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)

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

(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_{\text{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)

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

Eq. (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.

\textbf{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.

\textbf{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 crossover 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