbody>
</table>
Based on the presence of haemorrhages, microaneurysms, and neovascularization, grading score is mentioned regarding stage of diabetic retinopathy.

<table>
<thead>
<tr>
<th>Database</th>
<th>[Reference]</th>
<th>Images</th>
<th>Vessel Measurement</th>
<th>Angle</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Messidor</td>
<td>[24,25]</td>
<td>1200</td>
<td>1440x960, 2240x1488, and 2304x1536 pixels</td>
<td>45°</td>
<td>Based on the presence of haemorrhages, microaneurysms, and neovascularization, grading score is mentioned regarding stage of diabetic retinopathy.</td>
</tr>
<tr>
<td>REVIEW</td>
<td>[26]</td>
<td>16</td>
<td>193 segments of vessels</td>
<td></td>
<td>16 images with 193 segments of vessels are used for implementing and comparing various vessel measurement techniques. Ground truth of vessel edges marked by three experts is also available.</td>
</tr>
<tr>
<td>ROC</td>
<td>[27]</td>
<td>100</td>
<td>768x576, 1058x1061 and 1389x1383 pixels</td>
<td>45°</td>
<td>50 images are training images and 50 are test images.</td>
</tr>
<tr>
<td>VICAVR</td>
<td>[28]</td>
<td>58</td>
<td>768x584</td>
<td></td>
<td>Retinal images present in this dataset are helpful in the computation of the A/V Ratio. Ground truth of A/V marked by three experts is also available.</td>
</tr>
<tr>
<td>CHASE_DB1</td>
<td>[29]</td>
<td>28</td>
<td>1280x960 pixels.</td>
<td>300°</td>
<td>It contains retinal fundus images of both eyes of 14 children.</td>
</tr>
<tr>
<td>DIARETDB0</td>
<td>[30]</td>
<td>130</td>
<td>1500x1152 pixels</td>
<td>50°</td>
<td>It contains 110 abnormal images. Ground truth file for every</td>
</tr>
</tbody>
</table>
image is available containing the optic disc/macula centers and all lesions appearing in the specific retinal image. This database is known as "calibration level 0 fundus images".

<table>
<thead>
<tr>
<th>Database</th>
<th>Reference</th>
<th>Images</th>
<th>Resolution</th>
<th>50'</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIARETDB1</td>
<td>[31]</td>
<td>89</td>
<td>50°</td>
<td></td>
<td>84 images with non-proliferative signs and 5 images are normal images. This database is known as &quot;calibration level 1 fundus images&quot;.</td>
</tr>
<tr>
<td>DIARETDB1 v2.1</td>
<td>[32]</td>
<td>89</td>
<td>1500×1152 pixels</td>
<td>50°</td>
<td>It contains 28 training and 61 test images. Ground truths of fundus images are also available.</td>
</tr>
<tr>
<td>HRF</td>
<td>[33]</td>
<td>45</td>
<td>3504×2336 pixels</td>
<td></td>
<td>This database contains high-resolution images of three types of patients: healthy patients, patients suffering from diabetic retinopathy and glaucomatous patients. The number of images of each type is 15. Ground truth of blood vessels and masks are also available.</td>
</tr>
</tbody>
</table>

Mostly all retinal segmentation techniques and algorithms use DRIVE and STARE database which is the most popular databases. These databases are popular due to the two reasons: the presence of fundus images of high resolution and presence of gold standard images which are produced by experts using manually labeling method. In
proposed work, DRIVE database is used for extraction of vasculature map and for computation of different features points of the retinal fundus image.

1.9. Retinal performance metrics

Pixel base classification is used for segmentation of retinal vasculature map from the fundus image. Pixel classification is done on the basis of whether the pixel belongs to a vessel or some other tissue. So, four different possible events are possible which include pixel classifications and pixel misclassifications. True Positive (TP) and true Negative (TN) are the two-pixel classifications and False Positive (FP), and False Negative (FN) is the two-pixel misclassifications. The number of these four different classifications can be used for the evaluation of various performance metrics.

An event is classified as TP if a vessel pixel is correctly identified as vessel and TN if the non-vessel pixel or pixel in the surrounding tissue is correctly identified as a non-vessel pixel.

An event is classified as FN if the predicted pixel represents non-vessel pixel but actually, it was vessel pixel. An event is said to be FP if the predicted pixel represents vessel pixel but actually, it was a non-vessel pixel. The important performance metrics which can be derived from the above events are Sensitivity (SN), Specificity (SP) and Accuracy (Acc).

(i) Sensitivity

Sensitivity metrics represents the capability of a segmentation algorithm to identify the vessel pixels. Sensitivity is defined as the ratio of TP to the sum of TP and FN. The range of SN is between 0 and 1. More sensitivity means algorithm is able to identify vessel pixels correctly. SN measure is expressed as by Eq. (1.1)

\[ SN = \frac{TP}{TP + FN} \]  

(ii) Specificity

Specificity metrics represents the capability of a segmentation technique to identify background or non-vessel pixels. Specificity is also defined as the ratio of TN to the sum of TN and FP. The range of SP is also between 0 and 1. More SP means
algorithm can identify non-vessel pixels correctly. SP measures are expressed by Eq. (1.2)

\[ SP = \frac{TN}{TN + FP} \]  

(iii) Accuracy

Accuracy metrics is evaluated by taking the ratio of the total number of true events which is the sum of TP and TN, to the total population which is the total number of pixels actually present in the image. Accuracy measure is expressed by Eq. (1.3)

\[ Acc = \frac{\sum TP + \sum TN}{\sum Total \ Population} \]  

1.10. Organization of thesis

The structure of the thesis is organized as follows:

Chapter 2 presents the literature review of binary mask generation, vasculature map extraction, and feature point detection is presented in detail. Binary mask generation can be performed using various methods which mostly include thresholding methods and morphological methods. Vessel extraction methods can be performed using various vessel segmentation algorithms such as kernel-based algorithms, tracking based algorithms; morphology-based algorithms; model-based algorithms; thresholding base algorithms; and machine learning-based algorithms. Feature point detection is mostly performed using window-based techniques. Further, complete research methodology for the proposed work is also presented.

In Chapter 3, the methodology for the generation of a binary mask is presented. This methodology uses a bimodal masking technique for the generation of a mask of the fundus image.

Chapter 4 presents two different methods for extraction of vasculature map of fundus image which includes Pixel Level Snake (PLS) and Modified Pixel Level Snake (MPLS). Pixel level snake is iterative technique, in which counter evolution is performed using the external potential of the image which is computed using edge based techniques. This technique is modified further and modified technique known as MPLS is further used for evolution of vasculature map of the fundus image in four
cardinal directions. In MPLS, an external potential is computed using Black Top-Hat (BTH) or Bottom Hat transformation technique.

Chapter 5 presents the methodology for detection of bifurcation points and cross-over points of the vasculature map. The new approach used for identification of points is Modified Window Feature-point Detection (MWFD) technique in which 3x3 and 5x5 windows with alternative vessel pixel approach have been used for computation of feature points of the fundus image.

Chapter 6 presents the conclusion of the proposed work, which summarizes the findings of the work and discusses areas for future investigation.
CHAPTER 2

LITERATURE REVIEW

Early detection of various abnormalities of the fundus image is necessary for identification of various cardiovascular diseases. An effort has been made for the development of various algorithms that can extract various features of fundus image accurately and efficiently so that various eye related disorders can be identified on time. Certain Image processing techniques are applied on fundus images for the extraction of the binary mask of fundus image, vasculature map of retinal fundus image and detection of feature points. Different morphological attributes of vasculature map, like significant feature points, can be used for identification and treatment of different cardiovascular disorders.

Here, the literature review is divided into three sections. Section 2.1 represents the review of different masking algorithms used for the generation of the binary mask. The mask of the fundus image is used to get a region of interest (ROI) of fundus image which will reduce the analysis time and computational effort as operations will be focused only on the object pixels. Section 2.2 represents the review of different methodologies used for the extraction of vasculature map of the fundus image. Section 2.3 represents the review of different methods used for the detection of feature points of the vasculature map.

2.1. Literature based on binary mask generation

Different masking algorithms have been used in literature for extraction of the mask of the retinal fundus image. In the existing literature, various techniques have been used for the extraction of mask of the fundus image.

Gagnon et al. [34] developed a technique in which thresholding is applied on three channels separately to generate a binary image for each band. Thresholding is performed using a 4-sigma thresholding technique in which pixel values above the threshold are considered to be part of ROI. Logical operators are then used to
combine the binary results of all bands, identifying the largest common connected mask.

Englmeier et al. [35] developed a technique in which processing of black border of the image is avoided by employing the automatic mask generation technique.

Goatman et al. [36] developed a technique in which initially 5x5 median filtering is applied and after that mask is generated automatically by thresholding of the green band of the retinal fundus image.

Abramoff et al. [37] presented a technique in which mask is generated using image processing tools like ImageJ software.

Ter Haar [38] proposed a method in which threshold of t=35 is applied to the red color band and then the morphological operators were applied using a 3x3 square structuring element for generation of the ROI of the fundus image.

Youssif et al. [39] presented a methodology in which initially adaptive histogram equalization is applied. After that, thresholding and morphological operations using 3x3 square shape structuring element are applied on the red channel of fundus image to generate final ROI mask.

Akram et al. [40] presented a methodology in which background masks and noise masks are computed initially. For the generation of background pre-processing mask and noise mask, initially input retinal image I(i, j) is divided into 8x8 nonoverlapping blocks. For each block, local mean value M(I) and local standard deviation value std(I) is computed. Background mask is generated using std(I) and a threshold value. For the generation of noise pre-processing mask contrast enhancement has been performed using histogram equalization. Noise in the background region of the image is reduced using a 3x3 median filter. Parameters used for the generation of noise mask are noise factor and a threshold value. Final mask is generated by a combination of both background mask and noise mask. Further, morphological erosion is used for removal of white single pixel noise and morphological dilation is used for removing black single pixel noise.

Giancardo et al. [41] presented an algorithm in which region growing technique is used for the generation of a binary mask of the retinal fundus image. The green band
of the retinal image is taken for this task. Computational complexity is reduced by scaling down the image.

Zheng et al. [42] used mean filtering of fundus image initially. After that retinal mask generation and correction of illumination has been performed for segmentation of mask of the fundus image.

Manjiri et al. [43] presented an algorithm in which histogram equalization and thresholding function have been employed for the generation of a mask.

Hashim et al. [44] developed an automatic technique for the generation of a binary mask. For this purpose, the Gaussian filter has been used to define the ROI of the fundus image.

Chong et al. [45] presented an algorithm in which histogram equalization and thresholding function have been used for mask generation.

Santhakumar et al. [46] used Image processing tools like ImageJ software for the generation of the mask of the fundus image. Log transformation is applied to the green band of the fundus image. Otsu’s thresholding is used for conversion of produced log transformed image into a binary image. Mask of the fundus image is produced by applying closing operation with a disk-shaped structuring element having radius 20 on the binary image.

Aibinu et al. [47] developed an algorithm based on pseudo modeling for the generation of a binary mask of the fundus image. Synaptic weights of the neural network are used for the estimation of coefficients which are further used for the generation of the mask.

2.2. Literature based on retinal vasculature map segmentation

Retinal vascular network segmentation is a tedious task due to less contrast of vessels with respect to background tissue. Due to various challenges faced in the extraction of vasculature map robust segmentation techniques are required. The use of classical segmentation methods such as Sobel [48], Prewitt [49], gradient [50], and Krish and Robert differential operations [51] leads to inefficiency and inaccuracy of the system.
In literature, various vessel segmentation techniques are categorized according to the methodologies employed in image processing. Various segmentation techniques used for the detection of blood vessels are classified into two categories: Rule-based techniques or Machine learning techniques. In rule base learning, specific rules are followed in an algorithmic structure, whereas in machine learning, ground truth image is used to make a labeled dataset in the training process. Both these techniques are further classified into various techniques such as kernel-based algorithms, tracking based algorithms; morphology-based algorithms; multiscale based algorithms; model-based algorithms; thresholding base algorithms; machine learning based algorithms and hardware-based techniques.

Sometimes, a combination of various techniques known as hybrid techniques is also made to improve the accuracy of the system. Various hybrid techniques used for segmentation of vascular map are proposed in [52-57].

### 2.2.1. Matched filtering or kernel based techniques

Profiling of vessels has many applications in areas of vessel width measurement [58] and the classification of various vessel types [59]. The process of segmentation of retinal vasculature map is possible using various filtering based approaches or region growing approaches. Matched filters approach convolve a 2-D structural element (kernel) which is linear in nature with a retinal image for segmentation and extraction of the retinal vascular network. The kernel is designed to rotate at many different orientations to fit into vessels of different configurations and the presence of this feature is identified by the matched filter response (MFR). Due to the less contrast variation between a vessel and surrounding tissue or due to the presence of noise and pathologies in the fundus image, the number of false responses increases. So, MFR method in conjunction with various image processing techniques is found to be very efficient. Image enhancement is performed with the help of matched filters which do not perform in isolation, so this approach is followed by other approaches like thresholding for the correct identification of pixels belongs to vessel.
Chaudhuri et al. [60] presented a technique in which an operator based on the spatial and optical properties of objects is developed for the extraction of features. Piecewise linear extraction of vasculature map is detected using a matched filter technique. Searching for blood vessel segments is performed by constructing twelve unique templates along with all directions.  

KUMAR et al. [61] presented a technique in which a green band of the retinal fundus is used. Enhancement of blood vessels is performed using 2D matched filter. After that line detection technique is applied to the enhanced vessels for segmentation purpose. This line detection technique uses four directional filters for filtering. Finally, the vasculature map is extracted by taking the integration of the output of each directional filter.  

Wang et al. [62] developed a technique in which an approach based on matched filtering with multi-wavelet kernels has been used for vessel enhancement. Further, noise from the enhanced image is removed and vessels are localized using the decomposition process. Finally, the vasculature map is obtained by locally adaptive thresholding.  

Kochner et al. [63] proposed a model based technique for the segmentation of vasculature map of retinal fundus photographs. For efficient implementation purposes, the approach of steerable filters is used. Steerable filters [64] are those which are applied only in two basic directions. The response in other directions is calculated using combinations of individual responses produced from two basic directions [65].  

Villalobos-Castaldi et al. [66] presented an algorithm where matched filter is used along with adaptive thresholding technique for extraction of vessels. In this work initially, enhancement of vasculature map is performed using a matched filter approach. Then all grey-level variations were depicted using a co-occurrence matrix. After that background pixels are separated from the foreground pixels using entropy-based thresholding. The total time recorded for the segmentation process is 3sec.  

Chanwimaluang and Fan [67] used the same method for the extraction of blood vessels and optic disk as proposed in [66]. The algorithm was applied to images of
STARE database. Instead of only matched filtering approach and entropy-based thresholding approach, some post-processing steps were also applied in this algorithm. Morphological operation thinning is also used for identification of crossover and intersections. The total time recorded for the segmentation process is 2.5min.

Singh et al. [68] used a similar algorithm as proposed by [66, 67]. But the overall performance of the system was increased after modification of the parameters of Gaussian function.

Al-Rawi et al. [69] presented a technique which is applied to images of the DRIVE database. In this work also Gaussian-kernel parameters are modified for extraction of the vasculature map.

Kaur and Sinha [70] developed an algorithm in which 12 different Gabor filters oriented in different directions were used for enhancement of blood vessels. Use of this Gabor filter approach instead of Gaussian function improved the accuracy of the system.

Zhang et al. [71] used two matched filters on fundus images due to the symmetric nature of vessels and due to the asymmetric nature of non-vessles. Gaussian kernel and first-order derivative of Gaussian kernel is used for vessels and non-vessels structures respectively. Detection of vessels is performed using a matched filter which uses Gaussian kernel whereas dynamic threshold is adjusted using the local mean of the first-order derivative of Gaussian kernel.

Zolfagharnasab et al. [72] used a similar algorithm as proposed by [71]. The only difference is the use of Cauchy probability density function instead of a matched filter Gaussian kernel.

Kumar et al. [73] presented a technique in which initially enhancement of retinal vessels is performed by Contrast Limited Adaptive Histogram Equalization (CLAHE) algorithm. After that, 2D matched filters with Laplacian of Gaussian kernel functions has been used for extraction of vasculature map.
Singh and Strivastava [74] proposed a filtering approach in which Gumbel probability density function is used as a kernel function. All isolated pixels are removed using entropy-based optimal thresholding in the post-processing phase.

Fraz et al. [75] developed a technique in which methodology based on ensemble system of bagged and boosted decision trees are used. In this algorithm, a feature vector [76] is used to handle normal and pathological fundus images.

2.2.2. Vessel tracking based techniques

Vessel tracking algorithms use local information for segmentation of a vessel between two points. In tracking algorithms, vessel center lines are followed which are guided by local information.

Advantages of vessel tracking algorithm:

- It measures the width of vessels accurately.
- It provides individual information of vessels which can’t be extracted using other methods.
- It provides information about various branches and connections between branches.
- Computation time is less due to the existence of a connection between the vasculature structures.

In vessel tracking mechanism, based on starting seed-points, ridges of fundus image are traced. Selection of seeds is the initial step which is performed using a manual or automatic method. Vessels ridges are detected by inspection of the zero-crossing gradient. Ridge detection procedure needs some technique which involves complicated steps for enhancement of vessels due to various vessels sizes and orientations.

Liu et al. [77] presented an algorithm in which the detection of vasculature map is performed by taking some initial points on vessels. After that, trajectories are estimated recursively for segmentation of vasculature map.

Zhou et al. [78] developed a methodology for finding the vessel centerline course and for measurement of the tortuosity and diameter. New locations were searched
based on recent location using Gaussian matched filter [79]. In the final stage, estimation is verified and then the new location is re-estimated recursively. 

**Quek et al. [80]** proposed vasculature extraction technique based on wave propagation and traceback mechanism. The speed of the wave is fast when it propagates through low refractive index pixels known as vessel pixels and the speed of the wave is slow when it propagates through high refractive index pixels. Based on the speed of the wave, extraction of the vasculature map had been made. 

**Can et al. [81]** developed an algorithm in which vessel tracking operation is performed recursively using directional templates. Vessel tracking is performed by taking some initial seed points. The algorithm moves in maximum response direction calculated using the directional template for estimation of new location iteratively. 

**Poon et al. [82]** developed a semi-automatic technique for the extraction of vasculature map of fundus images. Multiscale vesselness filtering is also included in the conventional livewire framework [104]. Dijkstra's algorithm is used for determination of seed points and for finding contour which exists between these points. 

**Delibasis et al. [83]** developed a tracing algorithm for extraction of vessels and estimation of diameters. To define parameters, geometric properties are exploited using parametric model consists of “strip”. Seed pixels are initialized using vesselness filter [84] for tracking of vessels. Further, seed point, the orientation of strips, the width of strips and the measure of the match are used for identification of matching strip with the retinal blood vessel. 

**Wu et al. [85]** developed a vessel tracking technique based on a combination of Hessian matrix and matched filters for extraction of vasculature map. This method also used edge information at vessel boundaries as developed by Sofka and Stewart [86]. In this initially, contrast enhancement of vessels has been done and after that information of size and orientation of enhanced vessels is collected. After getting this information, ridges are used for tracing the vessels through vessel center lines along automatically selected seeds.
Yedidya and Hartley [87] developed a tracking based methodology for the detection of both thin and wide vessels. This algorithm consists of four operations. Initially, selection of seed points had been made. Secondly, vessel tracking is performed using a Kalman filter. Thirdly, the probability of vessel tracing had been checked. If the probability of tracing the vessels is less, then the tracing process gets ceased. Finally, the tracing of all segmentation results is also performed.

Yin et al. [88] developed a statistical-based method in which edge points are detected iteratively using a Bayesian approach [89]. After that, profiles and geometric properties of vessels are combined to improve the accuracy of the system.

2.2.3. Mathematical morphology based techniques

The word morphology deals with the shape and structures of objects. Two operators used in morphological processing are dilation and erosion. Structuring element of certain intensity and shape is used by these dilation and erosion operations. Expansion of an object is done by dilation operator and compression of an object is done by erosion operator. Two more operations are opening and closing of an image which is built up from dilation and erosion.

Opening operation on an image \(I\) using the structuring element \(S\) is defined by Eq. (2.1)

\[
I \circ S = (I \ominus S) \oplus S
\]  

(2.1)

Closing operation on an image \(I\) using the structuring element \(S\) is defined by Eq. (2.2)

\[
I \cdot S = (I \oplus S) \ominus S
\]  

(2.2)

Where \(\oplus\) represents the dilation operation and \(\ominus\) represents erosion operation.

Opening and closing operations are mostly used together in the image segmentation process for the selection of features. Features size can be enlarged and reduced repeatedly with the help of these operations, allowing the elimination of noise and very small details. Watershed and top hat transformations are the two methodologies which are related to the mathematical morphology and can be used in image segmentation for numerous medical applications.
Miri et al. [90] presented a technique in which curvelet transform coefficients are modified for enhancement of edges of the image. Image ridges are computed using morphological operations with various structuring elements. After that thresholding technique along with connected components analysis is used for indication of edges which belongs to vessels. To increase the efficiency, connected components analysis is applied locally instead of an entire image.

Hassan et al. [91] developed a technique in which initially, mathematical morphology is used for enhancement of vasculature map and for suppressing of background data. After that K-means clustering is used for extraction of the vasculature map which is further enhanced by the morphological operation.

Kundu et al. [92] proposed a morphological angular scale-space technique. In this technique, all connected components are determined using multiple structuring elements at multiple angles. The use of various structuring elements creates scale-space across the blood vessels. Information extracted from lower scales is further used to get information about higher scales.

Salem et al. [93] developed an algorithm using morphological tools which further used watershed transform [94], gradient, distance function, top-hat transform, and geodesic distance for segmentation purpose instead of only morphological operators. Lowest mean square error for DRIVE database had also been computed by this method which comes out to be 0.0363.

Frucci et al. [95] proposed a technique in which initially, the image is divided into multiple regions using watershed transformation. Then grey-level values are assigned to each individual region and computation of contrast is made by taking the difference between adjacent grey-levels. The directional map is made in 16 directions by taking 9x9 window around each pixel. After computation of contrast and direction map, segmentation is performed. The region with high contrast is known as non-vessel region and region with low contrast is termed as a vessel.

Jiang et al. [96] developed a global thresholding technique based on morphological operations for extraction of vasculature map. The system is designed to perform
multiple operations in parallel, which further reduced computational complexity and
time complexity of the system.

Mendonca et al. [97] developed an algorithm for extraction of retinal vasculature
map, in which for differential operators oriented in particular direction are used for
selection of points which can be further classified into centreline pixels. Final
extraction of the map is performed using region growing technique iteratively. In this
technique, segmentation is performed by taking the integration of information
retrieved from various binary images which are produced from various morphological
filters.

2.2.4. Multi-scale based techniques

In multi-scale techniques, the image is represented at multiple levels or scales [98]. In
these techniques, convolution is performed using smoothing kernels which includes
Gaussian kernel and its derivatives with increasing scales or widths [99]. Initially, the
framework for multi-scale image representation is scale-space. [100]; along with that
pyramid [101], and Quad-tree [102] are the two most used types of multi-scale
representation.

With the scale level, the size of the retinal image decreases exponentially which
further reduced the computation time. The structures of the eye, which are fixed in
size, are difficult to extract because these techniques are best suited for the
anatomical structures which show variation in width and length.

Budai et al. [103] presented a multi-scale algorithm for extraction of vasculature
map. Initially, Gaussian pyramid is generated on the green band of retinal fundus
image; then neighborhood analysis operation is performed in which, a Hessian matrix
is used to check belongingness of vessel pixels and finally, binarization is performed
using two thresholds and images fusion is performed using pixel-wise OR operation.

Martinez-Perez ME et al. [104] developed a technique based on a multiscale
approach for extraction of vasculature map of fundus image which includes vessels of
varying lengths, widths, and orientations. Segmentation is performed by taking
spatial information along with feature information. Branching angles and vascular diameters are also computed and verified using ground truth images.

**You et al. [105]** proposed a technique in which thin and wide vessels are extracted separately using a radial projection method and semi-supervised self-training method respectively. Enhancement of vessels at various scales is performed by using steerable complex wavelet. Also, a feature vector is constructed for representation of pixels of vessels by line strength. In the end, a union operation is applied to obtain the entire retinal vascular map.

**Moghimirad et al. [106]** presented a multi-scale based technique for segmentation of vasculature map. Initially, a weighted medialness function is used along with the hessian matrix eigenvalues and then final segmentation is performed using vessel reconstruction in which centerline and radius of vessels are extracted simultaneously.

**Abdallah et al. [107]** proposed an algorithm based on multi-scale for vasculature map segmentation. Initially, noise is removed by using anisotropic diffusion method. Multiple scales are computed for a multi-resolution image. After that, eigenvalues and vectors are analyzed for each scale. The net result is represented by taking the maximum values of the pixels computed over all scales.

**Rattathanapad et al. [108]** presented a technique which used multilevel line detection and line primitives for segmentation of retinal vasculature map. In multilevel line detection technique, retinal vessels are extracted by taking various values of Gaussian smoothing parameters. Finally, the line primitives extracted at various scales were merged to produce one single vessel.

**Zheng et al. [109]** proposed a technique in which multiscale Hessian-based filter has been employed for enhancement of vessels. Nonlocal mean filter and radial gradient symmetry transformation has been used for suppression of noise and non-vessel structures respectively. Graph cut has also been used for segmentation of vessels.

**Farnell et al. [110]** employed a multiscale line operator technique on the green band of retinal image for segmentation of vasculature map. After that, results produced using the median filter is compared with multiscale line operator results.
Enhancement is performed by using a region-growing technique having random initial seeds.

Saffarzadeh et al. [111] proposed an algorithm based on the linear features of the fundus image. The use of this technique is for detection of vasculature map for both normal and abnormal retinal images. Similar to [110], MSLO technique is used for detection of vasculature map of the retinal fundus image.

Yin et al. [112] developed a technique based on an orientation-aware detector for extraction of vasculature map. Modeling of vessels orientation is done by using Fourier transformation. Adaptive segmentation is used for extraction of thin and wide vessels. Bank of Gabor filters is used for small scale vessels and line operators are used for large scale vessels. A post-processing method is also used to remove various false responses.

2.2.5. Model based techniques
Model-based techniques are further classified into vessel profile models and deformable models. Different algorithms used in model-based techniques are mentioned below:

2.2.5.1. Vessel profile models
Lam et al. [113] used multi-concavity modeling for extraction of vasculature map of the fundus image. Bright lesions are removed by differentiable concavity measure and dark lesions are removed by the line-shape concavity measure. Unevenly distributed noise is handled using the locally normalized concavity measure. Combination of all these concavity measures is done according to their statistical distributions for extraction of retinal vasculature map of fundus images.

Wang et al. [114] developed a technique based on multi-resolution Hermite model for segmentation of vasculature map. Local model parameters are estimated by using expectation-maximization optimization scheme. An information theoretic test is applied to every area of the image for selection of the most effective scale model. Finally, Bayesian stochastic inference technique is applied to describe global vascular structure by making a link of all local features.
2.2.5.2. Deformable models

Deformable models are used for segmentation to search out the proper shape or boundary of the object by using initial contour. The main aim of the deformable model is to perform modeling of the objects of the image by describing a set of computer vision algorithms and techniques. Modeling of shape variation is the important version of these techniques, where the shape of an object [115] is deformed for matching purpose. Minimization of energy and evolution of curve are the two most important theories for deformation [116]. Two main categories of deformable models are geometric [117,118] and parametric deformable models [119].

Jin et al. [120] presented a vasculature map segmentation technique based on snake contours. Initially, parameters are initialized based on hessian boundaries of features. Then based on seeds of segmented linear structure, regions of fundus image are divided. Then snake energy function was calculated on each region for the realization of the snake’s locations. In the final stage, the final vessel area is extracted using a region growing technique.

Zhao et al. [121] developed an infinite perimeter active contour model technique for segmentation of vasculature map. Hybrid information of the region of the fundus image is used for this purpose which includes the local phase based enhancement map for the preservation of vessel edges and image intensity information for segmentation of features.

Zhao et al. [122] presented a technique based on retinex theory for addressing challenges faced by retinal image processing like inhomogeneities in the intensities and contrast of the image. Enhancement of vessels is performed using the local phase enhancement algorithm for the preservation of the vessel edges. Finally, segmentation is performed using an active contour model based on graph cut-based theory.

(a) Geometric Deformable Models (GDM)

GDM is the models which are independent of parameterization; no self-intersections are produced because they are numerically stable and topology changes can be permitted automatically. Level set techniques and curve evolution theory is the base of GDM. These surfaces and curves are evolved using geometric measures only, leading
to a contour evolution that does not depend on parameterization and can be represented implicitly. The starting of GDM arises from the curve evolution and surface evolution analysis which was first presented by the Sethian (mathematician) [123,124].

Caselles et al. [125] suggested the representation of the curve as a level set which depends on Euclidian distance instead of dependency on parameters. Accordingly, in level set theory, the contour is flexible in nature and it can break or join without the requirement of parameterization.

Gongt et al. [126] presented a level set based algorithm in which initialization of a level set function [127] is not required. Here initially, contour C is located and after that entire fundus image is divided into different parts based on whether the pixel lies in the area of contour or not. After that clustering technique is used on the sub-regions to produce new region information for redefining an energy function until the algorithm converged.

(b) Parametric Deformable Models (PDM)
Active contours models or snakes are used to locate the boundary of the object or extract the important feature from the fundus image by using the initial contour. The parametric curve is used for the modeling of active contour and this curve tries to find the minimum energy by moving the points of the contour to its minimum neighborhood. The energy of the snake is computed form internal and external energy, so the sum of both these energies is minimized to get minimum snake energy.

The snake energy is the combination of three terms: $E_{int}$, $E_{img}$, $E_{con}$. $E_{int}$ represents the internal energy of the snake, $E_{img}$ represents the energy of the image and the $E_{con}$ represents the energy of the external constraint forces.

Mathematically the internal energy of the snake is shown by Eq. (2.3)

$$E_{int} = \frac{1}{2} (\alpha(s) \| V_s(s) \|^2 + \beta(s) \| V_s(s) \|^2)$$

(2.3)

where alpha($\alpha$) and beta ($\beta$) are the two coefficients used for controlling the energy of the snake. $V(s)$ represents the position of snake given by the curve where $s \in [0,1]$. $\| V_s(s) \|^2$ and $\| V_{ss}(s) \|^2$ represent a measure of elasticity and curvature respectively.
$E_{img}$ is computed by taking the gradient of the image. Sometimes Gaussian filter is applied to the image for the noise removal operation if present. After that gradients are evaluated to find the energy of the image.

Mathematically image energy with 2D Gaussian filter can be expressed by Eq. (2.4)

$$E_{img} = \|\nabla[(G(x, y) * (I(x, y)))]\|^2$$

(2.4)

Sign of image energy may be different for different algorithms used for extraction of vessels.

As compared to other classical feature extraction techniques, active contour models have many advantages:

- Minimum state is searched adaptively and autonomously.
- Sensitivity is introduced with the use of Gaussian smoothing in the image energy function
- Dynamic objects can be tracked.

Along with the advantages, these models also possess some key drawbacks.

- Active contour models are sensitive to local minima states, but with the use of simulated annealing techniques, this effect can be counteracted.
- During energy minimization, minute features are ignored over the entire contour.

Al-Diri et al. [128] developed an algorithm based on “Ribbon of Twins” active contour model for segmentation and measurement of blood vessels. Vessels centerlines are identified using morphological order filter. Finally, all junction configurations are resolved using neural cost function.

### 2.2.6. Adaptive local thresholding based techniques

Thresholding technique is a simpler technique which is used in medical image analysis for segmentation of different structures like organs and tissues of the image. Further, these segmented structures can be used for the diagnosis of many diseases at an early stage. The basic of thresholding is the selection of optimal or threshold value/level which is used for separation of various classes of objects in the image. Technique becomes more effective when different objects of the image have a well-
defined area. The probability of segmentation error increases due to the following reasons:

- Uneven illumination
- Camera distortions/artifacts
- Inferior quality of the source material
- Presence of hybrid features
- Anatomical objects with multiple classes

These issues can be resolved by using region-based thresholding methodologies for segmentation of vasculature map from the retinal fundus images. Three major categories of a region-based technique are knowledge-based, statistical and fuzzy-based adaptive thresholding.

Christodoulidis et al. [129] presented a technique based on statistical-based adaptive thresholding. The main focus of this technique is on segmentation of small and thin vessels using multi-scale tensor voting scheme. Here, initially pre-processing was performed for extraction of a green band of the image. Then enhancement of vessels is made using multiscale line detection technique [130,131] and noise is removed using Dual-tree wavelet transform [132]. After that, adaptive thresholding [133] is performed for segmentation of large and medium blood vessels and a multi-scale tensor voting scheme is used for segmentation of small thin vasculature map. Finally, in the post-processing stage all non-vascular components are removed using morphological cleaning operation [134-135].

Akram et al. [136] proposed an algorithm which uses 2-D Gabor wavelet for enhancement of vasculature map and a multilayered thresholding algorithm for extraction of vasculature map of the fundus image. The benefit of the proposed technique is that it can perform well even in the presence of uneven illumination. This technique is also able to capture thin vessels.

Mapayi et al. [137] presented a method for pre-processing of retinal images which implements and various vessel segmentation algorithms based on global thresholding. Finally, a comparison of various algorithms has also been presented.
Akram et al. [138] developed a technique based on statistical-based adaptive thresholding for the creation of binary vasculature map. Pre-processing and adaptive thresholding are the two main phases of this technique. In pre-processing, pattern enhancement of vasculature map (specifically vessels which are less visible) of RGB retinal image is made using Gabor wavelet filter which is based on technique as proposed by [139]. After that, adaptive thresholding is used for segmentation of vasculature map using intensity information.

Jiang and Mojon [140] developed a technique based on knowledge-guided local adaptive thresholding. In this technique, based on a certain threshold, thresholding is performed and then classification is made to decide whether the binary image extracted contains any object or not. Different thresholds are taken to carry this operation and finally, segmentation is performed by taking combinations of different outputs of different thresholds.

Ravichandran et al. [141] presented a fully automatic enhancement/thresholding based vessel extraction method. The input image is enhanced by histogram matching and CLAHE techniques. Following CLAHE, Wiener filtering is carried in order to remove the background noise. A local entropy based thresholding technique is then used to extract blood vessel from the 2D Gabor filter response of CLAHE'd image.

2.2.7. Machine learning based techniques

Pattern recognition [142,143] and machine learning has become a more renowned and active research area in the detection and classification of vessel objects and non-vessel objects of the retinal fundus image. Supervised, unsupervised and reinforcement learning are the three main classifications of machine learning. Depending upon the input value x and output response y, these categories have been made.

In supervised learning, corresponding to each input, there is one output response which is not possible in another two categories due to lack of information. In unsupervised learning [144], without the requirement of any external supervision, patterns are generated. In reinforcement learning [145], a particular model is
followed by system dynamics. So, the major difference in supervised and unsupervised learning is that in supervised learning prior labeling knowledge of labels is required for classification of vessel pixels but in unsupervised learning, segmentation of vasculature map can be made without any prior knowledge.

Sun et al. [146] developed a technique based on the active contour model approach. This technique used local morphology fitting operation for segmentation of vasculature map. To minimize the energy which is associated with the contour model, a level set framework is used.

(a) Supervised methods

Supervised classification techniques require some labels to judge whether a particular pixel belongs to a vessel or surrounding tissue. Labeled training data is used by supervised segmentation methods for the training of the classifier. These classifiers can be used for the classification of pixels in a new field of view. Various classifiers used in segmentation are artificial neural networks (ANN), support vector machine (SVM), k-nearest neighbors (KNN), AdaBoost, Gaussian mixture models (GMM)).

An advantage of supervised classification is that the accuracy of the system is more due to the presence of labeled data. But the disadvantage is that it requires human effort in the loop.

Staal et al. [147] developed a technique based on segmentation of image ridges, which coincide with vessel centerlines. The main use of these ridges is to compose line elements which are further used to design patches. After that feature vectors are evaluated for every pixel. Finally, the classification of features is made with the help of neural network classifier and sequential forward feature selection.

Vilariño et al. [148] developed a technique based on the discretization of pixel-level and evolution of active contours. High speed in processing is achieved by implementing it on parallel Convolutional Neural Network (CNN) architecture. This pixel-level snake based algorithm is also used in managing the changes in the topology of contour.
Soares et al. [149] developed a supervised method for classification of pixels using Bayesian classifier. Filtering of noise and vessel enhancement is performed using Morlet wavelet.

Salem et al. [150] developed a technique based on KNN classifier for the classification of pixels as vessel pixels or non-vessel pixels.

Ricci et al. [151] developed a technique based on a line operator for extraction of vasculature map of the fundus image. A line detector is applied to the green band of the retinal image. For unsupervised classification, pixel classification is performed by thresholding the response of the line detector. Supervised classification is performed using SVM in which construction of feature vector is performed using line operators which are connected orthogonally.

Osareh et al. [152] developed a technique for automatic identification of vasculature map of the retinal fundus image. Computation of feature vectors is made using Gabor filters. Finally, classification of extracted features is made by using GMM and discriminative SVM classifiers.

Lupascu et al. [153] developed an algorithm for extraction of vasculature map of the fundus image. A 41-D feature vector is computed for each pixel of an image. Information is encoded on various scales based on geometrical and spatial properties. For classification purpose of pixels as vessels or non-vessels, AdaBoost classifier is used.

Xu et al. [154] developed a technique for segmentation of vasculature map of the fundus image. Here initially, adaptive local thresholding is used for segmentation of large blood vessels and SVM is used for segmentation of small blood vessels. This technique is also able to solve the problems produced due to contrast variations of blood vessels.

Marín et al. [155] developed a technique for classification of pixels which is based on neural network scheme. The 7-D feature vector is constructed for representation of pixels.

Fraz et al. [156] developed a supervised technique for extraction of vasculature map of the fundus image. For the generation of feature vectors, dual Gaussian, Gaussian,
and Gabor filters second derivatives, multiscale line strength measurements and morphological transformation are used collectively. Information is encoded using feature vectors for the handling of normal vessels and vessels affected with central flux.

Zhao et al. [157] presented an algorithm in which pre-processing is performed for enhancement of an image using CLAHE and two-dimensional Gabor wavelet. Smoothening of the image is performed by anisotropic diffusion. After that level set technique and region-growing technique has been used for extraction of wide vessels and thin vessels respectively.

Wang et al. [158] presented a technique based on classifier for extraction of retinal vasculature map. Integration of strengths of traditional classifier (random forest) and feature extractor (CNN) is used for automatic detection of features and prediction of patterns from raw images.

Roychowdhury et al. [159] presented a novel technique for segmentation of vasculature map having three stages of processing. Processing of green band of the fundus image is made for extraction of the binary image after using a high-pass filter. Then a binary image is extracted using morphological reconstruction. Regions which are common to both these images are considered as major blood vessels. Classification of all remaining pixels of a binary image is made using GMM classifier. Finally, a combination of major regions and vessel pixels classified is made for extraction of final vasculature map.

(b) Unsupervised methods

Unsupervised methods do not require any prior labeling information for the segmentation of retinal vasculature from the fundus image. Intrinsic patterns of vessels can find out using unsupervised classification methods which can be further used for classification of pixels. An advantage of unsupervised classification is that it requires minimal human effort in the loop. But the disadvantage is that the accuracy of the system is less due to the absence of labeled data.

Frucci et al. [160] developed an unsupervised technique for segmentation of retinal vasculature map. The green band of the fundus image is considered for further
processing. The algorithm is based on the construction of the direction map of retina images assigning each pixel one out of twelve discrete directions.

Saez et al. [161] presented a technique in which vascular tree structure is obtained using the segmentation algorithm and the extraction of all profiles is also made. Selection of arteries and veins is made based on best features. In the final stage, a clustering technique is used for classification of a vessel as artery or vein.

Tolias and Panas [162] presented a technique based on fuzzy C-means (FCM) clustering algorithm. Initially, optic nerve and its bounding circle are used for identification of an initial point of this algorithm. After that, the FCM technique is applied for segmentation of the points as vessel points and non-vessel points. If the number of points classified as vessels is more than three, then the segmented region is considered as an initial point of an iterative fuzzy vessel tracking technique. A drawback of this algorithm is that it is not able to detect vessels having a small diameter and less contrast.

Simo and de Ves [163] developed a technique based on Bayesian analysis for segmentation of vasculature map of the fundus image. Estimation of the statistical parameter is made in this segmentation process. Markov random fields’ is also used for getting information about the anatomy of the retinal fundus image.

Salem et al. [164] presented a radius based clustering algorithm for distributions of the image pixels. The main features used in this technique are the intensity of green channel, gradient magnitude of local maxima and large eigenvalue which are calculated using a Hessian matrix.

Kande et al. [165] presented a fuzzy based unsupervised technique for correction of uneven illumination problems by using information of intensities of pixels from green and red bands of the retinal image. Enhancement of the vessel is performed using matched filtering. Finally, segmentation of vasculature map is made using FCM clustering technique which is followed by connected component analysis method.

Ng et al. [166] proposed a technique for identification of vasculature map in which a generative model using a Gaussian-profiled valley is used. The noise and image models are also included in a maximum likelihood estimator for estimation of model
parameters like direction, contrast, and width of vessels. Along with the noise, likelihoods of the model are also produced which are used with estimated vessel parameters for detection of vessel centerline. Finally, the combination of centerline and the estimated width parameter is made for marking of vessels.

Villalobos-Castaldi et al. [167] proposed a technique in which gray-level co-occurrence matrix (GLCM) along with local entropy information is used for segmentation of vasculature map. After computation of GLCM, enhancement of vessels is made using a matched filter which is further used for calculation of statistical feature used as a threshold value. Finally, extraction of the vasculature network is done using local entropy thresholding technique.

Nekovei and Ying [168] developed an algorithm for segmentation of retinal vasculature map using backpropagation ANN vessel classifier. Grey values of pixels are considered as neural network input. The proposed technique is not complex in nature due to the absence of feature extraction algorithms.

Maji et al. [169] presented a hybrid technique based on deep learning and ensemble learning for detection of retinal vasculature map. Unsupervised learning of vasculature through denoising auto-encoder [170,171] is performed using Deep Neural Network (DNN) technique. Further, the response of DNN was utilized in the supervised learning process for identification of vasculature tissues.

Maji et al. [172] presented a technique based on DNN for classification of vessel pixels and non-vessel pixels. For this purpose, twelve convolutional neural networks are used each having three convolutional layers. Training of each layer is performed using 60,000 random patches. Final probability is formed by taking the average of individual responses of each convolutional network.

Gu and Cheng [173] used a two-step process which is iterative in nature for detection of vasculature map. The proposed algorithm was based on latent classification tree model. Thick and large vessels are generated by applying thresholding technique on the confidence map and thin vessels are generated using a latent classification tree. Finally, all structures are connected for the generation of complete vasculature map.
Maninis et al. [174] developed a supervised technique based deep conventional neural network for automatic extraction of retinal vasculature map. For this purpose, training of layers of the conventional neural network [175] is performed in a different way.

Salem et al. [176] developed an algorithm for segmentation of vasculature map using KNN clustering algorithm. The intensity of the green band, gradient magnitude of local maxima and large eigen value calculated using a Hessian matrix are the main features of the proposed algorithm. Clustering of image pixels is made using an only modified version of KNN algorithm.

Sharma and Wasson [177] presented a fuzzy-based segmentation for segmentation of retinal vasculature map. The input of this system is the difference between low-pass and high-pass filtered version of the fundus image. Fuzzy based rules are used for the selection of pixel values, which further leads to segmentation of vasculature map.

Akhavan and Faez [178] developed a technique which uses vessel tracking technique and fuzzy based technique for detection of vasculature map of the fundus image. All vessel centerlines of enhanced image are detected using tracking technique. Further, the detected centerlines worked as initial points for region growing algorithm which is based on FCM.

Xie and Nie [179] developed a technique in which a combination of FCM and genetic algorithm is made for segmentation of vasculature map. Green channel of the fundus image is taken for processing. Histogram equalization is performed for enhancement of the image. After that, two layers i.e. texture layer and smooth layer are separated from the fundus image. Processing is applied only on the texture layer as it contains the maximum amount of information. Finally, an approximate solution is obtained using a genetic algorithm which is further used as an initial point of the FCM algorithm.

Emary et al. [180] developed a technique which utilized the FCM technique optimized by Cuckoo search algorithm for segmentation of retinal vasculature map.
An optimal version of FCM is established by using possibilistic C-means method as proposed by [181] and possibilistic FCM method as proposed by [182].

### 2.2.8. Parallel hardware based techniques

Implementation of parallel hardware-based algorithms is used for addressing requirements of real-time performance. CNN which can be used with VLSI chips [183,184] is one of the important paradigms for real-time image processing representation.

**Alonso-Montes et al. [185]** developed an algorithm based on hardware in which CNN based histogram equalization, local adaptive thresholding, and morphological operation opening techniques are used for segmentation. PLS [186,187] technique is used for extraction of vasculature map.

**Alonso-Montes et al. [188]** developed a technique based on the CNN-based methodology for the extraction of retinal vasculature map. Estimation of vessel region, initial region and external potential and evolution of PLS are the building blocks of the proposed algorithm.

**Dudek et al. [189]** developed a technique based on PLS for segmentation of retinal vasculature map. Performance is optimized by implementing PLS on processor array. Contour is evolved in various directions for detection of vasculature map.

**Vilarino et al. [190]** developed a technique in which line strength is measured for extraction of vasculature map from the green band of the fundus image. This technique uses CNN algorithm [191-195] for segmentation of vasculature map.

**Vilarino et al. [196]** presented a technique in which cellular array based on the focal plane is used for the extraction of vasculature map. Filling of holes is also performed using non-propagative operations.

**Nieto et al. [197]** analyzed the implementation of various retinal vasculature techniques on various hardware architectures and platforms. Processor array and field-programmable gate arrays (FPGA) is used for mapping of retinal vasculature map.
2.3. Literature based on bifurcation and cross over point detection

The identification of feature points in the vasculature map increases the information about the vasculature map which can be used for diagnosis of various diseases related to the eye. Any pixel is considered as bifurcation point if the count of active pixels around the central active pixel is equal to 3 and any pixel is considered as a cross over point if the count of pixels around the central active pixel is equal to 4.

Martinez-Perez et al. [198] developed a geometric feature based methodology for identification of feature points of the vasculature network. This technique will fail, if the distance between two bifurcation points is too less and if one more vessel passes through the fixed-size window.

Bevilacqua et al. [199] presented a computational model for the extraction of feature points of the fundus image. In the first four steps, noise is removed with the help of various filters and then the optimized skeleton of vessels is produced. Finally, feature points are detected from the fundus image using a 3x3 window method technique. In this method, there was an issue for the detection of a cross point because sometimes one cross-point can turn into two bifurcation points.

Jung et al. [200] developed a technique for the extraction of vasculature map and vasculature landmarks of the fundus image. Various image processing operations like an acquisition of the image, enhancement of the image, reduction of background artifacts, etc. are performed for accurate identification of feature points.

Bhuiyan et al. [201] developed a technique based on geometrical features of vessels for the detection of vasculature landmarks of the fundus image. Here initially, vasculature map is segmented from the colored retinal fundus image and then vessel centerlines are generated using morphological thinning operation. Finally, a filter is applied to the vessel centerlines for the identification of feature points of the fundus image. Properties of vasculature map crossing through these points are used for identification of bifurcation points and cross over points.

Ardizzone et al. [202] developed a technique based on cross-correlation method for the identification of feature points of the fundus image. Initially, artifacts removal and enhancement is performed by using anisotropic diffusion and matched filter.
respectively. Thresholding is used for the extraction of binary vasculature map of the fundus image. After that, all isolated pixels are removed using connected pixels labeling concept for the generation of complete vasculature map. Finally, all feature points are identified using the cross-correlation technique.  

**Calvo et al. [203]** presented a technique for the identification and classification of feature points of the fundus image. Identification of feature points is performed with the help of filters or morphologic operations. Finally, classification of feature points is made by analyzing the environment of feature points.  

**Aibinu et al. [55]** proposed a hybrid technique known as combined cross-point number (CCN) method for identification of feature points of the retinal fundus image. In this algorithm, two techniques namely simple cross-point number (SCN) technique and modified cross-point number (MCN) technique are used for identification of feature points. SCN used 3x3 window and MCN used 5x5 window for the identification of feature points of the fundus image. In CCN method, feature points are computed by taking SCN and MCN both into consideration. According to CCN, any point is bifurcation point, if it is bifurcation point in both in SCN and MCN. A similar criterion is used for identification of cross over points of the fundus image.  

**Azzopardi et al. [204]** developed an algorithm for the identification of vascular feature points using a trainable Combination of Shifted Filter Responses or COSFIRE filters. In this technique, different shift and blur parameters are computed by selecting Gabor filters. The final response is evaluated by taking mean of all responses of the Gabor filters.  

**Nguyen et al. [205]** proposed a robust technique to separate crossover from non cross-over points by utilizing local information and geometrical features of the vasculature at the crossing points.  

**Yavuz et al. [206]** developed an algorithm for identification of feature points of fundus image which are further used in the image registration process. Here initially, the skeletonized map is obtained using thinning operation. Finally, a vascular network is extracted using a characteristic matrix.
Pratt et al. [207] developed a methodology for classification of bifurcation and cross over points of retinal fundus based on convolutional neural network approach. DRIVE database has been chosen for implementation of this algorithm.

Morales et al. [208] developed a technique for identification of feature points on the vasculature map using Hit or Miss Transformation (HMT). For identification of complex intersections, the post-processing stage is also used to differentiate a bifurcation point from a crossover.

2.4 Assessment and research gaps

From the literature studied, it is found that retinal vascular network segmentation is a tedious task due to less contrast of vessels with respect to background tissue. Due to various challenges faced in the extraction of vasculature map, robust segmentation techniques are required. Here, different methodologies used for the extraction of vasculature map of fundus image are studied. It has been observed that in recent years, the supervised approach is used generally for extraction of vasculature map of the fundus image. In supervised approaches, in spite of high levels of accuracy, it requires large volumes of clinical annotations to generate the requisite training data. In practical terms, this is often not very easy to source. Hence, an efficient unsupervised approach is required for segmentation of the vasculature map of the fundus image.

From further literature studied, it is found that the existing vasculature extraction algorithms lead to high computational complexity due to the presence of pixels other than object pixels. So, an efficient masking algorithm is required to remove background pixels and to focus only on the foreground pixels which will reduce the computational complexity of the algorithm.

It is also found from the literature studied that the existing feature point algorithms based on window techniques, lead to false detection of feature points. So, an efficient modified window technique is required which can further minimize the false detection of feature points.
2.5 Problem definition
Develop an advanced and robust algorithm for segmentation of retinal vessels and computation of feature points like bifurcation points and cross over points, for all types of images.

2.6 Thesis objectives
Based on the research gaps mentioned above, following objectives are proposed.

I. To propose an algorithm for pre-processing of the retinal fundus image.

II. To propose an accurate segmentation algorithm for evolution of retinal vascular network using Modified Pixel Level Snake.

III. To formulate an algorithm for automated detection of retinal vascular feature points i.e. Crossover and Bifurcation points.

IV. To evaluate and analyze the performance of developed algorithm using sensitivity, specificity and accuracy parameters.

2.7 Proposed research methodology
To improve the accuracy of the system, a novel algorithm based on MPLS for automated segmentation of retinal vasculature map is used in proposed work. Vascular feature analysis has also been done for detecting retinal abnormalities of the fundus image.

The basic research methodology used for the proposed work is represented by Fig. 2.1. Here, research methodology is divided into three stages: pre-processing, processing and post-processing.

2.7.1. Pre-processing
In the pre-processing stage, initially coloured retinal fundus image is read and then it is converted to a grayscale image. Then the binary mask is generated from the gray scale fundus image using proposed bimodal masking given in section 3.2. Evaluation of different performance metrics of the proposed bimodal masking technique is done
in section 3.3. Finally, CLAHE is applied to the masked fundus image for improving the contrast of the masked image.

2.7.2. Processing

In the processing stage, global thresholding technique is applied to the enhanced image for the extraction of vasculature map of the fundus image. Produced vasculature map is the initial contour of the image which is further used for evolution in the post-processing stage. Finally, the border of the vasculature map is removed with the help of the mask produced using bimodal masking technique.

2.7.3. Post-processing

In the post-processing stage, an evolution of the vasculature map is performed using the proposed MPLS algorithm given in section 4.3. In this algorithm, an external potential is computed using BTH based transformations. After that noise is removed by removing the pixels having the small area. Evaluation of different performance metrics is done using extracted vasculature map and ground truth vasculature map of fundus image which is given in section 4.4. After that, feature points like bifurcation points and cross over points are computed from the vasculature map using the proposed methodology as given in section 5.2. Finally, feature points are plotted on the vasculature map of fundus image which is shown in section 5.3.
Fig.2.1: Research methodology of proposed work
CHAPTER 3
GENERATION OF MASK USING BIMODAL MASKING

3.1. Introduction
Pre-processing is an important key in the automatic diagnosis of various disorders related to retinal fundus images because it improves the localization and segmentation of various retinal image features. The processing of the noisy and surrounding areas in the retinal fundus image is not necessary to perform because a lot of time is consumed at all stages of processing. The number of operations required for pre-processing can be minimized by focusing only on the retinal image feature region. To accomplish this task, a binary mask of the fundus image is generated using the proposed bimodal masking technique. The main parts of bimodal masking are RGB to gray conversion, histogram generation and gray to binary conversion using peaks and valleys of the histogram of the fundus image. The present study provides a simple and accurate method for the generation of a binary mask of fundus images. It can be helpful for the accurate extraction of vasculature map and other morphological attributes of the fundus image.

Segmentation of retinal image features [34] in an accurate manner is a tedious job due to fewer variations in the contrast between vasculature and surrounding tissue and due to the presence of noise in the retinal fundus image. So, pre-processing of the fundus image is required for the segmentation of features of the image in an accurate manner. Pre-processing is the initial step in the automatic diagnosis of various abnormalities present in retinal fundus image and it is performed to extract foreground from background. Each retinal fundus image is composed of two major regions-foreground regions which include the circular region and background region which includes the surrounding black region. Foreground region is further used for processing of images because this is the region, where all features of fundus image like arteries, veins, fovea, and optic disc appear. Presence of the background region
for processing may lead to false computation of features of the retinal image. Foreground region can be extracted from the entire fundus image by generating a mask of the fundus image. So, the generation of a mask of the fundus image is very important to concentrate only on the retinal image features.

A mask of the fundus image is basically a binary image which consists of 1's and 0's. Here, 1 represents the fundus image pixels corresponding to the foreground region and 0 represents the retinal image pixels corresponding to the background region or noisy region. The mask of an image is used to get ROI of fundus image which will reduce the analysis time and computational effort by excluding pixels that belong to background from further processing as operations will be focused only on the object pixels.

The aforementioned discussion reveals the importance of masking of the fundus image. Considering this view, several research works [36, 43, 45] have been conducted for accuracy assessment of generated mask for fundus images. Authors [36, 43, 45] applied thresholding technique and morphological operation one or the other ways for extraction of a mask of fundus images but they have not evaluated their performance metrics. Here, performance metrics are evaluated for thresholding technique as well as bimodal masking techniques for checking the performance of the system. Our proposed approach is different than existing due to the use of bimodal masking technique for extraction of a mask of the fundus image.

A bimodal histogram is just a histogram in which two peaks of data are present. The bottommost point (valley) which is between these two peaks denote the threshold point, as shown by Fig. 3.1 [209].

![Fig.3.1: Bimodal histogram](image)
Rigorous computational experiments are conducted by developing robust experimental environment. Hence, the proposed approach is efficient, robust and statistically sound.

### 3.2. Proposed methodology for the generation of mask

Bimodal masking is used for the generation of the accurate binary mask of the fundus image. In bimodal masking, the mask of the fundus image is generated from the grayscale image, obtained after RGB to gray conversion. Flow chart of the proposed methodology used for the automated generation of the binary mask of the fundus image is presented by Fig. 3.2. The main components of the methodology are: RGB to gray conversion, histogram generation, identification of peaks and valleys of the histogram of fundus image, gray to binary conversion and generation of a masked image.

![Flow chart for generation of a mask using the bimodal masking technique](image)

(i) **Read retinal fundus image**

For analysis purpose, color fundus image of the retina is chosen from the DRIVE database. Simulation is performed on 20 test images of the DRIVE database. Here,
the initially RGB fundus image (1_test of DRIVE database) is read and used for further processing.

(ii) **Convert RGB to gray image**

In this step, the RGB image is converted to a grayscale image. Conversion of RGB to the gray image also reduces the time required for processing of the image. Different weights for R, G & B components are selected for conversion purpose. RGB to gray conversion is performed using the following formula as represented by Eq.(3.1) [210].

\[
I_g = 0.2989 \times r + 0.5870 \times g + 0.1140 \times b 
\]

where \( r \), \( g \), and \( b \) symbolize the red, green, and blue bands of fundus image respectively and \( I_g \) is the gray image produced after conversion. The green channel is the channel which gives the maximum information of fundus image, so the weight of the green channel is chosen larger as compared to other channels. RGB image and the extracted gray-scale image are represented by Fig. 3.3(a) & 3.3(b) respectively.

![Fig.3.3: (a) Original image, and (b) Grayscale image](image)

(iii) **Generate a histogram of the image**

In the next step, intensity histogram of the grayscale image is plotted as shown by Fig. 3.4. The histogram is a graph which displays the number of pixels corresponding to each existing intensity value present in the image graphically. Intensity range
varies from 0 to 255 because there are 256 different intensities for an 8-bit grayscale image.

(iv) **Identify peaks and valleys of the histogram**

After generation of a histogram of the image, the next step is to identify dominant peaks and valleys from the histogram of the image [211]. The flow chart used for the identification of peaks and valleys of the histogram is shown by Fig. 3.5(a). In this work, the average filter having size 5 is applied to the histogram of the image for a smoothening purpose. After that peaks and valleys (local minima low and local minima high) are identified. Peaks are refined on the basis of area threshold and Minimum Peak Distance (MPD). MPD is chosen as 10 for this work. Finally, identified peaks and valleys of the generated histogram are plotted on the histogram of the image. In Fig. 3.5(b), peaks are represented by ‘*’, minima low are represented by red circles and minima high are represented by green circles.
Fig. 3.5: (a) Flowchart for identification of peaks and valleys of the histogram, and (b) Peaks and valleys representation

(v) Convert gray to binary Image
After identification of peaks and valleys of the histogram, the next step is to choose the threshold level for the conversion of a gray image into a binary image. Here, a
first minimum high is used as a threshold for conversion of gray to the binary image. This minima high behaves like a valley between two peaks of the histogram of the image. The binary image produced after thresholding is termed as the final mask as shown by Fig. 3.6(c).

**vi) Generate masked image**

Finally, the original input image is multiplied with the mask generated using bimodal masking for the generation of a masked image. In the masked image, all pixels other than object pixels have pixel value ‘0’ which reduces the computation time. Fig. 3.6 (a),(b) & (c) represents the original image of the DRIVE database, the corresponding mask of fundus image and masked image respectively.

![Original Image](image1.png)
![Mask Image](image2.png)
![Masked Image](image3.png)

Fig.3.6: (a) Original image, (b) Mask of the image, and (c) Masked image
Fig. 3.7 (a) & (b) represents the original image and small part of original image respectively. The matrix of the cropped part of the original image and masked image is represented by Fig. 3.7(c) and Fig. 3.7(d) respectively. It is observed that in the matrix of the original image, all pixels other than object pixels have also some intensity value which is of no concern. But, in the matrix of masked image only object pixels have some intensity value. All other pixels other than the object pixels have pixel value ‘0’ which further reduces the processing time.
3.3. Results and discussion

Different performance metrics like sensitivity, specificity, and accuracy of the mask of the fundus image with respect to ground truth mask have been evaluated. The accuracy of the proposed algorithm is better is due to the generation of an accurate binary mask of fundus image using bimodal masking technique which is simple and efficient in nature. Comparative analysis of proposed bimodal masking technique with existing Otsu’s thresholding technique is also made. Fig. 3.8(a) represents the original fundus image and Fig. 3.8(b) and 3.8(c) represent the mask obtained by Otsu’s thresholding method and bimodal masking method respectively.
Fig. 3.8: (a) Original retinal fundus image, (b) Binary mask generated using Otsu’s thresholding technique, and (c) Binary mask generated using the bimodal masking technique.

The algorithm can be applied to all images of the DRIVE database. Fig. 3.9 represents the results of the three normal images of the DRIVE database and their generated binary mask respectively.

Fig. 3.9: (a-c) Original images, and (d-f) Corresponding binary mask generated using the bimodal masking technique.
Evaluation of performance metrics have not been made by [36, 43, 45] authors but in proposed methodology evaluation of different performance metrics is also made for analysis purpose. Results of the proposed method are compared with the results obtained using Otsu’s thresholding technique. Table 3.1 represents a comparative analysis of different performance metrics for the DRIVE database. Fig. 3.10(a), (b) and (c) represents the comparative analysis of sensitivity, specificity and accuracy of DRIVE database for Otsu’s thresholding technique and bimodal masking technique graphically. It is observed that for bimodal masking, all performance metrics i.e. average sensitivity, specificity, and accuracy of proposed methodology comes out to be better than sensitivity, specificity, and accuracy of Otsu’s thresholding technique.

**Table 3.1: Comparative analysis of bimodal masking technique with Otsu’s thresholding technique**

<table>
<thead>
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<th>Image No.</th>
<th>Otsu’s thresholding technique</th>
<th>Bimodal masking technique</th>
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<td>SP</td>
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<td><strong>Average</strong></td>
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</tr>
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</table>
Fig. 3.10: Comparative analysis of Otsu’s thresholding technique and bimodal masking technique (a) Sensitivity, (b) Specificity, and (c) Accuracy
3.4. **Summary**

Segmentation of vasculature map of fundus image plays an important role in the diagnostic procedure of various retinal disorders. For accurate segmentation of vasculature map, the focus is made only on the object pixels. Different masking techniques have been used in literature for mask generation but in the proposed method, bimodal masking technique is used for the generation of the binary mask of the fundus image. Comparative analysis of performance metrics of mask produced using the proposed bimodal masking technique is also made with Otsu’s thresholding technique. It is observed that the average sensitivity, specificity, and accuracy for existing Otsu’s thresholding technique come out to be 97.93%, 98.96% & 98.25% respectively. But in bimodal masking technique, the average sensitivity, specificity, and accuracy come out to be 99.46%, 99.77% and 99.56% respectively which shows the improved performance of the proposed method.
CHAPTER 4

EXTRACTION OF VASCULATURE MAP USING MODIFIED PIXEL LEVEL SNAKE

4.1. Introduction

The accurate extraction of vasculature map of retinal fundus image has become an important and challenging task for analysis of different pathologies. The present study offers an unsupervised method for segmentation of vasculature map from retinal fundus images. This work presents the methodology for the evolution of vessels using MPLS algorithm based on BTH transformation. In the proposed method, initially adaptive segmentation and global thresholding are applied to the masked image to find an initial contour image. Finally, MPLS is used for evolution of contour in all four cardinal directions using external, internal and balloon potential. The present study provides a simple and accurate method for the detection of vasculature map for normal fundus images as well as pathological images. It can be helpful for the assessment of various retinal vascular attributes like length, diameter, width, tortuosity, and branching angle. In proposed work, global thresholding is used for segmentation of vasculature map of fundus image and MPLS based on BTH transformation is proposed for the evolution of map in four cardinal directions.

PLS is an iterative technique, in which internal and external forces are used for evolution of pixels of contour. Normally, in PLS, an external potential is computed using edge based techniques. In our proposed methodology, BTH transformation is used for the computation of external potential, which results in improved accuracy of the extracted vasculature map.

4.2. Pixel Level Snake

PLS is an active contour technique which is iterative in nature. In PLS, the evolution of contour of the image is performed in four cardinal directions iteratively according to the potential of an image, which is computed by the addition of internal, balloon
and external potential of the image. The use of external potential is to guide the contour in a particular direction. Internal potentials are computed to maintain the smoothness of contour. The evolution of contour is performed by a pixel-by-pixel shifting of the contour, towards a position where the potential is minimum. Contour expansion and compression are performed using balloon potential. After computation of the external potential of the image, collisions are also detected and avoided using a collision detection module. This operation is performed to avoid merging of two different vessels of the vasculature map of the fundus image. In [189], PLS has been used for the identification of an object in traffic, where the object is identified from the sequence of traffic. Here, PLS is used for the evolution of vasculature map of fundus image which results in improvement of different performance metrics of the system.

4.3. Proposed methodology for extraction of vasculature map

In literature, different methodologies have been used for the extraction of vasculature map of the fundus image. In our proposed work, initially, PLS is used for the evolution of vasculature map of the fundus image. To improve the accuracy of the system, further PLS is modified for the evolution of vasculature map. In PLS, the external potential of the image is computed using simple edge based techniques but in MPLS external potential is computed from the green channel of fundus image using BTH transformation method. It is observed that the Modified PLS leads to improved accuracy of the system as compared to the PLS method.

In proposed work, the methodology used for extraction of the vasculature map is shown by Fig. 4.1. Here, initially masked image produced in section 3.2 is chosen for further processing. Then adaptive histogram equalization is applied on the masked image to get the equalized image. After that, the average filter having size 9 is applied to the equalized image to get the filtered image. Global thresholding technique is applied on filtered image for the extraction of the vasculature map of the fundus image. The border of the fundus image is removed with the help of the mask
produced using bimodal masking. Finally, vasculature map is evolved using MPLS technique.

(i) **Enhancement of image using adaptive histogram equalization**
CLAHE operation is performed for enhancement of contrast of an image. In CLAHE operation, small areas of the image are taken for equalization purpose instead of the entire image. These small regions are termed as ‘tiles’. Using bilinear interpolation technique, all neighboring tiles are combined. In this work, two-level enhancements have been done using the CLAHE technique. It means CLAHE is applied two times for getting the proper enhanced image. Size of tiles used for CLAHE is [8 8] and the number of bins is 128. The enhanced image produced after applying the CLAHE technique two times on the fundus image is shown by Fig. 4.2.
(ii) Extraction of vasculature map using global thresholding technique

The main component of the processing is a global thresholding technique which is used for extraction of the initial contour of the fundus image. The enhanced image produced using CLAHE is further used to produce a segmented image. For the generation of a segmented image, initially, adaptive segmentation is required because gray values along vessels in retinal vasculature are non-uniform. The input of adaptive segmentation is preprocessed image (enhanced image) and output is a segmented image. Fig. 4.3(a) represents the block diagram of adaptive segmentation. The average filter having size 9 is applied to the preprocessed image to produce averaged image as shown by Fig. 4.3(b). After that subtracted image is generated by taking the difference between the averaged image and preprocessed image. Then global thresholding technique is applied to the subtracted image for computation of the threshold level of the image. Algorithm for global thresholding technique is stated by Algo. (4.1).

**Algo. 4.1: Algorithm for global thresholding technique**

1. Choose an initial random threshold \( T \) for segmentation. This threshold is called the global threshold.
2. Using threshold \( T \), segment the fundus image. Two groups of pixels are produced:
   (i) All pixels having value more than \( T \) belongs to group \( G_1 \).
   (ii) All pixels having a value less than or equal to \( T \) belongs to group \( G_2 \).
3. Evaluate the average intensities \( m_1 \) and \( m_2 \) of both the groups \( G_1 \) and \( G_2 \) respectively.
4. Again compute threshold using \( T = \frac{(m_1+m_2)}{2} \).
5. Repeat steps 2-4 until the successive iterations threshold difference is smaller than the already defined value.
6. Segment the image by taking \( T \) as a threshold.
Using global thresholding technique algorithm, a threshold level is obtained. Using this threshold, the subtracted image is converted to a binary image, which is called a segmented image as represented by Fig. 4.3(c). This segmented image will be used to find initial contour which is defined implicitly as a region boundary. After that morphological operation closing is applied on a segmented image by taking disk shape structuring element having size 1. Then small areas having the pixel size less than 35 are removed from the closed image. The image produced after performing closing and small area pixel removal operation is termed as contour image as shown by Fig. 4.3(d).

(iii) Removal of border using the bimodal masking technique
Next task is to remove the border from the contour image because the retinal vasculature map does not include the outer border. So, the border is removed from the initial contour image as shown by Fig. 4.3(d) with the help of binary mask produced using bimodal masking technique. To perform this operation, the contour image is subtracted from the complement of the mask. If after subtraction, some pixels contains a value greater than 0, the value 1 is assigned to that pixels and if some pixels contains value less than zero, then 0 is assigned to that pixels. So after subtraction and assigning values to the subtracted image, a contour image without border is produced. After that morphological operation dilation is performed on the image using disk shape structuring element with size 1 as shown by Fig. 4.3(e). This is the image which is further used for evolution using MPLS.

(iv) Evolution of vasculature map using MPLS
The main components of the post-processing are the evolution of contour using MPLS and removal of noise. Fig. 4.4 shows the flowchart of the MPLS algorithm. MPLS is different from PLS in terms of computation of external potential of the image. In PLS, the external potential of the image is computed using simple edge based techniques like Sobel edge detection and canny edge detection whereas in MPLS external potential is computed using BTH method. In PLS as well as in
MPLS, the method for computation of internal potential and balloon potential is the same. MPLS is used for the evolution of vasculature map of fundus image in four cardinal directions. Topologic Transformations module is also used to handle merging and splitting of contours. This module is basically used to avoid collisions between contours. The details of each module of MPLS algorithm is mentioned in the coming sections.

Fig. 4.3: (a) Block diagram of adaptive segmentation, (b) Average image, (c) Segmented image, (d) Contour image, and (e) Contour image without border
(a) External potential computation
The external potential of the image can be computed using PLS as well as using MPLS. In normal PLS algorithm, original gray image is used as an input image for computation of external potential. But in MPLS algorithm, the green channel of the masked image is used as an input image for computation of external potential of the image. Here, the green channel is used because it contains maximum information about the vasculature map of the fundus image. Details of the methodology used for the computation of external potential using PLS and using MPLS are mentioned in the following sections.

I. External potential computation using PLS
For the evolution of PLS, a suitable external potential image is computed. Low-level image processing is applied to the fundus image for computation of external potential of the image. Flow chart for computation of external potential using PLS is shown by
Fig. 4.5. In this technique, the edge detection technique using a Sobel filter is applied to the gray image and contour image to get actual vessel edges. The produced edge detected images are further combined and then diffused using anisotropic diffusion method. The diffused image and combined edge detected image is further used to get an appropriate potential field of the image known as an external potential image. This potential image is used to properly guide the PLS evolution in a particular direction.

![Flow chart for computation of external potential using PLS](image)

**Fig. 4.5: Flow chart for computation of external potential using PLS**

Evolution of PLS will be done by an external potential which is stronger in areas close to the edges. If the image is static, then the external potential is computed once. Applications like real-time computer vision, in which moving images are there, an external potential is computed for every frame of the image.

**II. External potential computation using MPLS**

In PLS, an external potential is calculated from the gray image of the original retinal fundus image using edge based techniques like Sobel and Canny edge detection. But in MPLS, the external potential of the image is calculated by using the BTH transformation method on the green channel of the fundus image. The external
potential produced using BTH method contains more information about the vasculature map resulting in higher accuracy of the system. Using this external potential, the total potential of the image is computed which evolve the contour in an efficient way as compared to the PLS technique. Flow chart for computation of external potential for MPLS is shown in Fig. 4.6(a). Here, BTH transformation is applied on the green channel of retinal fundus image (as shown by Fig. 4.6(b)) by taking three different structuring elements.

The BTH transform is the transformation which is evaluated by subtracting the input image from the closing of the input image. The BTH transform of an image $I$ is given by Eq.(4.1).

$$T_{b}(I) = I \odot s - I$$

Here, $I$ is the input image; $s$ represents the structuring element and $\odot$ represents the closing operation. Output $T_{b}(I)$ represents the BTH transformed image.

Three different disk shapes structuring elements having size 2,7,11 are used for closing operation in the computation of external potential. Then the sum of all three BTH transformed images is taken to produce the external potential image as represented by Fig. 4.6(c). For the evolution of contour towards minimum potential, the complement of external potential is taken. The complement of an external potential image ($P_e$) is represented by Fig. 4.6(d). This is the external potential which guides the contour of the image towards edges of vasculature map.
(b) Internal potential computation

This potential is useful in maintaining the smooth shape of the contour. During the evolution of PLS, all vessel discontinuities are avoided using internal potential. The internal potential is computed from the initial contour image. Flow chart for computation of internal potential of the image is shown by Fig. 4.7(a). Here initially, the binary contour edge image is produced from a contour image using the Eq.(4.2) [189].

\[ C = I_C \text{ AND NOT } (I_{CN} \text{ AND } I_{CS} \text{ AND } I_{CW} \text{ AND } I_{CE}) \]  \hspace{1cm} (4.2)
Here $I_C$ represents the initial contour of the image, $I_{CN}$ represents active region pixels in north direction from the current pixel, $I_{CE}$ represents active region pixels in east direction from the current pixel, $I_{CW}$ represents active region pixels in west direction from the current pixel, $I_{CS}$ represents active region pixels in south direction from the current pixel.

In terms of coordinate representation, if individual pixel $I_C$ is represented by $I_{C(x,y)}$, then pixels $I_{CN}$, $I_{CE}$, $I_{CW}$ and $I_{CS}$ are represented by $I_{C(x,y-1)}$, $I_{C(x,y+1)}$, $I_{C(x-1,y)}$ and $I_{C(x+1,y)}$ respectively.

Edge image produced from the initial contour image is shown by Fig. 4.7 (b). Diffusion of the image is performed on an edge image of initial contour using anisotropic diffusion method. Anisotropic diffusion also called Perona–Malik diffusion is a method which is used to reduce noise present in the image without removing important information of the image. Here, anisotropic diffusion of contour image is performed to obtain an internal potential field.

The expression used for computation of anisotropic diffusion is defined by Eq. (4.3(a)) and Eq. (4.3(b)) [212].

$$\frac{\partial I}{\partial t} = \text{div} (c(x,y,t)\nabla I)$$  \hspace{1cm} 4.3(a)

$$\frac{\partial I}{\partial t} = c \nabla I + c(x,y,t)\Delta I$$  \hspace{1cm} 4.3(b)

Where $\nabla$ denotes the gradient, $\Delta$ denotes the laplacian, div is the divergence operator and $c(x,y,t)$ is the diffusion coefficient. Two functions for diffusion coefficient are proposed by Perona and Malik which are represented by Eq. (4.4(a)) and Eq. (4.4(b)) [212].

$$c(||\nabla||) = e^{-\left(\frac{||\nabla||}{K}\right)^2}$$  \hspace{1cm} 4.4(a)

and

$$c(||\nabla||) = \frac{1}{1+\left(\frac{||\nabla||}{K}\right)^2}$$  \hspace{1cm} 4.4(b)

Constant $K$ is used to control the sensitivity to edges. Here the value of $K$ is chosen as 40. The diffused image as shown by Fig. 4.7(c) is multiplied with weight ($W_{IP}$). The weight is adjusted using hit and trial method. Here, the value of weight selected for
generation of an internal potential of the image is 0.1. After that complement of the weighted internal potential image ($P_i$) is taken for contour evolution in the proper direction. Fig. 4.7(d) and 4.7(e) represents the weighted internal potential image and complemented image respectively.

![Flow chart for computation of internal potential](image)

**Fig. 4.7:** (a) Flow chart for computation of internal potential, (b) Edge of contour image, (c) Diffused image, (d) Weighted internal potential image, and (e) Complemented image
(c) Balloon potential computation

The external potential is not able to guide the contour in all directions if initial contour lies far from the vessel edges. In that case, there is one potential i.e. balloon potential which produces forces, to guide the contour towards object pixels. To get balloon potential image, contour image is multiplied with some weight which is computed using hit and trial method. Value of weight used for this purpose is 0.1. Fig. 4.8(a) and Fig. 4.8(b) represent weighted balloon potential and complement of weighted balloon potential image (P_b) respectively.

(d) Guiding force extraction module

Guiding Force Extraction Module is helpful in the computation of Guiding Force Extraction Potential (GFPE) which is further used for the evolution of contour. This GFPE is computed as the weighted sum of external, internal and balloon potential as represented by Eq. (4.5). All internal and external forces are produced through the GFPE that guides the evolution of contour towards minimum energy level.

\[ P_T = P_e + P_i + P_b \]  

Here \( P_T \) represents the GFPE of an image, \( P_e \) represents the external potential of the image, \( P_i \) represents the internal potential image and \( P_b \) represents the balloon potential of the image. Fig. 4.8(d) represents the potential field image produced after the addition of all potential images.
(e) Directional contour evolution with topological transformation and collision detection module

Evolution of directional contour is the most important part of MPLS technique. It is performed in four prime directions: North, East, West, and South (NEWS). Different numbers of iterations are performed for the evolution of contour of vasculature map. Contour image (Fig. 4.3(e)) is evolved according to GFPE image (Fig. 4.8(c)) so that it can acquire minimum potential.

The expansion of contour may result in merging and splitting of contours. But in the segmentation of retinal vasculature, it's required to prevent the collision between the contours. For preventing the collision, a collision detection module is used. When contour expands in each direction, the two vessels may combine with each other. So, this expansion of contour is done in such a way that there should be no danger of collision. Fig. 4.9(a) represents the different cases for dangers of collisions. Considering the danger of collision point in mind, the algorithm for expansion in north, south, east, and west directions are described in detail.
Algorithm for expansion of contour in north direction is given by Algo. 4.2. Pixel-wise expansion of initial contour is performed in a north direction based on the potential of the image.

**Algo. 4.2: Algorithm for contour expansion in north direction**

```
SET R = NOT(I_C) AND I_CN
SET D = R OR R_E OR R_W
IF NOT(D) = 1 AND I_CS = 1 AND (P_T < P_TS)
SET I_C = 1
```

Here, $P_T$ represents the total potential field, $P_{TS}$ represents potential field in south direction, $R$ represents the active/background pixel pairs in the vertical direction, $D$ represents the danger of collision which is determined by taking logic ‘OR’ of the current pixel of $R$ with its east ($R_E$) and west ($R_W$) neighbor.

Expansion of initial contour in the north direction is represented by taking a matrix of 10x10 elements. Fig. 4.9(b) represents the 10x10 matrix of the contour image. Fig. 4.9(c) and Fig. 4.9(d) represents the binary matrix and potential matrix of Fig. 4.9(b) respectively. In both matrices, intensity values and potential field for the same pixels are highlighted with a red box. It is observed that the potential of a pixel having pixel value ‘0’ is less than the potential of a pixel which lies in its south direction. So, value 1 is assigned to that pixel as represented by Fig. 4.9(e). Fig. 4.9(f) represents the expanded version of Fig. 4.9(b) in the north direction.
Similarly, the following expressions are used for the computation of initial contour of the image by considering all cases of danger of collisions. Expressions used for computation in other directions like the south, east and west are represented by Algo. (4.3), Algo. (4.4) and Algo. (4.5) respectively. Images produced after expansion in N, S, E, W directions are represented by Fig. 4.10(a)-(d).
Algo. 4.3: Algorithm for contour expansion in south direction

\[
\text{SET } R = \text{NOT}(I_C) \text{ AND } I_{CS} \\
\text{SET } D = R \text{ OR } R_E \text{ OR } R_W \\
\text{IF NOT}(D)=1 \text{ AND } I_{CN}=1 \text{ AND } (P_T < P_{TN}) \\
\text{SET } I_C = 1
\]

Algo. 4.4: Algorithm for contour expansion in east direction

\[
\text{SET } R = \text{NOT}(I_C) \text{ AND } I_{CE} \\
\text{SET } D = R \text{ OR } R_N \text{ OR } R_S \\
\text{IF NOT}(D)=1 \text{ AND } I_{CW}=1 \text{ AND } (P_T < P_{TW}) \\
\text{SET } I_C = 1
\]

Algo. 4.5: Algorithm for contour expansion in the west direction

\[
\text{SET } R = \text{NOT}(I_C) \text{ AND } I_{CW} \\
\text{SET } D = R \text{ OR } R_N \text{ OR } R_S \\
\text{IF NOT}(D)=1 \text{ AND } I_{CE}=1 \text{ AND } (P_T < P_{TE}) \\
\text{SET } I_C = 1
\]
(f) Inversion

The image produced after expansion in all directions is inverted to ensure contour evolution towards minimum potential. Inversion of active region produces new contour shifted by one pixel. So, inverted image is not simply calculated by \((\text{not } I_C)\) but using Eq. (4.6)

\[
\text{Inv} = (\text{NOT } I_C) \text{ OR } c
\]

Here, \(c=I_C \text{ AND NOT } (I_{CN} \text{ AND } I_{CS} \text{ AND } I_{CW} \text{ AND } I_{CE})\)

Inverted image computed using the above expression is represented by Fig. 4.11(a).

After inversion, an evolution of the contour image is again performed in all directions. So, expansion and contraction of active and background regions are performed in each iteration. Also, the steady direction of forces which are inflating and deflating in nature can be maintained by inverting balloon potential after each iteration. Fig. 4.11(b-e) represents second iteration results of contour expansion in the north direction, south direction, east direction and west direction respectively.

(g) Removal of noise

In the last stage, small objects (extracted from retinal vasculature obtained after MPLS evolution) having pixels less than 30 are removed. The noise which is present outside the border is removed by multiplying the final vasculature map with a
complement of the mask. After that morphological closing with a disk-shaped structuring element having size 1 is applied on the image. This is the final vasculature map as shown by Fig. 4.12(a), which can be further used for identification of various diseases.

Fig.4.11: (a) Inverted image, (b) Expanded image in the north direction, (c) Expanded image in the south direction, (d) Expanded image in the east direction, and (e) Expanded image in the west direction

4.4. Results and discussion

Various performance matrices like sensitivity, specificity, and accuracy have been computed by using extracted vasculature map and ground truth map. The accuracy of the proposed algorithm is better due to the extraction of an accurate binary mask of fundus image; better enhancement of image and evolution of vasculature map using MPLS technique in all four cardinal directions. Fig. 4.12(a) and Fig. 4.12(b) represent the extracted map and ground truth map of original retinal fundus image respectively.
The algorithm can be applied to all images of the DRIVE database. Fig. 4.13 represents the results of the three normal images of the DRIVE database and their extracted vasculature map respectively.

Table 4.1 represents a comparative analysis of sensitivity, specificity and accuracy metrics for DRIVE database. For analysis purpose, results of the proposed method are compared with the results obtained by Staal et al. [147], Soares et al. [149], Mendonca et al. [97], Martinez-Perez et al. [104], You et al. [105], Fraz et al.[75], Ravichandran et al. [141], Zhao et al. [157], Yin et al. [112], Frucci et al. [160] and Zhang et al. [76]. Fig. 4.14(a), (b) and (c) represents the comparative analysis of sensitivity, specificity and accuracy of DRIVE database respectively graphically. It is observed that for MPLS, average sensitivity (76.959%) comes out to be better than existing methodologies except [76] and specificity (98.34%) comes out to be better than existing methodologies except [160] because there is a trade-off between sensitivity and specificity of the image. Accuracy (96.30%) of the proposed methodology is better than the accuracy of all existing methodologies.
Fig. 4.13: (a,c,e) Original images, and (b,d,f) Corresponding extracted vasculature map
This methodology is applied to all test images and pathological images of the DRIVE database. Fig. 4.15 represents the results of the two pathological images of the DRIVE database and their extracted vasculature map respectively. Table 4.2 represents performance metrics results for 5 pathological images of the DRIVE database. It is observed that average sensitivity, specificity, and accuracy for pathological images are 70.80%, 96.40%, and 94.41% respectively. Extracted vasculature map represents the robust nature of the proposed algorithm.

### Table 4.1: Comparative analysis of Sensitivity, Specificity and Accuracy

<table>
<thead>
<tr>
<th>Method</th>
<th>Year</th>
<th>SN</th>
<th>SP</th>
<th>Acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staal[147]</td>
<td>2004</td>
<td>0.7194</td>
<td>0.9773</td>
<td>0.9442</td>
</tr>
<tr>
<td>Soares[149]</td>
<td>2006</td>
<td>0.7230</td>
<td>0.9762</td>
<td>0.9446</td>
</tr>
<tr>
<td>Mendonc-a[97]</td>
<td>2006</td>
<td>0.7344</td>
<td>0.9764</td>
<td>0.9452</td>
</tr>
<tr>
<td>Martinez-Perez [104]</td>
<td>2007</td>
<td>0.7246</td>
<td>0.9655</td>
<td>0.9344</td>
</tr>
<tr>
<td>You[105]</td>
<td>2011</td>
<td>0.7410</td>
<td>0.9751</td>
<td>0.9434</td>
</tr>
<tr>
<td>Fraz [75]</td>
<td>2012</td>
<td>0.7406</td>
<td>0.9807</td>
<td>0.9480</td>
</tr>
<tr>
<td>Ravichandran [141]</td>
<td>2014</td>
<td>0.7259</td>
<td>0.9799</td>
<td>0.9574</td>
</tr>
<tr>
<td>Zhao [157]</td>
<td>2014</td>
<td>0.7354</td>
<td>0.9789</td>
<td>0.9477</td>
</tr>
<tr>
<td>Yin [112]</td>
<td>2015</td>
<td>0.7246</td>
<td>0.9790</td>
<td>0.9403</td>
</tr>
<tr>
<td>Frucci [160]</td>
<td>2016</td>
<td>0.670</td>
<td>0.986</td>
<td>0.959</td>
</tr>
<tr>
<td>Zhang [76]</td>
<td>2017</td>
<td>0.7861</td>
<td>0.9712</td>
<td>0.9466</td>
</tr>
<tr>
<td><strong>Proposed Method</strong></td>
<td><strong>2018</strong></td>
<td><strong>0.76959</strong></td>
<td><strong>0.9834</strong></td>
<td><strong>0.9630</strong></td>
</tr>
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</table>
Fig. 4.14: Comparative analysis of (a) Sensitivity, (b) Specificity, and (c) Accuracy
Fig. 4.15: (a,c) Pathological images of DRIVE database, and (b,d) Corresponding extracted vasculature map

Table 4.2: Sensitivity, Specificity and Accuracy values of pathological images of DRIVE database

<table>
<thead>
<tr>
<th>Image</th>
<th>SN</th>
<th>SP</th>
<th>Acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6769</td>
<td>0.9941</td>
<td>0.9664</td>
</tr>
<tr>
<td>2</td>
<td>0.7617</td>
<td>0.9646</td>
<td>0.9519</td>
</tr>
<tr>
<td>3</td>
<td>0.7483</td>
<td>0.9220</td>
<td>0.9083</td>
</tr>
<tr>
<td>4</td>
<td>0.7012</td>
<td>0.9868</td>
<td>0.9702</td>
</tr>
<tr>
<td>5</td>
<td>0.6522</td>
<td>0.9523</td>
<td>0.9239</td>
</tr>
<tr>
<td>Average</td>
<td>0.7080</td>
<td>0.9640</td>
<td>0.9441</td>
</tr>
</tbody>
</table>
4.5. Summary

Accurate segmentation of vasculature map of fundus image plays an important role in the diagnostic procedure of various retinal disorders. In proposed work, initially, vasculature map is extracted from the masked image using global thresholding technique. MPLS technique based on BTH transformation method is used for the evolution of contour in all directions to extract the vasculature map in an accurate manner. Simulated results demonstrate that the proposed technique is an efficient approach that can segment vessels of normal images accurately; reaching the average sensitivity of 76.959%, average specificity of 98.34% and average accuracy of 96.30% for DRIVE database. This technique can also segment vessels of pathological images accurately; reaching the average sensitivity of 70.80%, average specificity of 96.40% and average accuracy of 94.41%. Since the methodology used for the extraction of vessels is unsupervised, so no training is required. Vessels connectivity is also done without any danger of collision. The proposed algorithm is an effective technique because it is used for extraction of vasculature map from normal as well as pathological images. Further extracted vasculature map can be used to find the features of the retina such as macula or fovea or optic disk or for the automatic identification of pathological elements like haemorrhage, microaneurysms, exudates or lesions accurately.
CHAPTER 5
IDENTIFICATION OF FEATURE POINTS OF FUNDUS IMAGE

5.1 Introduction

Characterization of the retinal structure is a basic component of automatic retinal disease screening systems [213] which is used for identification of different pathologies. In general, fundus image processing is used to segment anatomical structures of the eye. Further, important features are extracted from these structures for the characterization of various pathologies. Vascular network is the most crucial structures of the retinal fundus image. Different morphological attributes of vasculature map, like length, width, significant feature points, tortuosity, branching angles, and patterns can be used for diagnosis and treatment of different ophthalmologic and cardiovascular diseases [214]. Significant feature points of the vasculature map are mainly classified as terminal, bifurcation and cross over point. Identification of such feature points in fundus images is useful for predicting the various cardiovascular diseases e.g. Neovascularization and bleeding in retinal fundus images are related to changes in bifurcation point and crossover point. Neovascularization can be termed as the irregular growth of retinal vessels in some regions of the eye including the retina. Due to abnormal growth of retinal vessels, the number of bifurcation points and cross over points get exceeded beyond the actual count. This abnormal growth lifts the retina away from the back of the eye. This particular condition is termed as retinal detachment which can cause severe vision loss, including blindness. Hence, the count of these feature points is important for the early diagnosis and treatment of retinal detachment.

Bifurcation point and cross over points are the unique features of retinal vasculature map. Bifurcation point is basically a point where a vessel is split into two vessels. Whereas, when two vessels cross at a point, then it is known as a crossover point. The detected feature points can also be used for authentication purpose or for image
registration purpose [200]. Table 5.1 represents different cases of bifurcation points and cross-over points respectively.

Table 5.1: Different cases of bifurcation points and cross over points

<table>
<thead>
<tr>
<th>Bifurcation points</th>
<th>Cross over points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 1 1 1 0 1 1 0 1</td>
<td>0 1 0 1 0 1 0 1</td>
</tr>
<tr>
<td>1 1 0 1 1 1 1 1</td>
<td>1 1 1 0 1 0 1</td>
</tr>
<tr>
<td>1 1 0 1 1 1 1 1</td>
<td>0 1 0 1 1</td>
</tr>
</tbody>
</table>

5.2 Proposed methodology for the detection of feature points

In previous window method technique [55], two windows i.e. 3x3 and 5x5 have been used for computation of feature points of the fundus image. According to that technique, sum of pixels with value ‘1’ around the center pixel is computed in both 3x3 window and 5x5 window separately. If in both windows, the count of foreground pixels around the central pixel is equal to three, then the detected point is bifurcation point and if the count is equal to four, then the detected point is cross over point. In previous window techniques, a 3x3 and 5x5 window is taken around the central pixel. In a 3x3 window as shown by Fig. 5.1 (a), if we simply count the numbers of 1’s around the central pixel P, the number of 1's will be three i.e. P1, P5 and P8. Similarly, in a 5x5 window as shown by Fig. 5.1(b), if we simply count the numbers of 1’s around the central pixel P, the number of 1's will be three i.e. P1, P9 and P16. So, according to this, it is a bifurcation point. But actually, it's not a bifurcation because pixel P is the junction of only two vessels.

In proposed work, Modified Window Feature-point Detection (MWFD) is used to identify the vascular feature points in the fundus image. The MWFD technique makes use of two different windows 3x3 and 5x5 with alternative vessel pixel property for the detection of all feature points where only alternative 1's are considered around a central pixel P. In Fig. 5.1(a) both P1 and P8 are ‘1', so only one ‘1' will be considered. Hence, the total number of 1's around the central pixel P are only two i.e. P1 and P5. Similarly, in Fig. 5.1 (b), both P1 and P16 are ‘1’, so only one ‘1’ will be
considered. Hence, the total number of 1's around the central pixel P are only two i.e. P1 and P9. So, pixel P is neither a bifurcation point nor a cross over point in both windows. So, this pixel will not be considered a feature point, which is actually not a feature point.

![Figure 5.1: (a) 3x3 window, and (b) 5x5 window](image)

According to previous window technique [55], any point is considered as a feature point, if any of the following Window Feature-point Detection (WFD) condition is satisfied.

1. **Bifurcation point condition (WFD1):** In 3x3 window AND 5x5 window, count of 1’s around the central pixel should be equal to 3.
2. **Cross over point condition (WFD 2):** In 3x3 window AND 5x5 window, count of 1’s around the central pixel should be equal to 4.

According to proposed work, any point is considered as a feature point, if any of the following MWFD condition is satisfied.

1. **Bifurcation point condition (MWFD1):** In 3x3 window OR 5x5 window, count of alternative 1’s around the central pixel should be equal to 3.
2. **Cross over point condition (MWFD 2):** In 3x3 window OR 5x5 window, count of alternative 1’s around the central pixel should be equal to 4.

In proposed work, methodology as shown by Fig. 5.2 is used for the detection of feature points of the fundus image. In this technique, initially, skeletonization is
performed on vasculature map to obtain one-pixel wide vasculature map. Then spurs are removed to reduce the error generated due to skeletonization. After that, feature points are computed by applying alternative vessel pixel property approach on skeletonized vasculature map. Adjacent feature points are removed by checking the 8-adjacency between all points. The problem of conversion of one cross over point into two bifurcation points due to skeletonization is also resolved in this work.

Fig.5.2: Proposed MWFD methodology used for detection of feature points

Delete feature points detected as terminal points

Plot all crossover points and bifurcation points on vasculature map
(i) Read vasculature map
For the identification of bifurcation and cross over points of the complex network, initially, vasculature map of the retinal fundus image is read. The image chosen for simulation of proposed MWFD methodology is ground truth vasculature map of the image of DRIVE database (Im01) as shown by Fig. 5.3(a).

(ii) Fill holes in vasculature map
Several holes are present in the vasculature map of fundus image which leads to false identification of feature points. Fig. 5.3(a) represents the vasculature map with holes where a highlighted box is the small part of vasculature map having a hole as shown by Fig. 5.3(b). So, the next task is to fill the vessel hole for the correct identification of points. For hole filling operation, a 3x3 window is taken around each pixel of the image. If the central pixel of the window is binary ‘0’ and sum of pixels in the window around that central pixel is greater than or equal to 7, then binary ‘1’ is assigned to the central pixel. Fig. 5.3(c) represents the small part of the vasculature map with filled holes. Fig. 5.3(d) represents a complete vasculature map of fundus image with filled holes.
(iii) Perform skeletonization

Along the entire structure of complex vasculature map, properties like vessel width are not constant. So, without changing the direction of vessels and connectivity of the vessels, vessel width is reduced to one pixel. The conversion of a vessel having wide width to a vessel having one-pixel width is termed as skeletonization. The skeletonization of an image $I$ is expressed by the Eq. (5.1) [215].

$$S(I) = \bigcup_{m=0}^{K} S_m(I)$$

(5.1)

Where, $K$ is the last iteration before image $I$ erodes to an empty set and $S_m(I)$ is given by Eq. (5.2)

$$S_m(I) = (I \ominus mB) \ominus (I \ominus mB) \odot B$$

(5.2)

Here, $B$ is 3x3 structuring element of 1’s and $(I \ominus mB)$ indicates $K$ times erosions of image $I$ with structuring element $B$ which is expressed by Eq. (5.3).

$$(I \ominus mB) = (\ldots (I \ominus B) \ominus B ) \ominus \ldots ) \ominus B$$

(5.3)

In this work, skeletonization operation is performed infinity times; it means the process is repeated until the output image no longer changes. Fig. 5.4(a) represents the skeletonized image of vasculature map.
(iv) Remove spur

The image produced after skeletonization contains too many spurs. All these spurs lead to false identification of feature points. So, spurs should be removed from the skeletonized image. For this purpose, the pruning method is applied to the skeletonized image.

In pruning method, initially thinning is performed on an image $I$ using structuring element $B$ as represented using Eq. (5.4) [215].

$$P_1 = I \otimes \{B\} \quad (5.4)$$

Here, $\otimes$ symbol indicates thinning operation with the structuring element $B$. $\{B\}$ denotes the sequence of 8 structuring elements shown in Table 5.2. In this work, thinning is repeated two times to remove the spurs having pixel length less than or equal to two.

**Table 5.2: Structuring elements used for pruning**

<table>
<thead>
<tr>
<th>Element 1</th>
<th>Element 2</th>
<th>Element 3</th>
<th>Element 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>X</td>
<td>1</td>
<td>1</td>
<td>X</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>X</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

After that, all endpoints in $P_1$ are retained by using Eq. (5.5).

$$P_2 = \bigcup_{k=1}^{8} (P_1 \ominus B^k) \quad (5.5)$$

Here, $\ominus$ symbol indicates HMT with the above specified structuring element $\{B\}$.

Now next step is to perform dilation operation two times on $P_2$ using an image $I$ as delimiter as shown by Eq. (5.6).

$$P_3 = (P_2 \oplus H) \cap I \quad (5.6)$$
Here, $\oplus$ symbol indicates dilation of $P_2$ with H which is 3x3 structuring element of 1’s. Finally, the union of $P_1$ and $P_3$ is performed to get the desired result as shown by Eq. (5.7).

$$P_4 = P_1 \cup P_3$$  \hspace{1cm} (5.7)

Fig. 5.4(a) represents the skeletonized image of vasculature map with spurs where a highlighted box is the small part of vasculature map having spurs as shown by Fig. 5.4(b). Fig. 5.4(c) represents that small part of the image without spur and Fig. 5.4(d) represents the complete image of vasculature map with spur removed.

Fig.5.4: (a) Skeletonized image, (b) Small part of skeletonized image with spurs, (c) Small part of the skeletonized image with spur removed, and (d) Complete skeletonized image with spur removed
(v) Find feature points using 3x3 Window

In [55], the window technique is taken around the central pixel for identification of bifurcation and cross over points of vasculature map. In proposed work, two different windows of size 3x3 and 5x5 have been used for identification of feature points. The alternative vessel pixel property of window is also applied to reduce the false identification of feature points. Algo. (5.1) is used for detection of all feature points using a 3x3 window.

Algo. 5.1: Algorithm for point detection using 3x3 window

```
FOR itr=1:7
    IF P(itr) EQUALS ‘0’ AND P(itr+1) EQUALS ‘1’
        count=count+1;
    END
END

IF count EQUALS 3
    THEN Bifurcation Point

IF count EQUALS 4
    THEN Cross Over Point
```

According to this algorithm, numbers of ‘1’’s that does not have any neighboring pixels as ‘1’ are computed around the central pixel (from P1-P7). After that, if the ‘count’ is equal to 3, then only that central pixel is considered as the bifurcation point and if the ‘count' is equal to 4, then the central pixel will be cross-over point according to a 3x3 window. Fig. 5.5 (a) and Fig. 5.5 (b) shows the different cases of bifurcation points and cross over points respectively using alternative vessel pixel property. Fig. 5.5 (c) and Fig. 5.5 (d) represents the bifurcations points (shown by green dots) and cross over points (highlighted by red boxes) plotted on skeletonized vasculature map respectively using the above algorithm.
(vi) Find feature points using 5x5 Window

After detection of points using a 3x3 window, the next step is to use a window of size 5x5 around the central pixel for the detection of feature points as shown in Fig. 5.6(a). Here also alternative vessel pixel property is applied on a 5x5 window to reduce false identifications. Algo. (5.2) is used for detection of all feature points using a 5x5 window.
Algo. 5.2: Algorithm for point detection using 5x5 window

\[
\begin{align*}
\text{FOR } & \text{itr}=1:15 \\
& \text{IF } P(\text{itr}) \text{ EQUALS } '0' \text{ AND } P(\text{itr}+1) \text{ EQUALS } '1' \\
& \text{count} = \text{count} + 1; \\
& \text{END} \\
\text{END} \\
\text{IF } & \text{count} \text{ EQUALS } 3 \\
& \text{THEN Bifurcation Point} \\
\text{IF } & \text{count} \text{ EQUALS } 4 \\
& \text{THEN Cross Over Point}
\end{align*}
\]

According to this algorithm, numbers of ‘1’’s that does not have any neighboring pixels as ‘1’ are computed around the central pixel (from P1-P16). After that, if the ‘count’ is equal to 3, then that central pixel is bifurcation point and if the ‘count’ is equal to 4, then the central pixel is considered as cross-over point according to a 5x5 window. Fig. 5.6 (b) and (c) represents the bifurcations points (shown by green dots) and cross over points (shown by red color dots) plotted on skeletonized vasculature map respectively using 5x5 window.

(vii) Combine bifurcation points of 3x3 and 5x5 windows

In the above two steps, bifurcation and cross over points are computed using two different windows. Now the next task is to combine all feature points obtained from different windows. In [55], bifurcations points are computed with 3x3 and 5x5 window using Eq.(5.8).

\[ B = B_3 \times B_5 \quad (5.8) \]

In the proposed MWFD technique, all bifurcations points are computed with 3x3 and 5x5 window by using Eq.(5.9).

\[ B = (B_3 + B_5) \quad (5.9) \]

Here B represents combined bifurcation points of all windows; B3 and B5 represent the bifurcation points computed from 3x3 and 5x5 windows respectively. Combined
bifurcation point image obtained using [55] and proposed MWFD method is shown by Fig. 5.7 (a) and (b) respectively.

Fig. 5.6: (a) 5x5 window, (b) Bifurcation points produced using a 5x5 window, and (c) Cross over points produced using a 5x5 window

Fig. 5.7 (a) Bifurcation points detected using [55], and (b) Bifurcation points detected using proposed MWFD method
(viii) Combine cross over points of 3x3 and 5x5 window
Similarly, in [55], cross over points are computed with 3x3 and 5x5 window using Eq. (5.10).

\[ C = C_3 \ast C_5 \]  \hspace{1cm} (5.10)

In the proposed MWFD technique, all cross over points is computed with 3x3 and 5x5 window using Eq. (5.11).

\[ C = (C_3 + C_5) \] \hspace{1cm} (5.11)

Here C represents combined cross over points of all windows; C3 and C5 represent cross over points computed from 3x3 and 5x5 windows respectively. Combined cross over point image obtained using [55] and proposed MWFD method is shown by Fig. 5.8 (a) and (b) respectively.

![Fig.5.8: (a) Cross over points detected using [55], and (b) Cross over points detected using proposed MWFD method](image)

(ix) Convert bifurcation points having 8-adjacency into single bifurcation point
Two pixels p and q with values from set V will be 8-adjacent if q lies in the set \( N_8(p) \).
So, if two bifurcation points are having 8-adjacency, then out of adjacent bifurcation points, only one point is picked. Fig. 5.9(a) represents the small part of the image produced after combining bifurcation points of all windows. Here, three bifurcation points are shown by three red dots which are 8-adjacent. So, only one bifurcation
point is picked from all these points as represented by Fig. 5.9(b). Fig. 5.9(c) represents the complete image produced after checking the 8-adjacency of all bifurcation points.

![Diagram](image1.png)

**Fig.5.9:** (a) Small part of the image represents bifurcation points, (b) Small part of the image represents bifurcation point produced after checking 8-adjacency, and (c) Complete image of bifurcation points after checking 8-adjacency

(x) **Convert two connected bifurcations points into a single cross over point**

During skeletonization, some cross over points is converted to two bifurcation points. In Fig. 5.10(a), a small part of vasculature map is shown where only one cross over point is present but after skeletonization, this cross over point is converted into two bifurcation points as shown by Fig. 5.10(b).

![Diagram](image2.png)

**Fig.5.10:** (a) Small part of vasculature map with one cross over point, and (b) Small part of skeletonized vasculature map with two bifurcation points
This issue is also resolved in this work. Two bifurcation points will be converted to one cross over point if the following two conditions are satisfied.

(1) If chessboard distance between two bifurcation points is less than a certain threshold value. Here, $D_8(p,q)$ threshold value is taken as 3.

(2) If 8-connectivity exists between two bifurcation points.

Here, chessboard distance i.e. $D_8$ distance between the two points $p(m,n)$ and $q(r,s)$ is defined by Eq.(5.12).

$$D_8(p,q) = \max(|m - r|,|n - s|)$$  \hspace{1cm} (5.12)

Fig. 5.11(a) represents the image which satisfies only first condition and Fig. 5.11(b) represents the image which satisfies the only second condition. So, in both these figures, these points are bifurcation points not cross over points. But Fig. 5.11(c) represents the image which satisfies both conditions, so these are not two bifurcation points, this is one cross over point.

![Fig. 5.11: (a) First condition satisfied, (b) Only second condition is satisfied, and (c) Both conditions satisfied](image)

Fig. 5.12(a) represents a small part of the image which contains two bifurcation points highlighted with red boxes on the skeletonized map. Fig. 5.12(b) representing the small part of the actual vasculature map which is showing that it has only one cross over point. So, the two bifurcation points are converted into one cross over point after applying the above-mentioned conditions as shown by Fig. 5.12(c). Fig.
5.12(d) represents the complete image produced after the combination of two false bifurcation points into one cross over point.

Fig. 5.12: (a) Two bifurcation points on the small part of the skeletonized map, (b) Small part of original vasculature map, (c) Two bifurcation points converted to one cross over point, and (d) Complete image produced after conversion of two connected bifurcations into a single cross over point.

(xi) Convert cross over points having 8-adjacency into single cross over point

After production of all cross over points, 8-adjacency is checked between different cross over points. If two cross over points is having 8-adjacency, then out of adjacent cross over points, only one point is picked. Fig. 5.13(a) represents the small part of the image produced after combining cross over points of all windows. Here, three cross over points is shown by three red boxes which are 8-adjacent. So, only one cross over point is picked from all these points as represented by Fig. 5.13(b). Fig.
5.13(c) represents the complete image produced after checking the adjacency of all cross over points.

![Fig.5.13: (a) Small part representing cross over points having 8-adjacency, (b) Small part representing the conversion of cross over points having 8-adjacency into single cross over point, and (c) Complete image of cross over points after checking 8-adjacency](image)

(xii) **Delete feature points detected as terminal points**

In the above steps, two different images of bifurcation points (Image B) and cross over points (Image C) are created. So, final feature points are computed by combining image B with image C. Fig. 5.14(a) represents the image produced after
combining all feature points on the vasculature map. Now next step is to detect all terminal points of vasculature map using HMT. Terminals points are the end pixels of each retinal blood vessel in the vasculature map.

The HMT transformation of an image $I$ by $B$ is given by Eq.(5.13) [22], where $B$ is a pair of structuring elements $B = (B_1, B_2)$.

$$ I \ominus B = (I \ominus B_1) \cap (I^c \ominus B_2) \quad (5.13) $$

Here, $I^c$ is the set complement of $I$. It locates all pixel points that match $B_1$ structure called hit but do not match $B_2$ structure called miss. Here, HMT is performed on the skeletonized image with the help of the ‘$n$' pairs of structuring elements $B_{n1}$ and $B_{n2}$ where $n$ varies from 1 to 8. Different structuring elements used for HMT in this work are shown in Table 5.3.

<table>
<thead>
<tr>
<th>$B_{11}$</th>
<th>$B_{21}$</th>
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<td>0 0 0</td>
<td>0 0 0</td>
<td>0 1 0</td>
<td>0 0 0</td>
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<td>0 1 1</td>
<td>0 1 0</td>
<td>0 1 0</td>
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<table>
<thead>
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<th>$B_{32}$</th>
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<td>0 0 0</td>
<td>1 1 1</td>
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</tr>
<tr>
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<td>1 1 0</td>
<td>1 1 1</td>
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<table>
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<table>
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<th>$B_{72}$</th>
<th>$B_{82}$</th>
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<td>1 0 1</td>
</tr>
<tr>
<td>0 1 1</td>
<td>1 1 0</td>
<td>1 1 0</td>
<td>0 1 1</td>
</tr>
</tbody>
</table>

Fig. 5.14(b) represents all terminal points detected using HMT transformation (highlighted by blue stars) along with all feature points (highlighted by green dots). Fig. 5.14(c) represents the small part of the map which contains one feature point and one terminal point. For deletion of feature points which are also terminal points, a 5x5 window is considered around each terminal point. If any feature point exists in
that window, then that feature point is deleted from the vasculature map. Fig. 5.14(d) represents the small part of the map which shows the deletion of feature point which is actually a terminal point. Fig. 5.14(e) represents the complete map of the fundus image with actual feature points.

Fig. 5.14: (a) Combined feature points image, (b) Terminal points shown by blue stars along with feature points, (c) Small part of map showing both terminal point (blue box) and feature point (green box), (d) Small part of map with feature point deleted, and (e) Complete image of final feature points.
(xiii) Plot all crossover points and bifurcation points on vasculature map

Now, all detected feature points are plotted on the vasculature map of ground truth image to compare proposed methodology results with the ground truth results. In Fig. 5.15, red points represent the points of ground truth image and green points represent the feature points computed from the proposed MWFD methodology.

![Fig.5.15: Calculated points plotted over ground truth points](image)

Points computed by [55] and proposed MWFD techniques are plotted on skeletonized vasculature map as shown by Fig. 5.16(a) and (b) respectively. Fig. 5.17(a) and (b) represents the true positives of [55] and proposed MWFD technique respectively. It is analyzed that some points (highlighted with yellow boxes) which are actually feature points are not detected using previous technique but these points are detected using proposed MWFD technique which shows that proposed technique works well in the identification of feature points. Similarly, Fig. 5.17(c) and (d) represents the false positives of [55] and proposed MWFD technique respectively. It is analyzed that some points (highlighted with yellow box) which are not feature points actually are detected using previous technique but these points are not detected using proposed MWFD technique which further improves the accuracy of the system. Justification of
highlighted points identified by [55] and proposed MWFD technique is shown in Table 5.4.

Fig. 5.16: (a) Combined feature points obtained using [55], and (b) Combined feature points obtained using the proposed MWFD method
Fig. 5.17: (a) True positives of [55], (b) True positives of proposed work, (c) False positives of [55], and (d) False positives of proposed work

Table 5.4: Justification of feature points of previous WFD method and proposed MWFD method

<table>
<thead>
<tr>
<th>(a) Previous WFD Method</th>
<th>(b) Proposed MWFD method</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Count in 3x3 window=4</td>
<td>Count in 3x3 window=2</td>
</tr>
<tr>
<td>Count in 5x5 window=5</td>
<td>Count in 5x5 window=3</td>
</tr>
<tr>
<td>Neither WFD1 nor WFD2 is satisfied. So, it's not a feature point, but actually, it's a feature point.</td>
<td>MWFD1 condition is satisfied. So, it’s a feature point.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Count in 3x3 window=4</td>
<td>Count in 3x3 window=2</td>
</tr>
<tr>
<td>Count in 5x5 window=5</td>
<td>Count in 5x5 window=4</td>
</tr>
<tr>
<td>Neither WFD1 nor WFD2 is satisfied. So, it's not a feature point, but actually, it's a feature point.</td>
<td>MWFD2 condition is satisfied. So, it's a feature point.</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Count in 3x3 window=6</td>
<td>Count in 3x3 window=1</td>
</tr>
<tr>
<td>Count in 5x5 window=4</td>
<td>Count in 5x5 window=4</td>
</tr>
<tr>
<td>Neither WFD1 nor WFD2 is satisfied. So, it's not a feature point, but actually, it's a feature point.</td>
<td>MWFD2 condition is satisfied. So, it's a feature point.</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Count in 3x3 window=3</td>
<td>Count in 3x3 window=2</td>
</tr>
<tr>
<td>Count in 5x5 window=3</td>
<td>Count in 5x5 window=2</td>
</tr>
<tr>
<td>WFD1 is satisfied. So, it's a feature point which is actually not a feature point.</td>
<td>Neither MWFD1 nor MWFD2 is satisfied. So, it's not a feature point and actually, it's not a feature point.</td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Count in 3x3 window=3</td>
<td>Count in 3x3 window=2</td>
</tr>
<tr>
<td>Count in 5x5 window=3</td>
<td>Count in 5x5 window=2</td>
</tr>
<tr>
<td>WFD1 is satisfied. So, it’s a feature point which is actually not a feature point.</td>
<td>Neither MWFD1 nor MWFD2 is satisfied. So, it's not a feature point and actually, it's not a feature point.</td>
</tr>
</tbody>
</table>
5.3 Results and discussion

Different results of true positive and false positive of proposed method and [55] method are represented by Table 5.5 and Table 5.6 respectively.

In Table 5.5, the first column represents the true positives of [55] and the second column represents the true positives computed by the proposed MWFD technique by green dots. In both columns, red dots represent the ground truth feature points on the vasculature map. For comparison purpose, some points are highlighted with yellow color boxes on same locations of vasculature map in both columns of Table. In the first column of this Table, only one ground truth feature point is present in each yellow box. No feature point is computed at this location using the algorithm proposed by [55]. But in the second column of this Table, yellow box is showing ground truth feature point as well as feature point computed using proposed MWFD technique which proves that proposed MWFD technique overperforms the algorithm proposed in [55] in terms of true positives.

Similarly, in Table 5.6, the first column represents the false positives of [55] and the second column represents the false positives computed by the proposed MWFD technique by green dots. In both columns, red dots represent the ground truth feature points on the vasculature map. For comparison purpose, some points are highlighted with yellow color boxes on same locations of vasculature map in both columns of Table. In first column of Table 5.6, each yellow box is showing the detected feature points using [55] which do not actually match with ground truth feature points whereas in the second column of this Table, yellow boxes corresponding to that location are not showing any false feature points which further proves that proposed MWFD technique overperforms the algorithm proposed in [55] in terms of false positives also.

It is analyzed that some ground truth feature points are not detected using the previous technique but these points are detected using the proposed MWFD technique. Similarly, some points which are not ground truth feature points actually, are detected using the previous technique but these points are not detected using the proposed MWFD technique which further improves the performance of the system.
Table 5.5: True Positives of [55] and proposed MWFD method

<table>
<thead>
<tr>
<th>True Positives of [55]</th>
<th>True Positives of Proposed MWFD Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image9.png" alt="Image" /></td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
<tr>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>
### Table 5.6: False Positives of [55] and proposed MWFD method

<table>
<thead>
<tr>
<th>False Positives of [55]</th>
<th>False Positives of Proposed MWFD Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Image of False Positives of [55]" /></td>
<td><img src="image2" alt="Image of False Positives of Proposed MWFD Method" /></td>
</tr>
<tr>
<td><img src="image3" alt="Image of False Positives of [55]" /></td>
<td><img src="image4" alt="Image of False Positives of Proposed MWFD Method" /></td>
</tr>
</tbody>
</table>
Comparative results of previous WFD method and proposed MWFD method is also made with TP, FP and FN values as represented by Table 5.7. In this Table, results of previous WFD method [55] and proposed MWFD method are represented using small windows; where red points represent ground truth feature points and green points represent detected feature points. In this Table, the first column is showing the results obtained by previous WFD method; the second column is showing the results obtained by proposed MWFD method and the third column is showing the values of TP, FP, and FN in comparison to actual ground truth feature points.

Table 5.7: Comparative results of previous WFD method and proposed MWFD method with TP, FP and FN values

<table>
<thead>
<tr>
<th>Ground truth feature points=4</th>
<th>Previous WFD Method Results [55]</th>
<th>Proposed MWFD Method Results</th>
<th>Comparative Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>Ground truth feature points=4</td>
</tr>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>WFD</td>
</tr>
<tr>
<td>TP</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ground truth feature points=1</th>
<th>Previous WFD Method Results [55]</th>
<th>Proposed MWFD Method Results</th>
<th>Comparative Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>Ground truth feature points=1</td>
</tr>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>WFD</td>
</tr>
<tr>
<td>TP</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ground truth feature points=3</th>
<th>Previous WFD Method Results [55]</th>
<th>Proposed MWFD Method Results</th>
<th>Comparative Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>Ground truth feature points=3</td>
</tr>
<tr>
<td></td>
<td><img src="image1" alt="Previous WFD Method Results" /></td>
<td><img src="image2" alt="Proposed MWFD Method Results" /></td>
<td>WFD</td>
</tr>
<tr>
<td>TP</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>FP</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FN</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
5.4 Summary
Identification of feature points of vasculature map of fundus image leads to early detection of various diseases present in fundus image. In literature, different window techniques have been used for identification of points but these techniques lead to false detection of feature points of vasculature map. So, this research proposed a modified window technique that works effectively in the identification of feature points. Simulated results show quantitative improvements by increasing the number of true positives and reducing false positives and false negatives. These results prove that the proposed technique is an efficient and reliable technique for the detection of feature points of the retinal fundus image.
CHAPTER 6

CONCLUSION

Segmentation of vasculature map of fundus image plays a vital role in the diagnostic procedure of various retinal disorders. For accurate segmentation of vasculature map, the focus is made only on the object pixels. In the proposed method, the bimodal masking technique is used for the generation of a binary mask of the fundus image. It is observed that average sensitivity, specificity, and accuracy of mask generated using bimodal masking technique comes out to be 99.46%, 99.77%, and 99.56% respectively. Comparative analysis of mask produced using the proposed bimodal masking technique is also made with Otsu’s thresholding technique showing the improved performance of the proposed method.

For extraction of vasculature map of the fundus image, initially global thresholding is used for segmentation of vasculature map and after that MPLS is used for evolution of map in four cardinal directions. Simulated results demonstrate that the proposed technique is an efficient approach that can segment vessels of normal images accurately; reaching the average sensitivity of 76.959%, average specificity of 98.34% and average accuracy of 96.30% for DRIVE database. This technique can also segment vessels of pathological images accurately; reaching the average sensitivity of 70.80%, average specificity of 96.40% and average accuracy of 94.41%. Since the methodology used for the extraction of vessels is unsupervised, so no training is required. The proposed algorithm is an effective technique because it is used for extraction of vasculature map from normal images in an accurate way. Comparisons have also been made with other existing techniques showing the higher performance of the proposed method.

Identification of bifurcation and cross over points in an efficient way is a very tedious task due to the complex nature of the retinal vasculature map. In this work, a novel method called the MWFD method is proposed for the identification of feature points from images of the complex vasculature map. Here, two different windows with alternative vessel pixel property have been used for the detection of all feature points.
The proposed MWFD methodology is applied on DRIVE database and various pixel classifications and misclassifications are computed for the identification of feature points of the fundus image. The results prove that the modified window technique detect feature points with high accuracy as compared to previously existing window techniques.

In the future, extracted vasculature map can be used for measurement of different attributes of vasculature map like width and diameter of blood vessels that plays a vital role in the diagnosis of various cardiovascular diseases.

Also, the algorithm could be developed for the detection of various abnormal attributes of retinal fundus image such as haemorrhages, exudates, and microaneurysms, etc.

Further, the detected feature points especially cross over points can be used for localization of AV crossing locations. So, the quantitative and objective assessment of arteriovenous nicking can also be performed in the future for the prediction of various eye and cardiovascular diseases.
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