AUTOMATIC SUBLINGUAL MICROCIRCULATORY IMAGE ANALYSIS AND QUANTITATIVE ASSESSMENT OF THE MICROCIRCULATION

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Abstract
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Automatic sublingual microcirculatory image analysis and quantitative assessment of disease severity
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Microcirculation of cardiovascular system is the place where the blood flow is regulated, tissue oxygen is delivered. Microcirculatory dysfunction results in maldistribution of blood flow and hypoperfusion which can lead to organ dysfunction and death. Organ failure will cause sepsis, shock, hypertension, hemorrhage, diabetics and other diseases. These diseases can be reflected in the dynamic changes in microcirculation, especially the microcirculation in capillaries. Early diagnosis and treatment of these diseases can produce favorable outcomes based on computed hemodynamic parameters. Investigation and quantitative assessment of microcirculation play an important role in monitoring human physiological health. These hemodynamic parameters assessing microcirculation are obtained qualitatively and semi-quantitatively by trained image analysts. In this work, our objective is to develop a fully functional, automatic computer assisted diagnosis tool providing quantitative microvascular measurements for the characterization of sublingual microcirculation in healthy subjects and infected patients and assist doctors in determining pathological conditions of patients.
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Chapter 1

Introduction

1.1 Significance of This Study

Microcirculation is the blood circulation in small blood vessels. The microcirculation is composed of a network of arterioles, capillaries, and venules that connect the cardiovascular system. The blood flows from arterioles into the capillaries and then flows to venules. Microcirculation of cardiovascular system is the place where the blood flow is regulated and nutrients and oxygen are delivered to tissues, and waste products are removed. Microcirculatory dysfunction has been observed in patients and animal models with certain acute cardiovascular and infectious diseases, such as sepsis [1, 2, 3, 4, 5, 6, 7, 8], hypertension [9, 10], hemorrhagic shock [11], diabetics [12], and cardiac arrest [13]. These diseases can be reflected in the dynamic changes in microcirculation, especially the microcirculation in capillaries [14]. In the most severe cases, maldistribution of blood flow leads to hypoperfusion which can result in organ dysfunction and death. Sepsis is a system-wide inflammatory response to infection that strikes about 750,000 Americans per year. Sepsis can progress rapidly, with mortality rates greater than 30 percent [15]. Severe sepsis and septic shock disrupts the normal function of the vascular endothelium and is associated with the concomitant activation of the inflammatory and coagulation cascades [16]. The host response to sepsis causes leukocyte adhesion to microvascular endothelium and reduced vasomotor tone resulting in alterations in microvascular hemodynamics, such as reduced density of perfused capillaries, and heterogeneity of
Chapter 1. Introduction

regional perfusion, with vascular shunting [2, 8]. Likewise during hemorrhagic shock, microcirculation changes include the presence of leukocyte rolling and adhesion, the reduction of blood flow and arteriolar diameter, and impaired functional capillary density [17]. Aggressive, early diagnosis and treatment has been shown to improve clinical outcomes in these diseases [1, 5, 6, 18, 19]. Therefore, investigation of microcirculation has the potential to play an important role in monitoring human physiological health. Moreover, continuous monitoring and quantitative assessment of microcirculation is very helpful to assist doctors in the diagnosis of the pathological recovery conditions during and after the treatment for patients with these diseases, especially for patients under the critically ill conditions. Resuscitation is a general term for reviving a patient with acute illness, such as infection or circulatory shock. Our main concern with microcirculation is inadequate perfusion that results from shock. Leukocyte adhesion and rolling may be due to endothelium damage. Bedside real-time assessment of microcirculation can help determine when a treatment endpoint has been reached [1, 5, 6, 18, 19, 20].

1.2 Problem Statement

The purpose of this research is to develop a fully functional, automatic framework to quantitatively measure the functionality of microcirculation and assess pathological conditions of patients based on quantitative microvascular measurements from sublingual microcirculatory videos. Finger tip nailfold skin and sublingual mucosa are two main locations for optical observation of microcirculation at the bedside. Quantitative measurements of microcirculation may provide valuable prognostic information of pathological conditions of patients. A variety of non-invasive image acquisition and semi-quantitative scoring systems have then been developed to monitor and evaluate the microcirculation at the bedside. Due to the high variability in image analysis, a round table conference was organized aiming to achieve a consensus on microcirculatory image acquisition and analysis [21]. According to this consensus conference, the standardized evaluation of microcirculation should consider three different characteristics. They are vessel density, including total vessel density (TVD) and perfused vessel density (PVD) [2], capillary perfusion including proportional of perfused vessel (PPV)
and microcirculatory flow index (MFI) [5], and heterogeneity index (HetIndex) [8]. Semi-quantitative assessment of TVD, PVD, MFI, and PPV is time consuming and requires significant user interaction. Development of robust automated microcirculation analysis tools at the bedside is necessary to standardize microcirculatory measurements and assist in clinical decisions based on these measurements [22]. Geometry of vessels and velocity of red blood cells (RBCs) need to be estimated first in order to obtain these semi-quantitative microvascular measurements and investigate quantitative microvascular measurements. Algorithms to automatically extract vessels and estimate RBC velocity are developed and tested through real sublingual microcirculatory videos. A few quantitative microvascular measurements are automatically calculated based on extracted vessels and estimated RBC velocities. These microvascular measurements can be used to quantitatively characterize sublingual microcirculation.

1.3 Challenges

Tackling all these tasks in order to achieve the ultimate goal is not a trivial problem. Many challenges exist due to limitations of imaging devices. Even though current microcirculatory imaging techniques provide reasonable quality videos of microcirculation with respect to venular contrast, sharpness, and resolution, microcirculatory images are still noisy and have low contrast. The camera may jitter or the subjects may move during the video recording. The movement can be compensated by performing video registration techniques. Microcirculatory video registration is difficult. Thousands of moving objects exist in microcirculatory videos, and camera focus also changes rapidly during imaging. RBC occlusions limit capabilities of 2D rigid registration. Intensity based image registration will not produce good registration results, because images are noisy and illuminance of images varies over space and time. For control point based image registration, distinct features, such as edges, corners, are hard to identify in microcirculatory images.

None of the existing techniques can produce zero registration errors. The presence of RBCs depends on the intensity change of each pixel over time. If pixels in videos appear in different positions at different frames with uneven illumination, velocity estimation of RBCs will not
be very accurate. Moreover, at least three consecutive frames are required in order to visualize the moving objects. The velocity measurement is restricted by the imaging rate and the length of vessels where the flow is assessed. With low video frame rates, some RBCs are blurred and can be barely recognized, and the transition of RBCs and plasma is fuzzy thereby distinguishing different clouds of RBCs very difficult. These image artifacts make the velocity estimation of RBCs a challenging problem. Not only these image artifacts make the velocity estimation of RBCs difficult, but also for microcirculation at sublingual surfaces, RBCs occlude with each other. With current resolution limitations, it is not possible to track single RBC. RBCs travel along the axial direction of vessels, but they travel at different speed along the cross section of vessels. Furthermore, speed and direction of RBCs may vary at different times during the video sequence. Densities of RBCs in each vessel also differ.

Vessel geometry is hard to extract. Vessels have low contrast to the background in video sequences. They are embedded at various depths to the surface with different diameters. Vessel segments in good focus are clear in videos, while other vessels out of the plane of focus cannot be resolved. Vessels are visualized mainly due to the passage of RBCs, thus, perfused vessels are easier to be identified. However, because of the transition of RBCs and plasma gaps, vessels at each frame are always interrupted and discontinuous. Vessel walls are not visualized either, especially in small vessels with RBCs separated by long plasma gaps. It then makes hard to determine vessel diameters. All these make the extraction of microvasculature network, especially capillaries, more difficult. The smallest capillary blood vessels have been shown to be the most sensitive indicators of microcirculatory dysfunction [2, 6].

1.4 Contribution

Existing approaches for vessel segmentation and RBC velocity estimation from sublingual microcirculation videos are mostly manual and semi-automatic. Manual and semi-automatic work is time-consuming and requires significant human interaction. No automated analysis has been widely adapted. In light of difficulties in microvascular image analysis, we propose a fully automatic computer-assisted tool to automatically, accurately, and rapidly perform these
tasks from even noisy and low quality human microcirculatory videos. A novel line detection method using nonparametric theoretic measurements and order statistics to automatically estimation the orientation of RBC or plasma gap traces in noisy space-time images is proposed. Moreover, this is the first study to investigate quantitative microcirculatory measurements. Development of robust automated microcirculation analysis tools at the bedside is necessary to standardize microcirculatory measurements and assist in clinical decisions based on these measurements.

1.5 Organization

The following provides a brief explanation of each chapter:

**Chapter 2**, presents methods of microcirculation visualization and collection, and existing microvascular image analysis techniques.

**Chapter 3**, introduces microcirculatory image pre-processing and video stabilization.

**Chapter 4**, explains our proposed microcirculatory vessel extraction method.

**Chapter 5**, explains our proposed RBC velocity estimation method.

**Chapter 6**, introduces quantitative microvascular measurements and characterization of disease severity based on these measurements.

**Chapter 7**, draws the conclusion for the entire thesis and future work.
Chapter 2

Related Work and Literature Review

2.1 Visualization of Microcirculation

Several noninvasive imaging techniques for real-time clinical observation and investigation of the intact microcirculation have been developed, such as video microscopy/capillaroscopy [23, 24, 25], fluorescence videomicroscopy [26], ultrasound imaging [27], orthogonal polarization spectroscopy (OPS) [28, 29, 30, 31], and sidestream dark field (SDF) [6, 32, 33]. A video microscopy/capillaroscopy system is a light microscope combined with a video camera to record microcirculation in real time at specified imaging rate. It is commonly used to image the microcirculation in nailfold skin area [23, 24, 25]. A fluorescence microscopy system consists of a fluorescence microscope, appropriate filters, a video camera, and a recording system imaging the fluoresced light illuminated from the injected fluorescent dyes [26]. For ultrasound imaging technique, a piezoelectric transducer transmit ultrasound waves at the desired frequency. The waves penetrate the skin and then are reflected at tissue boundaries or blood flow back to the transducer and are detected as echoes. The transducer turns the echoes into electrical pulses, and the ultrasound scanner then transforms the electrical pulses into images. The strength of the echo determines the brightness of image pixels, the higher the intensity value, the stronger the echo. Moving RBCs change the frequency of the reflected pulses, thus, the echo will be weaker. RBCs appear dark in ultrasound images compared
to stationary tissues. However, ultrasound imaging suffers from the limited resolution and contrast [27].

OPS and SDF are two of the few non-invasive clinical bedside imaging techniques for real-time observation of the intact microcirculation. They have been applied to visualize microcirculation at various sites, including sublingual mucosa. Figure 2.1 shows the implemented hand-held OPS imaging probe and the schematic of the OPS imaging technique [28]. Green filtered light is guided to the first polarizer through an optical fiber first. The polarized light is reflected by a half pass mirror to provide dark field illumination to the tissues. Some of the lights are reflected and some others are scattered by the tissues. These scattered lights travel through the mirror and reach the second polarizer. The second polarizer is orthogonal to the first polarizer, called analyzer. Reflected lights lose the polarization characteristics blocked by the analyzer, while scattered lights are depolarized and transmitted by the analyzer and are imaged onto a CCD camera. Scattered green lights can be absorbed by hemoglobin in RBCs. The tissues embedding the microcirculation lack of absorption appear white/grayish, and RBCs appear dark/black against a bright tissue background. Video sequences are recorded on a digital video recorder and displayed in a black and white monitor [29, 30, 30, 31] (Figure 2.1(b)). This imaging technique has been implemented into a small hand-held device (Figure 2.1(a)). This small hand-held imaging device can be used at the bedside allowing the direct visualization and monitoring of the microcirculation in patients in real time, especially critically ill patients [1, 2, 3, 4, 5, 7, 34, 35]. Thus, OPS imaging has made a great impact in clinical microcirculatory research. OPS imaging technique suffers from several drawbacks.
For example, flowing RBCs and movement of the OPS device cause the smearing of moving objects over video sequences. A more advanced imaging technique, SDF imaging technique, was developed [6, 32, 33]. Figure 2.2 shows the SDF imaging device [33] (Microscan, B. V., Amsterdam, The Netherlands). SDF imaging technique is primarily used to observe the microvascular bed beneath the thin, transparent epithelial layer of the sublingual mucosa. The device has a 5 X magnifying objective lens system-containing probe attached to the sublingual surface. A concentric ring of green pulsed light emitting diodes (LEDs) outside of the optical path of the microscope objective illuminates SDF. The light penetrates the mucosal tissue, scatters from interior tissue boundaries, and illuminates the tissue-embedded microcirculation from behind. Video frames are recorded using a digital recorder and visualized on a monitor (Figure 2.2(a)). The illumination has a central wavelength of 530 nm, corresponding to an isosbestic point in the absorption spectra of deoxy- and oxyhe-moglobin, to ensure uniform absorption by RBCs. RBCs appear dark against a light background. The CCD chip in the SDF probe can be axially translated with respect to the fixed lens system to adjust focus (Figure 2.2(b)). The LEDs are strobed for short duration in sync with the CCD duty cycle to avoid motion blur and prevent smearing of flowing RBCs. Because the magnifying objective lens system is 5 X, a single RBC spans approximately 4-5 pixels. Early implementations of

![Figure 2.2: SDF imaging device.](image)
bedside SDF devices present several practical challenges inherent to the clinical environment: The video microscope is hand-held by the operator; focus and illumination are adjusted manually; and the operator must hold the tip of the microscope steady to within approximately 0.1 mm to observe the same microvascular bed over 10-20 seconds. Pressure applied to the probe tip can cause plastic deformation of the microvascular bed and/or iatrogenic occlusion of vessel flow.

2.2 Microcirculatory Image Analysis

2.2.1 Assessment of Microvascular Scores

Qualitative and quantitative investigation of microcirculatory dysfunction may play an important role in detection and diagnosis of certain diseases. A variety of non-invasive image acquisition and semi-quantitative scoring systems have then been developed to monitor and evaluate the microcirculation at the bedside. Due to the high variability in image analysis, a round table conference was organized aiming to achieve a consensus on microcirculatory image acquisition and analysis [21]. According to this consensus conference, the standardized evaluation of the microcirculation should consider three different characteristics. They are VD, including TVD and PVD, capillary perfusion including PPV and MFI, and HetIndex.

Vessels with different diameters should be differentiated before the microcirculatory assessment. Capillaries are defined as vessels that carry a single RBC, measuring < 10µm. We classify small vessels as those with diameter < 20µm. Vessel density, perfusion, and heterogeneity index are then analyzed in capillaries and venules, respectively. Small vessels are important because they are site of oxygen transport to tissue and they clog up with sticky leukocytes and/or fibrin more easily than large vessels. VD is calculated as the number of vessels crossing the three equidistant horizontal and three equidistant vertical lines on the image divided by the total length of the lines [2]. Several research groups developed different semi-quantitative scoring systems for assessing OPS/SDF sublingual microvascular flow patterns [36], such as 0 = no flow, 1 = sludging, 2 = moderate flow, 3 = normal flow [1]; No flow, intermittent flow, continuous flow [3]; 0 = no flow, 1 = intermittent flow, 2 = sluggish flow,
3 = continuous flow [5]; low, normal, high flow [37]. PPV is calculated as the percentage of the length of vessels where flow is considered perfused divided by the total length of vessels. FCD is defined as the length of perfused capillaries per observation area. However, surface is proportional to the square of the vessel radius. If errors in determination of vessel diameters, especially the diameter in small vessels, are high, it will result in large errors in the calculation of the blood flow. PVD, an estimate of FCD, can then be calculated by multiplying VD by PPV. The semi-automated assessment of TVD, PVD, and PPV is time consuming and requires significant user interaction. Bezemer [38] developed a rapid automatic assessment of microvascular density in SDF images. First a mean image and a temporal variance image were obtained from the stabilized image sequences. Vessel centerlines were then detected on the mean image, and the false vessel centerlines were removed by thresholding the centerline contrast. The contrast at each centerline pixel was calculated as the standard deviation divided by the mean of intensity values in a $25 \times 25$ pixel area centered on the centerline pixel, multiplied by 100. Vessel segments with the centerline contrast scores above a predefined threshold were included in the TVD assessment. The temporal variance image allows the assessment of microvascular perfusion, because flowing RBCs result in pixel intensity fluctuations in time. This method is rapid and fully automatic for the assessment of microvascular density at the bedside. However, it is only applicable to high quality microcirculatory videos or low cell density, high imaging rate microcirculatory videos.

MFI is commonly used to semi-quantitatively characterize the velocity of microcirculatory perfusion. Calculation of MFI is based on previously mentioned flow pattern scorings. Pozo [39] summarized and compared three different methods for the calculation of MFI, including MFI by quadrants [5], MFI point of care [18], and MFI vessel by vessel [17]. MFI by quadrants was proposed by Boerma [5]. The microcirculatory video images were divided into four quadrant. The predominant flow rate for each cohort of vessels per quadrant is assessed. MFI was the average over the four quadrants. MFI by point of care was a direct real-time assessment at bedside [18]. Dubin [17] used the scoring system developed by Boerma and assigned a score to each individual vessel by eye. MFI of each video was calculated as the average of the individual scores. Based on the experimental results, Pozo [39] concluded that all three methods for the calculation of MFI were significantly correlated with RBC velocities and PPV for small vessels, and MFI vessel by vessel method had the best correlation. Instead
Chapter 2. Related Work and Literature Review

of OPS, SDF microcirculatory videos are used in this study. Flow rate scoring systems in OPS are still applicable in SDF. Our semi-quantitative SDF flow rate scoring system is 0 = no flow, 1 = intermittent flow, 2 = sluggish flow, 3 = continuous flow. A score is assigned to each vessel. MFI is then an overall score that is an average of all the vessels, or an average of the visual assessment of four quadrants. MFI indicates flow rate. MFI above a threshold is considered perfused.

The HetIndex is defined as the highest site flow index minus the lowest site flow index divided by the mean of flow indices across all sites [8]. PVD and PPV are estimated to be a measure of the average perfusion. They do not provide information about the flow heterogeneity. PPV is independent of the total vessel density. PVD is the density of perfused vessels by area. It distinguishes between perfusion and non-perfusion. It is a measure of required diffusion distance for oxygen to reach cells. The higher the PVD, the lower the required diffusion distance. MFI can differentiate among flow patterns, but does not provide information about FCD. The microcirculation is heterogeneous in many disease states. Septic patients may have more heterogenous flow than healthy subjects [21, 31]. Therefore, PPV, PVD, MFI, and HetIndex should be all be measured to comprehensively assess the microcirculation.

2.2.2 Video Stabilization

Frame-to-frame image registration to stabilize video sequences is a necessary and important pre-processing step. More accurate microcirculatory image analysis can then be achieved if motion artifacts of the video sequences are well compensated. Many approaches have been proposed to register video sequences [40]. These techniques may not be practical for registration of microcirculation video sequences, because thousands of moving objects with different speed exist in the video, and the video images are very noisy and illuminance uneven. The motion of microcirculation videos is mostly due to the movement of the hand-held camera and the subjects while imaging the microcirculation. Therefore, most existing techniques for microcirculation video stabilization only try to estimate 2D translation components by optimizing a similarity metric to preserve scale. Several approaches calculated the global translation between the reference frame and the current frame by maximizing the normalized cross correlation [41, 42, 43, 44, 45]. Distinct control points, such as edge points, corners,
vessel intersections, were selected first in the reference. Sub-blocks with control points as the centers in the reference frame were tracked in the current frame by searching the blocks with the maximal normalized cross correlation. The global translation was then the distance of corresponding blocks between the reference frame and the current frame. Goobic [46] proposed a registration system to stabilize intravital microscopy videos using template sub-regions selected from the stable background regions to match the corresponding locations in the reference frame for translation determination. Using the stable background regions reduces the registration errors caused by the moving objects. Zhang [47] estimated the transformation matrix between two consecutive frames using the matching feature points on both images detected by Scale-Invariant Feature Transform (SIFT). These features are detectable under changes in image scale, rotation, noise, and illumination. After matching points in both images being found, RANdom SAmple Consensus (RANSAC) [48] for homography was utilized to remove outlier matching pairs. The remaining inlier corresponding points were then used to obtain the translation component. Besides translation, the hand-held imaging device or the subjects could move during the imaging process, thus rotation motion should also be taken into consideration while compensating the motion artifacts. Wu [49] performed video sequence registration by finding maximal mutual information between the reference image and the current image. Two translation and rotation parameters were initially estimated. Based on the initial guess of these transformation parameters, these parameters were optimized by maximizing the mutual information between two images iteratively. The current image was then registered to the reference image based on the optimized transformation parameters. Song [50] proposed a probabilistic mesh model to register microscopic iris video sequences. Microscopic iris video sequences have high local non-linear deformations. Their proposed probabilistic mesh model was flexible to measure local similarity between images.

2.2.3 Vessel Geometry Extraction

In order to quantitatively measure vessel diameter, TVD, PPV, PVD, MFI and enhance RBC tracking accuracy, vessels need to be segmented and the centerlines of each vessel segment extracted to generate space-time images for RBC velocity estimation. Numerous approaches
for vessel extraction [51, 52], especially retinal vessel extraction, have been developed previously. Niemeijer [53] summarized several vessel segmentation algorithms developed in the past several decades and evaluated and compared them on a common database. Chaudhuri [54] proposed a matched filter approach to detect retinal vessels. Because the intensity profiles of the cross sections of vessels can be modeled by a Gaussian function, the filter was designed as a Gaussian model at different orientations and scales to convolve with the image. The vessels can be detected at the maximum response location. Perez [4] presented the scale-space analysis and region growing approach using the maximum gradient magnitude and the larger eigenvalues of the Hessian at different scales as features. However, finding the eigenvalues of the Hessian is subject to noise due to the second-order derivatives. Niemeijer [53] used supervised classification by extracting features from each pixel for training and a classifier trained to classify testing data. Mathematical morphology [55], thresholding approaches [56] are also popular for retinal vessel extraction. Other than these approaches, vessel tracking is another widely studied retinal vessel extraction method [57, 58, 59, 60]. Generally speaking, tracking based approaches start from one or more seed points, and move along the tracing direction by analyzing the local information around the current point. Some tracing algorithms also depend on the edge detection for accurate tracing [10]. If the image is noisy and has low resolution, no clear or robust edges will be detected, and the algorithm cannot achieve sufficient performance. Vessel tracking may also be complicated by vessel crossings and bifurcations [60]. Since the image intensity across the vessels can be approximated as a Gaussian, tracking the centerlines equals finding the ridges of the Gaussian model [59].

However, vessels, especially capillaries, in microcirculation videos are hard to identify, because microcirculatory images are noisy and have uneven illuminance. Passage of RBCs and plasma gaps makes the intensity of vessel pixels change over time. Due to the presence of plasma gaps or white blood cells, vessels appear interrupted and discontinuous. A few methods have been proposed to segment vessels in microcirculatory videos. Sato [41] presented the temporal variance of each pixel method to extract a vessel region. Due to the passage of RBCs and plasma along vessels, the intensity of each vessel pixel varies frame by frame. Therefore, the intensity variation is expected to be large in the vessel region and small in tissue regions. The variance image is defined as \( V(x, y) = \sum_{n=1}^{N} \frac{1}{N} (I_n(x, y) - \bar{I}(x, y))^2 \), where \( N \) is the number of frame, \( I_n(x, y) \) is the \( n \)-th frame, and \( \bar{I}(x, y) \) is the mean image. The vessel
region was then extracted by thresholding the variance image after the Gaussian smoothing. The vessel contours can then be detected. However, the variance image is noise sensitive in the video image, because it only represents a single sample of light intensity at a given pixel [61]. A high variance will appear at tissue regions if microcirculatory videos are not well stabilized, and video sequences are illuminance uneven. Vessels which are in poor or out of focus have similar intensity values to background tissues, then variance of vessels in poor or out of focus will be low. The variance of vessel pixels will also be low if trains of RBCs are not distinctly separated by plasma gaps. Furthermore, vessels with stopped flow cannot be identified in the variance image. Thus, building a variance image is not a robust way to extract vessel regions. Japee [61] proposed a difference image to determine the capillary wall and the diameter. Difference image is defined as the cumulative sum of the square of intensity differences between consecutive frames, 

\[ D(x, y) = \frac{1}{N-1} \sum_{n=2}^{N} (I_n(x, y) - I_{n-1}(x, y))^2. \]

The difference image emphasizes the capillaries with distinct RBCs well separated by plasma gaps. Thus, the perfused capillary regions have high values in the difference image while the surrounding stationary tissue regions appear darker in the difference image. Japee estimated a cut-off frequency to classify a pixel as belonging to the background tissue or to the capillary lumen. The cut-off frequency was determined as the 3 standard deviation of tissue intensities from the mean background tissue intensity obtained from the histogram of the difference image. A line-by-line scanning strategy was then used to detect the left and right walls of capillaries. Scanning started from the left to right and up to down. If the intensity values of all three adjacent pixels were above the cut-off frequency, the left wall was encountered. Continuing the scanning towards right, if at least one of the three pixels had an intensity value less than or equal to the cut-off frequency, the right wall was then encountered. Once the capillary wall was identified, the centerline positions, width, diameter, and orientation angle of the capillary can then be determined. However, the difference image is also sensitive to noise in real microcirculatory videos. Eden [42] claimed that temporal variance for segmentation yielded unsatisfactory results in their microcirculatory videos. They then trained artificial neural networks to classify each pixel into vessel or non vessel regions based on color, texture, and temporal variance features. Dobbe [43] applied the scale-space analysis [62] to segment vessels in the average image and the eigenvector and eigenvalue analysis of the local Hessian matrix to extract the centerline points [59]. The vessel wall was detected as the points in the perpendicular directions of the centerline with the maximum steepness. An
anisotropic diffusion kernel was used to close possible interruptions caused by plasma gaps and white blood cells. Edge points with the distance to the centerline larger than two standard deviations from the mean distance were removed. This method is applicable to extract vessels in retinal images and other high quality images but not in low contrast, noisy microcirculatory images. Mirshahi [45] proposed a method to segment capillaries and small blood vessels in the average microcirculatory image. Gaussian pyramid was applied to create a series of images at different resolution levels. These rescaled images highlighted vessels at different sizes and at different levels of proximity to the surface of tongue. Matched filter [54] was used to enhance blood vessels and then an entropic-based adaptive local thresholding method was applied to segment vessels in different levels of images. The final segmentation result was combined from the segmentations at each image level. Shahidi [44] applied adaptive local thresholding to segment vessels in the conjunctival microvascular circulation images. The segmentation results were refined by the morphological operations and then skeletonized to obtain vessel centerlines. Linear regression analysis was then used to determine the slope, the offset, and the line perpendicular to the centerline of each vessel segment. The vessel diameter was determined from the full width at half maximum (FWHM) of the cross-sectional intensity profile.

2.2.4 Velocity Estimation of Red Blood Cells

Several categories of techniques have been proposed for the measurement of the microcirculatory flow including instrument based approaches and video processing based approaches. Laser Doppler Flowmeters are popular instruments to quantify blood flow in human tissues, and the technology is called Laser Doppler Flowmetry (LDF) [63, 64]. Laser light from a low power laser penetrates the skin. It is then scattered with a Doppler shift by moving RBCs, and transmitted back to a photo detector. Optical heterodyning occurs at the photo detector and produces an heterodyned electrical signal. This signal is proportional to the Doppler shift frequency, by which the velocity value can be measured. This technique measures the mean velocity of moving objects in all the vessels but not the true flow which is the product of the velocity and the vessel cross-sectional area. If the cross-sectional area of flow is constant, the
velocity is proportional to the flow. Therefore, the heterogeneity of the microcirculation is ignored [63, 64].

RBCs flow through vessels with plasma gaps between them. Assuming that these gaps remain constant over short distances. The velocity of RBCs or plasma gaps can then be estimated from the measured transit time of plasma gaps over a known distance. Wayland [65] developed a two-slit photometric method and cross correlation method to measure the transit time of plasma gaps. Microcirculation was imaged using the microscope and projected onto the screen. The screen was penetrated by two parallel slits connected to photomultiplier tubes. The slit separation distance was known. The photomultiplier signals caused by passage of RBCs or plasma gaps across the two slits were recorded. They were called upstream and downstream photometric signals. The same RBCs or plasma gaps passing through these two slits would produce similar signal patterns. Cross correlation was applied to find the signal pattern from downstream signals to match the similar signal pattern from upstream signals. The delayed time can then be estimated as the time difference between the similar pattern in the downstream and upstream signals. This technique was called temporal cross correlation and later was commonly used to measure velocity of RBCs [23, 66, 67, 68].

A variety of video processing approaches have been presented to quantitatively estimate the RBC velocity, such as tracking, optical flow. For tracking based velocity estimation methods, centroids of moving objects are detected and then tracked at each video sequence. Average velocity is then estimated based on the displacements of the tracked centroids at two consecutive frames. This method has been applied to track molecular particles [69, 70], fluorescently labelled red blood cells [71, 72], leukocytes [42], RBCs in liver [73]. Spatial correlation method is one of the video based frame-frame tracking techniques. RBC displacement is recorded at different video sequences. The spatial displacement of the same RBC can be estimated using the cross correlation method by searching the region in the consecutive video sequence with the maximum normalized cross correlation. The instance velocity is then measured as the ratio of the spatial displacement to the transit time between consecutive frames [68, 74, 75, 76]. Cross correlation method has also been applied to measure fluid velocity in images obtained by particle image velocimetry (PIV) technique [77, 78, 79]. It is a method developed to visualize flow and obtain two dimensional velocity vector fields. The fluid is seeded with small particles. Small particles become visible in the illuminating fluid. The
motion of the seeding particles can then be studied. For cross correlation based method, if the video frames are noisy, and the frame-to-frame intensities vary substantially, cross correlation method is not robust to estimate the RBC displacement. Furthermore, the shape and size of the region window are hard to determine. In general, for tracking based velocity estimation, spatial and temporal resolutions need to be reasonably high in order to detect and track the moving objects.

Optical flow method can be applied to estimate both $x$ and $y$ components of the RBC velocity with the assumption that the image brightness of pixels remains constant within a small region of these pixels in the consecutive frame. Local information on the brightness gradient and the rate of change of brightness with time provides only one constraint on the components of the optical flow vector. The flow velocity is on the line perpendicular to the direction of the brightness gradient. However, the component of the optical flow in the direction orthogonal to the spatial image graident is not constrained by the image brightness constancy equation. Therefore, there will exist infinite components of the optical flow. This is known as the aperture problem. The two velocity components can be solved by looking at the spatial and temporal variations of the image brightness over a neighborhood of each image point [80]. This method was applied to PIV to measure velocity [81, 82]. This method has also been applied to estimate the RBC velocity in finger nail-fold capillaries [49, 83], in vivo [84, 85], and in the simulated vessels [86]. However, the brightness constancy assumption for calculating optical flow is difficult to satisfy if RBCs move fast and do not appear sufficiently large. This method for velocity estimation of RBC is also limited by low resolution, low frame rate, and noise of the recorded microcirculatory videos.

Another tracking and velocity estimation method is the space-time image analysis. It was first introduced by Ellis [87] to study the RBC oxygenation. Their method placed a fixed sample window aligned along axial direction of the vessels. Intensities of the fixed sample window were extracted at each sequential video sequence to form a space-time image. Thus, one axis of the space-time image is the time instances, and the other axis of the space-time image is the vessel spatial positions where RBCs flow through. Instead of the fixed sample window, passage of RBCs in the centerline of the vessels can also be used to generate the space-time image [43]. Generating space-time images becomes a popular technique to estimate the RBC velocity in vessel axial direction with the assumption that RBCs flow along the vessel axial
Chapter 2. Related Work and Literature Review

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direction parallel to the vessel walls. Flowing RBCs appear as dark traces in the spatiotemporal images establishing a connection between RBCs in one frame to the next. Individual RBC or trains of RBCs can be automatically tracked and their velocities can be estimated as the orientation of these traces. A variety of methods have been presented to calculate the orientation. Hough transform [88] is a popular method to estimate the slope of the straight lines and has been applied to detect traces in the space-time images. Kempczynski [89] applied sobel filter to get RBC trace fragments in the space-time image, applied Hough transform on the segments, and then found the angle with the maximum histogram of the accumulator to estimate the RBC aggregate velocity. However, Hough transform for line detection is noise sensitive, and accuracy of edge detection also affects the efficiency of the Hough Transform. Line detection by Hough transform directly on the gray-scale images was implemented [90]. Dobbe [43] applied gray-scale Hough transform to the space-time diagrams, thresholded the voting counts, and smoothed the Hough count curve with a Gaussian filter. The optimal global orientation corresponded to the highest peak in the filtered curve. Radon transform is an alternative to the Hough transform for line detection [91]. The Radon transformed image consists of the integral of a function of the object over straight lines. This method is more robust to noise compared to the Hough transform because the noise is attenuated through integration. If there are many lines in the image corresponding to the similar orientation, bright points will appear in the Radon transformed image at the similar angle across the radius range. The variance of the transformed image along the radius will be maximal at that orientation [92]. Drew [92] also compared the velocity estimation from the space-time image by Radon transform to Singular Value Decomposition (SVD) [93]. Experimental results showed that Radon transform based algorithm was faster and more robust to noise compared to SVD based algorithm. Duncan [94] calculated the signal-to-noise ratio (SNR) in the projection at each angle at the Radon transformed image. SNR was defined as the ratio of the standard deviation along the radius at a specific angle to the standard deviation in the entire space-time image. The peak of the SNR corresponded to the orientation of the traces. Sato [41, 95] proposed an automatic velocity estimation of moving leukocytes. Their method first generated a spatiotemporal image, and then filtered the spatiotemporal image with a Gabor filter, and then thresholded and skeletonized the enhanced leukocyte traces in the spatiotemporal image. The velocity of each leukocyte was computed from a tangent direction at each point of traces which was estimated by fitting a straight line to trace points [41] or from the direction of the principal axis of the
grouped traces [95]. Gabor filters were used in estimating one or two components of RBC velocities from ultrasound images [96]. Combination of Gabor filters and Radon transform were applied to speed up the processing time for detecting traces [97]. Shahidi [44] presented an orientation estimation method by thresholding the spatiotemporal image, and determining the slope of the longest continuous bands using linear regression analysis. Japee [98] binarized the spatiotemporal image through region growing and identified the connected tracks corresponding to the same RBC or train of RBCs. The location of the centroids of the RBCs was identified with those connected bands. Individual RBC velocities were estimated as the Euclidean distance between the RBC centroids divided by the time elapsed between two consecutive frames. In general, we assume that RBCs flow along the central axis of the vessels, the space-time image is then generated from the centerline of the vessel segments. However, there are situations that RBCs do not exactly move along the central axis. Thus, interrupted traces will appear in the space-time images. Chen [99] proposed a method to extract the traces based on the space-time images generated from the centerline, inner, and outer contour of the microvessels. The velocity of each cell was computed from a tangent direction at each point of the extracted traces. Most methods described above estimate global orientation of the traces on a spatiotemporal image. However, flow of RBCs is not constant in reality. It varies at different time instances. Therefore, it is necessary to estimate local orientation in order to track spatial and temporal variations. Sato [41] proposed a method to extract each leukocyte trace from the spatiotemporal image. The velocity of each leukocyte was computed from a tangent direction at each point of traces which was estimated by fitting a straight line to trace points within a local window along the time axis. Drew [92] also estimated the average velocity in a temporal window of the spatiotemporal image. Sourice [100] proposed the curve fitting method and the moment of inertia method to estimate local orientation on the region of interest of the autocorrelation selected using a watershed algorithm. RBC velocities estimated by these methods are all based on thresholded traces. Satisfactory results of these methods require clear traces in original or enhanced space-time images. In most cases, due to the aforementioned imaging artifacts, RBC or plasma gap traces are not clear in most space-time images generated from microcirculatory videos. Vessel crossing also causes horizontal artifacts in space-time images. The traditional orientation estimation methods do not work well in noisy space-time images with horizontal artifacts.
2.2.5 Computer Software for Assessment of Microcirculation

A few software packages have been developed to analyze microcirculatory videos. CapImage [101] was the first commercially available software for the analysis of microcirculatory images. It was originally developed for intravital microscopy but can also be used for analysis of OPS and SDF images. Capillary diameter, length, torquation index, and capillary density can be measured with this software. However, vessel geometry extraction was performed manually which is time-consuming.

CapiScope was a newer version of CapImage. It had been developed mainly for the semi-automatic analysis of OPS images. It provided measurements of FCD, vessel diameter, and RBC velocity in straight vessels. However, the velocity estimation was only restricted to straight vessel segments. It could not identify vessels and measure vessel length automatically. CapiScope tended to over estimate vessel diameter slightly over the entire range, and was not able to measure velocities below 50 pixels/s. Both CapImage and CapiScope did not provide video stabilization, therefore, only stabilized videos without any motion artifacts can be analyzed with either softwares.

Another software package for microcirculatory analysis, called AVA (Automated Vascular Analysis), was developed by Dobbe et al [43]. This was the first software capable of analyzing microcirculatory videos with motion artifacts and estimating RBC velocity in both straight and curved vessel segments. This software stabilized microcirculatory video sequences first and then averaged the stabilized video frames to fill up interruptions in capillaries caused by plasma gaps or leukocytes. Vessel centerlines were subsequently detected and linked in the averaged, smooth image. Vessel length could then be estimated from the traced vessel centerlines. Vessel walls were detected as the points in the perpendicular directions of the centerline with the maximum steepness. Vessels that were out of focus were removed to avoid over estimation of diameters in these vessels. The user was able to exclude vessels with a focus score below a manually adjusted limit. The software automatically cutted vessels at bifurcations into separate vessel segments. The user was also able to manipulate these intermediate results by deleting, cutting, or connecting vessel segments. Undetected vessel segments can be manually drawn in where the software suggested a present vessel segment, given a user-selectable
scale. If computer-assisted vessel detection failed, one can add remaining vessels by manual tracing with a user-selected diameter. Velocity of RBCs can be determined based on the space-time image generated from the centerline points of each vessel segments across video sequences. The utility and accuracy of this software was evaluated compared its performance to CapiScope. AVA obtained comparable accuracy with regard to vessel diameter, length determination, FCD calculation, and velocity estimation. Compared to CapiScope, AVA was able to measure vessel length automatically. Manual velocity analysis with CapiScope took 3 hours compared to 20 mins by AVA. AVA significantly reduced analysis time and user interaction, and increased accuracy. In sum, AVA can be used to determine vessel length, diameter, calculate perfused capillary density, and measure velocity of RBCs in individual capillary segments semi-automatically with high accuracy. Even though AVA is capable of analyzing the videos semi-automatically with high accuracy, it fails in measuring higher velocities > 1250 pixels/s, and it is still time-consuming and requires user interaction, such as identifying the vessels of interest, filtering of many false positives, adding of many false negatives, to produce acceptable results. Therefore, it is not appropriate for real-time clinical applications of microcirculatory analysis.

2.3 Data Collection

Videos processed by this research are obtained with a SDF device imaging the microcirculation at the sublingual surface. Our microcirculatory videos are captured by a SDF video microscope (Microscan, B.V., Amsterdam, The Netherlands). It is a 720 x 480 DV digital video with a 1:1.1 pixel aspect ratio and captures at the bedside with a laptop and custom software. Because the magnifying objective lens system is 5 X, the resolution at the image sensor is approximately 1.5 $\mu m$/pixels horizontally and 1.4 $\mu m$/pixels vertically. The hardware features a point spread function similar to a Gaussian distribution with a standard deviation of approximately 1 pixel in the x and y direction. Capillaries have a diameter of about 7.5 $\mu m$. That is approximately 3-4 pixel wide in standard SDF images. A single RBC then spans approximately 3-4 pixels in the images. The focal depth is set approximately 0.5 mm, and the imaging rate is 30 fps. The exposure time is set approximately to 1 ms per frame, corresponding to a maximum measurable blood flow rate of 2 mm/s.
2.4 Proposed Methodology

Figure 2.3 shows the flowchart of the proposed framework for microcirculatory image analysis consisting of 9 steps:

1. Video sequences are first pre-processed to remove noise and enhance the vessel contrast.
2. Videos are then stabilized to compensate the movement of the camera and/or subjects.
3. After stabilization, the minimum projection image is obtained.
4. Vessel segmentation is performed on the minimum image.
5. The segmented binary image is skeletonized to extract vessel centerline. Vessel skeletons are divided into the vessel segments based on bifurcation points.
6. Space-time images are generated using intensity values of centerline points in each vessel segment across frames.
7. RBC velocity is determined from the orientation of space-time images.
8. Semi-quantitative microcirculatory measurements, PVD, PPV, MFI, and HetIndex, are calculated. Quantitative microcirculatory measurements are investigated.
9. Semi-quantitative and quantitative microcirculatory measurements are used to assess microcirculation in patients diagnosed with and without infections.
FIGURE 2.3: Flowchart for microcirculatory image analysis.
Chapter 3

Pre-Processing and Video Stabilization

3.1 Pre-Processing

Due to limitations of the imaging device, the sublingual microcirculatory images have low resolutions. Vessels have low contrast compared to the tissue background, thus, capillaries are very hard to identify. Furthermore, images are noisy and have uneven illuminance across the images. Pre-processing steps to reduce the noise and enhance the vessel contrast in the images are necessary for better microcirculatory analysis in the following steps. The proposed pre-processing step is shown in Figure 3.1: (1) Contrast-limited adaptive histogram equalization (CLAHE) [102] is first applied to enhance vessel contrast; (2) uneven illuminance is then corrected by background division; (3) intensities are adjusted to further enhance the vessels; (4) an anisotropic diffusion filter is finally used to smooth the image sequences.

Figure 3.1: Flowchart for pre-processing step.

CLAHE is first applied to enhance vessel contrast to the background and bring out more details in the image. Different from the ordinary histogram equalization, CLAHE redistributes
the intensity histogram of the local region by effectively spreading out the most frequent intensity values. Local histogram adjustment instead of global histogram adjustments prevents the over-amplification of noise in relatively homogeneous regions of an image. Histogram of each pixel within a square region centered on that pixel is obtained first. Cumulative distribution function (CDF) of each pixel value in the image is then calculated. Each pixel is finally transformed according to a distribution function proportional to the CDF of pixel values in the neighborhood. This procedure may also cause the over-amplification of noise. This can be prevented by clipping the histogram at a predefined value before computing the CDF. Some bins larger than the predefined clip limit can be redistributed equally among all histogram bins. The neighboring regions are combined using bilinear interpolation to eliminate artificially induced boundaries. CLAHE locally transforms highly peaky histogram in the homogeneous region into a more evenly distributed histogram with contrast limit thereby improving the contrast in the image.

Intensities in the background of each video frame are not evenly distributed. Some regions appear dark, and some regions appear bright. Uneven illuminance can be corrected by dividing the image with the background image. The background image can be estimated by performing morphological closing on the image with a big radius disk-shaped structuring element. Here the radius is set up to 40.

The intensity values in these gray-scale video frames are adjusted such that 1% of data is saturated at low and high intensities of these frames. After the intensity adjustment, vessels are further enhanced. An edge preserving filter, anisotropic diffusion filter, is then applied to remove the noise in the image without smoothing vessel edges. Commonly used Gaussian kernels $G(x, y; t)$ convolving the original image $I_0(x, y)$ for smoothing remove some important details, such as boundaries of objects of interest. $I(x, y, t) = I_0(x, y) * G(x, y; t)$, where $t$ is the variance of the Gaussian kernel. Perona and Malik [103] proposed an edge detection method using anisotropic diffusion. According to the Fick’s laws of diffusion, flux moves from the high density area to the low density area, with a magnitude proportional to the local density gradient. $J = -D \nabla \phi$, where $J$ is the flux, $D$ is the diffusion coefficient, and $\phi$ is the local density. This is the Fick’s first law. Fick’s second law predicts how diffusion causes the density to change with time, $\frac{\partial \phi}{\partial t} = D \nabla^2 \phi$. If the diffusion coefficient is not a constant, but
depends on the coordinate or the density, \( \frac{\partial \phi}{\partial t} = \nabla \cdot (D \nabla \phi) \). The solution of the isotropic diffusion equation is equivalent to filtering the image with a Gaussian filter, \( \frac{\partial I(x,y,t)}{\partial t} = \text{div}(D\nabla I) \), \( I(x,y,0) = I_0(x,y) \) is the initial condition, \( (x,y) \) is the spatial coordinates of the image, \( t \) is a time parameter, and \( \nabla I \) is the image gradient. In order to keep the region boundaries sharp and smooth intraregion preferentially over interregion, Perona and Malik replaced the diffusion coefficient with an edge-stopping function \( g(\|\nabla I\|) \), \( \frac{\partial I(x,y,t)}{\partial t} = \text{div}(g(\|\nabla I\|) \nabla I) \), where \( \|\nabla I\| \) is the image gradient magnitude. This function is chosen to satisfy \( g(x) \to 0 \) when \( x \to \infty \) so that the diffusion is stopped at the boundary. The solution of the anisotropic diffusion function can be solved via a partial differential equation through iterations. The number of iterations controls the degree of image smoothness.

Figure 3.2 shows the pre-processing results.
Chapter 3. *Pre-Processing and Video Stabilization*

3.2 Video Stabilization

Sublingual microcirculatory videos are taken with the hand-held camera. It is not avoidable that the camera jitters or the subjects move during the imaging process. Video sequences have to be registered first in order for the more precise microvascular quantitative analysis. Image registration is a process to convert two images into the same coordinate system for comparison and analysis [40]. This conversion is obtained through a transformation matrix. The transformation matrix can be estimated by optimizing a similarity metric between the intensities or the corresponding points in the reference image and the moving image. The registration can be rigid where two images are rotated and shifted with respect to each other. It
can also be non-rigid where two images are related through non-rigid transformation. Group-wise image registration transforms a group of images to the same coordinate system. One reference image needs to be picked first, and then the rest images are aligned with respect to the reference image. For time lapse videos, two consecutive frames have a large overlap. Thus, video sequences can be registered sequentially by registering next frame onto the previous one. Sequential image registration improves the processing speed compared to the fixed reference image approach. However, registration errors in the previous frames can be accumulated over time. In order to accurately estimate global motion, static background or fixed edges or points, such as vessel edges, bifurcation points, should be used. Figure 3.3 shows the steps for video stabilization: (1) Kanade-Lucas-Tomasi (KLT) Feature Tracker [104] is utilized to track feature points across frames; (2) the tracked feature points are then classified into the background and moving foreground trajectories by RANSAC; (3) the corresponding background trajectories in the reference frame and the rest frame are then used to estimate the transformation matrices; (4) the rest frames are registered to the reference frame according to the estimated transformation matrices. The video sequences are then stabilized.

KLT tracker detects salient points in the initial frame that are easy to be tracked in subsequent frames, such as corners. The most commonly used corner detector is Harris corner detector [105]. Harris defined a Harris matrix which is the autocorrelation matrix of the second derivatives of the image intensity over a small window centered at point \((x, y)\),

\[
M = \sum_{x,y} w(x,y) \begin{bmatrix} I_x^2 & I_x I_y \\ I_x I_y & I_y^2 \end{bmatrix}.
\]

If both eigenvalues of this Harris matrix are large, point \((x, y)\) is then a corner. Corners are then determined by thresholding a corner response \(R = \det M - k(\text{trace} M)^2\), where \(k\) is an empirically determined constant. Eigenvalues of this autocorrelation matrix are invariant to rotation which is important for the object tracking under rotation. Shi [104] found out that good corners can be located by only examining...
the minimum eigenvalue. KLT tracker also refines the corner locations to subpixel accuracy. Detected features are then tracked using a Newton-Raphson method of minimizing the difference between the two windows centered at the feature points.

The tracked trajectories include background and moving foreground trajectories. Background trajectories need to be chosen in order for transformation matrix estimation. Sheikh [106] proposed a background subtraction method to identify rigid and non-rigid foreground moving objects from freely moving camera videos. In this method, RANSAC was applied to classify feature point trajectories into inliers corresponding to the background trajectories and outliers corresponding to the foreground trajectories. Let \( P \) feature points tracked over \( F \) frames, and let the trajectory of the \( i \)-th point be \( \mathbf{w}_i = [\mathbf{x}_{i1}^T \cdots \mathbf{x}_{iF}^T] \in \mathbb{R}^{1 \times 2F} \), where \( \mathbf{x}_{fi} \) is the mean subtracted coordinates in each frame \( f \), 

\[
\mathbf{x}_{fi} = [\bar{u}_{fi}, \bar{v}_{fi}] = [u_{fp} - \frac{1}{P} \sum_{p=1}^{P} u_{fp}, \bar{v}_{fp} = v_{fp} - \frac{1}{P} \sum_{p=1}^{P} v_{fp}]^T.
\]

Stacking together all \( P \) feature points at all \( F \) frames gives the registered measurement matrix [107]:

\[
\mathbf{W} = [\mathbf{w}_1^T \cdots \mathbf{w}_P^T] = \\
\begin{bmatrix}
\bar{u}_{11} & \cdots & \bar{u}_{1P} \\
\vdots & \ddots & \vdots \\
\bar{u}_{F1} & \cdots & \bar{u}_{FP} \\
\bar{v}_{11} & \cdots & \bar{v}_{1P} \\
\vdots & \ddots & \vdots \\
\bar{v}_{F1} & \cdots & \bar{v}_{FP}
\end{bmatrix}_{2F \times P}
\]

Let \( \mathbf{s}_p = [x_p, y_p, z_p]^T, p = 1, \ldots, P \) be the corresponding \( P \) points in the world space. Since the origin of the world reference system is arbitrary, we can assume that the origin is at the centroid of these \( P \) points in space, that is \( \frac{1}{P} \sum_{q=1}^{P} \mathbf{s}_q = 0 \). Let a pair of unit vectors \( \mathbf{i}_f, \mathbf{j}_f \) pointing along the rows and columns of the image be the orientation of the camera reference system corresponding to the frame \( f \) with respect to the world reference system. Here, we have an assumption that the camera model is orthographic. Thus, all projection rays are parallel to the optical axis, that is also in the direction of the cross product of \( \mathbf{i}_f \) and \( \mathbf{j}_f \), 

\( \mathbf{k}_f = \mathbf{i}_f \times \mathbf{j}_f \). Let \( \mathbf{R}_f, \mathbf{t}_f \) be the rotation matrix and the translation vector of the camera coordinate system with respect to the world coordinate system. The orthographic projection
is obtained as follows:

\[
\begin{pmatrix}
u_{fp} \\
v_{fp}
\end{pmatrix}
= \begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} \left( R_{fs} s_p + t_f \right) = \begin{bmatrix} i_f^T \\ j_f^T \end{bmatrix} s_p + v_f
\]

where \( v_f = [v_{1f}, v_{2f}]^T \). Then

\[
\tilde{u}_{fp} = u_{fp} - \frac{1}{P} \sum_{p=1}^{P} u_{fp} = i_f^T s_p + v_{1f} - \left( \frac{1}{P} \sum_{q=1}^{P} i_f^T s_q + v_{1f} \right) = i_f^T (s_p - \frac{1}{P} \sum_{q=1}^{P} s_q) = i_f^T s_p
\]

Similarly, we can have \( \tilde{v}_{fp} = j_f^T s_p \). Therefore, the registered measurement matrix can be rewritten as

\[
W_{2F \times P} = [w_1^T \cdots w_P^T] = \begin{bmatrix}
\tilde{u}_{11} & \cdots & \tilde{u}_{1P} \\
\vdots & \ddots & \vdots \\
\tilde{u}_{F1} & \cdots & \tilde{u}_{FP} \\
\tilde{v}_{11} & \cdots & \tilde{v}_{1P} \\
\vdots & \ddots & \vdots \\
\tilde{v}_{F1} & \cdots & \tilde{v}_{FP}
\end{bmatrix}
= \begin{bmatrix} i_1^T \\ \vdots \\ i_F^T \\ j_1^T \\ \vdots \\ j_F^T \end{bmatrix}
\]

\[ [s_1 \cdots s_P]_{3 \times P} = MS \]

M is called the motion matrix representing the orientations of the horizontal and vertical camera reference axes throughout the stream, and S is called the structure matrix representing the world coordinates of the \( P \) feature points in the scene with respect to their centroid.

The maximum rank of \( W \) is 3. This is because M has three columns and the columns of W are linear combinations of these [108]. If we do not place the centroid of the objects in the scene, the translation factor will not be removed. In this case, the rank will be maximum 4. Since the motion of the freely moving camera is a full 3D motion, and the objects lie in a 3D structure, the rank of the motion matrix M and the shape matrix S are both 3. The registered measurement matrix W is the product of the motion and shape, then the rank of W is 3.

There is an underlying assumption that the background is in the dominant rigid motion. If all trajectories in W are on the rigid objects, the rank is three. This paper uses this rank constraint to find the background trajectories. There exists three background trajectory basis that span a subspace where all the rest of the background trajectories lie, \( w_i = \sum_{i=1}^{3} a_i \hat{w}_i \),
where $\hat{w}_i$ is the $i$-th basis trajectory. If there are independently moving objects in the scene, the rank of $W$ will be greater than three. Therefore, all trajectories of the foreground moving objects will not adhere to the subspace spanned by the background trajectories.

RANSAC is used to iteratively estimate the three dimensional trajectory subspace from a set of mixed background and foreground trajectories. Since we have the assumption that the background is the spatially dominant entity in the image and a majority of the image motion is the camera motion, most columns in $W$ will lie in the background subspace. The 3D background subspace can be estimated by RANSAC thereby highlighting the outliers as the foreground trajectories of the moving objects. Therefore, sparse labeling of the foreground and background pixels can be produced. Given a set of mixed background and foreground trajectories, a set of three trajectories $w_i, w_j, w_k$ is randomly selected. These three trajectories are used to span the three dimensional subspace. The model is then built, $W_3 = [w_i^T \ w_j^T \ w_k^T]$. The fitting function that is used to measure how well the model fits the data. It is calculated by projecting the given data to the subspace and comparing the Euclidean distance error between the projected data and their matching data in the subspace. The projection matrix is defined as $P = W_3(W_3^T W_3)^{-1} W_3^T$. The projection error is $e = \|Pw_i - w_i\|_2$. If the model is only constructed from the background trajectories, the error should be less than any errors arising from the model built by the foreground trajectories or a mix of foreground and background trajectories. Therefore, if the error $e$ is below than a predefined threshold, then the given trajectory fits the model. If there are enough data in the consensus, the routine terminates. Otherwise, randomly select another subset of three trajectories and repeat the process.

After RANSAC, background trajectories are selected. These trajectories at each frame are used to estimate the transformation matrices with respect to the reference frame. According to the transformation matrices, frames are aligned into the same coordinate system as the reference frame. The videos are then stabilized. For sustained labeling in longer videos, a sliding window approach is taken. For example, each time the trajectories within 30 frame window are used to compute the trajectory basis. This ensures that erroneous parts of trajectories due to occlusion, exit, and illumination change do not render the entire trajectory.
Chapter 4

Vessel Segmentation

4.1 Vessel Segmentation in Retinal Images

Vessel extraction is an important step for identifying the regions where RBCs flow. Assessing TVD, PVD, PPV, and blood flow all depends on the vessel geometry. Microcirculatory images are noisy, and illumination is not uniform. Vessels have low contrast to the background. Vessels in retinal images have similar conditions. A principal curve based retinal vessel segmentation approach was proposed in our group [109]. The vessel radius can be estimated using an isotropic Gaussian kernel Frangi filter [110]. The principal curve projection and tracing approach is applied to extract the vessel networks recursively using the underlying kernel smoothing interpolation model for intensities. The tracing step is implemented in the output image of the Frangi filter instead of the original image because of the enhanced vessels, reduced noise, and no central light reflex affect in the Frangi filtered image. Furthermore, the kernel width of the kernel smoothing interpolation is obtained from the vessel radius estimated from the Frangi filter for each pixel [111].

The proposed approach consists of three steps: (1) The original image is preprocessed to reduce the noise, enhance the vessels, and eliminate the background; (2) The Frangi filter is used to enhance the tubular structures and estimate the vessel radius using isotropic Gaussian kernels; (3) A kernel interpolation of intensity is then obtained using the estimated vessel
radius from Frangi filter as the kernel bandwidth, and then the principal curve projection algorithm moves the pixels onto the ridges of the Gaussian kernel. The principal curve tracing algorithm is then applied to trace the vessel trees recursively.

4.1.1 Preprocessing

Our developed approach is implemented in the green channel of the original color image because retinal vessels in that channel present the maximum contrast between vessels and the background. The red and blue channels are noisy or in low resolution. Preprocessing of the green channel image to reduce the noise and enhance the vessels is still necessary for more accurate and reliable extraction of retinal vessels.

The green channel image is first smoothed by the anisotropic filter. Then adaptive histogram equalization is applied to enhance the contrast of the retinal images. The vessels are enhanced by subtracting the image with the approximate background. The background can be obtained using a large size median filter on the original image. Here we use the $25 \times 25$ window size median filter. In the background subtracted image, the dark vessels appear brighter than the background [112]. Figure 4.1 compares the original green channel image with the preprocessed image.

4.1.2 Frangi Filter

To enhance retinal vessels and to estimate the vessel radius, we employ the vessel enhancement filter, the Frangi filter [110]. The intensity of the input image is first interpolated using an isotropic Gaussian kernel at multiple scales. The Frangi filter then analyzes the eigenvalues of the Hessian matrix of the image intensity at each scale, $\sigma$, of the Gaussian smoother to obtain tubeness measure. Let $p$ be the pixel location in the image. If a scale is approximate to the radius in $p$, the maximum filter response in $p$ is obtained at that scale. For bright vessel tubes and dark background, the Frangi filter response is $w_r(\lambda(p)) = \exp(-\frac{R^2}{2\sigma^2})(1 - \exp(-\frac{S^2}{2\sigma^2}))$ if $\lambda_2(p) < 0$. $\lambda_1, \lambda_2$ are the eigenvalues corresponding to two orthonormal directions of the Hessian matrix of the image intensity, and their magnitudes are sorted in an ascending
Chapter 4. Vessel Segmentation

(a) The minimum image  (b) Preprocessed image

FIGURE 4.1: Preprocessing

order, $|\lambda_1| \leq |\lambda_2|$. $R_B = |\lambda_1|/|\lambda_2|$ distinguishes blob-like structures and accounts for the eccentricity of the second order ellipse; $S = \sqrt{\lambda_1^2 + \lambda_2^2}$ eliminates background noise; $b, c$ are normalizing constants. The filtered image has maximum response along the centerlines of the tubular structures, reduced near the boundary and close to zero outside the tubular-like regions.

The vessels are usually in a wide range of diameters. The Frangi filter returns the scales at each pixel where the maximum response is obtained. The vessel radius can be approximated by the scales estimated from the Frangi filter. Therefore, the Frangi filter is widely used in the vessel analysis. However, the ratio of the eigenvalues cannot distinguish the edges from the vessels, therefore, no clear boundaries are able to be obtained in the Frangi filtered image. In retinal images, some vessel branches are very close to each other, after filtered by the Frangi filter, two close vessels may be merged into one single vessel. In this paper, therefore, we apply the Frangi filter to the preprocessed image and restrict the Frangi filter response to within the boundaries of the vessels. The locally defined principal curve projection and tracing algorithm will be implemented in the Frangi filtered image because all the vessels are
enhanced, background noise is reduced, and no central light reflex of the vessels affect our further processing. Figure 4.2 shows the output of the Frangi filter.

![Figure 4.2: The output of the Frangi filter applied to the preprocessed image](image)

4.1.3 Principal Curve Analysis

4.1.3.1 Principal Curve Projection

Locally defined principal curves are discussed in previous papers by Ozertem and Erdogmus [113, 114]. Let \( \{p_i\}_{i=1}^N \) be the pixel locations of the filtered image, where \( p_i \in \mathbb{R}^n \). The kernel interpolation of the tubeness measure is given as,

\[
f(p) = \sum_{i=1}^N w(p_i)k_{\Sigma_i}(p - p_i)
\]

where \( w(p_i) \) is the tubeness measure for each voxel in the filtered image; \( \Sigma_i = \sigma_i^2 I \) is the covariance of the isotropic variable Gaussian kernel \( k_{\Sigma_i}(p) = C_{\Sigma_i}e^{-\frac{1}{2}p^T\Sigma_i^{-1}p} \). \( \sigma_i \) is achieved at the maximum Frangi filter response at pixel \( i \).

The gradient and the Hessian of the kernel interpolation are:

\[
g(p) = -\sum_{i=1}^N w(p_i)c_iu_i
\]
\[ H(p) = \sum_{i=1}^{N} w(p_i) c_i(u_i u_i^T - \Sigma_i^{-1}) \]

where \( c_i = k_{\Sigma_i}(p - p_i) \), \( u_i = \Sigma_i^{-1}(p - p_i) \). The local covariance inverse based on the gradient and Hessian is defined as:

\[ \Sigma^{-1}(p) = -f(p)^{-1}H(p) + f(p)^{-2}g(p)g^T(p) \]

\( (\lambda_1(p), v_1(p)), \ldots, (\lambda_n(p), v_n(p)) \) are the eigenvalue-eigenvector pairs of local covariance inverse, where the eigenvalues are sorted such that \( \lambda_1(p) < \lambda_2(p) < \ldots < \lambda_n(p) \) and \( \lambda_i \neq 0 \). Here \( n = 2 \). \( v_1(p) \) corresponding to the smallest eigenvalue forms a tangent subspace. It indicates the direction along the tubes with the minimum intensity variation. The normal subspace is spanned by the remaining eigenvectors, \( V_\perp = [v_2(p)] \). Mean-shift updates constrained in the normal subspace, \( m_\perp(p) \), iteratively force \( p \) to converge to the principal curve, where \( m_\perp(p) = V_\perp V_\perp^T m(p) \); \( m(p) = (\sum_{i=1}^{N} k_{\Sigma_i}(p - p_i) \Sigma_i^{-1})^{-1} \sum_{i=1}^{N} k_{\Sigma_i}(p - p_i) \Sigma_i^{-1} p_i \).

A point is on the principal curve iff the gradient \( g(p) \) is orthogonal to the normal subspace.

Figure 4.3 shows the principal curve projection of the vessels. Pixels are hill climbing to their respective ridges of the underlying kernel interpolation by principal curve projection. Ridges correspond to the center points of the vessels, however, no branch information of the vessel tree can be extracted. Therefore, in the following, centerlines of the vessel trees are to be locally traced.

![Figure 4.3: The principal curve projection of the vessels overlayed on the original color image](image)
4.1.3.2 Principal Curve Tracing

Following the locally defined principal curve definition, we employ a subspace constrained tracing method to trace each branch of the vessel trees in the Frangi filtered image [111]. Given a seed point inside the vessel and an initial tangent direction, a gradient ascent to the nearest local ridge returns a corrected seed point which is on the principal curve. Starting from the corrected seed point, vessel centerlines can be traced along the tangent space eigenvector of the local covariance inverse locally identified by the smallest eigenvector. Therefore, propagating through the tangent subspace with proper directions and step length will trace the locally defined principal curve at \( p \). However, each step taken in the direction of the vessel orientation will cause the trajectory to diverge from the principal curve. We therefore have to move the candidate point back to the principal curve iteratively in the constrained normal subspace \( V_\perp \). Once the point is close to the principal curve, the tracing will continue along the center of the vessels in the tangent subspace. If \( p \) has been previously traced, is out of the image boundary, or the kernel interpolated tubeness measure is lower than a threshold, then the tracing terminates. This iterative tracing algorithm combines the correction in the constrained normal subspace and propagation in the tangent subspace with proper directions to trace the locally defined principal curves. However, this method only traces one branch of the vessels so far. Since vessels are tree-like structures, and therefore bifurcations exist, we have to recursively trace all the branches of the vessel trees.

After each termination of the tracing, we define this proximity as the encapsulating curvilinear cylinder envelop having inner radius \( 1.5 \times R(p) \), and outer radius \( 3 \times R(p) \), where \( R(p) \) is the local radius estimate of the Frangi filter. Everything within the inner radius are deleted from the next tracing. The traced branch and other outliers can be removed. We locally search the bifurcations of the previous traced branch by analyzing the clustering of the principal curve points within the inner and outer radius. Medoid-shift method [22] is used to estimate the modes of these clusters, and each mode is assumed as the bifurcation candidates. For every candidate, the center is selected as the seed point, and we shoot two trajectories from the center in the opposite direction along the vessel using the tracing method defined above. If a trajectory converges on a previously traced branch, then the new branch is detected, and the mode is simply connected to the junction sample. Otherwise, we conclude that the tested trajectory diverges from the local principal curve. This will be recursively executed to trace
all the remaining branches. If the number of the members of one cluster group is less than a threshold, this cluster is ignored and deleted from the tracing, because for the disease analysis, the main branches are more important, and tiny branches are less likely to affect the disease measurement result.

For this tracing algorithm, we assume that all the branches are connected with each other or have only small gaps among them, and all the branches can be traced given an initial seed point at the beginning. However, for the retinal images, it is possible that there are separated branches away from the main branches, as shown in the red circle in Figure 4.4. In this case, our algorithm is not able to trace these branches. We then detect the centers of the these branches and use them as new seed points to trace these separated branches. Figure 4.4 shows the vessel tracing results. Each color represents one single trace. All the branches are iteratively traced.

![Figure 4.4: The principal curve tracing of the retinal vessels. The big red circle shows the branches far away from the main branches](image)

### 4.2 Vessel Segmentation in Microcirculatory Images

In general, vessel geometry extraction is performed in the mean image over several frames. The mean image is less noisy, and discontinuous vessels caused by plasma gaps are bridged into complete vessels in the mean image. High intensity values in the mean image represent tissue regions, while low intensity values represent arterioles and venules and capillaries with
high density. However, capillaries with low density have similar contrast to surrounding tissues. Vessels are visualized mainly due to the passage of RBCs. The imaging frame rate and speed of moving objects determine how frequent RBCs flow through vessels. Therefore, which vessels are highlighted in the mean images is somehow based on the number of frames and frames at which time instances to be averaged. Multiple mean images created by averaging frames at different time instances are needed in order to have as many vessels being highlighted as possible. The number of frames is hard to determine as well. Small vessels are blurred if more images are averaged, while vessels are still not continuous if less images are averaged. Moreover, mean images only highlight vessels that have high RBC density. Therefore, mean images are not very useful to obtain detailed information about capillary network structures [61]. In our method, we use minimum projected images to study vessel geometry. Since RBCs appear dark in microcirculatory videos and plasma gaps and tissue regions appear bright, all vessels can be visualized as long as there are RBCs flowing through them. Capillaries with stopped flow can be identified in minimum projected images as well. Thus, compared to mean images, minimum projected images are better tools to study network structure and to identify interconnections between capillaries and other vessels. Figure 4.5 shows a mean and a minimum projection image. Anisotropic diffusion filter is applied to reduce the noise in the minimum image.

![Image](image.jpg)

(a) The mean image  
(b) The minimum image

**Figure 4.5:** Comparison of the mean image across 30 frames and the minimum projection image from all frames

Vessels in our analyzed videos are manually labeled in the following steps:
1. Edit videos using VirtualDub to select clips that satisfy a minimum image quality criteria [115]. Criteria includes rejection of iatrogenic pressure artifacts which are caused by excess pressure applied at the probe tip. Clipped videos have 3-5 second duration.

2. Visually adjust gain and bias to optimize image contrast and brightness.


4. Create mean image over approximately 30-60 frames.

5. Manually trace and label vessel segment centerline and 80% density edges. Cut at bifurcations if necessary.

6. Assign flow classification to each labeled vessel segment:
   
   (a) Play stabilized video loop at 30 fps
   
   (b) Select labeled vessel segment
   
   (c) Assign classification

7. For each vessel segment, output label index and extracted data: includes centerline (x,y) locations, vessel orientation matrix, and vessel radius.

With prior microcirculatory vessel information, vessels are segmented in a supervised fashion.

### 4.2.1 Feature Extraction

1) Gray-level based features:

Chaudhuri designed a matched filter to detect and enhance blood vessels [54]. The matched filter should have the same shape as intensity profiles of vessels in order to have the maximum filtered response. Because vessels can be considered as piecewise linear segments, a number
of matched filters can be used to match intensity profiles of the cross section of a vessel along its length. Such matched filter can be expressed as

\[
K(x, y) = \begin{cases} 
\exp\left(-\frac{x^2}{2\sigma^2}\right) & \text{if } |y| \leq L/2 \\
0 & \text{otherwise}
\end{cases}
\]

where \(\sigma\) is the spread of the intensity profile, and \(L\) is the length of the segmented vessel determined empirically. Vessels appear in different directions, thus, the basic matched filter needs to be rotated to highlight vessels in all the directions. Let \(q = [x; y]\) be a point. Assuming the center position of \(K(x, y)\) is at the origin \([0; 0]\), \(q = [x; y]\) can be rotated through an angle \(\theta\) by the rotation matrix

\[
R = \begin{bmatrix} 
\cos \theta & -\sin \theta \\
\sin \theta & \cos \theta
\end{bmatrix}
\]

Point \(q = [x; y]\) becomes \(\bar{q} = [u; v] = Rq\) in the rotated coordinate system. \(K(x, y)\) is restricted within a neighborhood \(N\) such that \(N = \{(u; v) | |u| \leq 3\sigma, |v| \leq L/2\}\). \(K(x, y)\) then becomes

\[
K(x, y) = \exp\left(-\left(x \cos \theta - y \sin \theta\right)^2/2\sigma^2\right) \forall \bar{q} \in N
\]

In order for the mean to be zero, the mean is subtracted in the final matched filter,

\[
\overline{K}(x, y) = K(x, y) - \sum_{\bar{q} \in N} K(x, y)/A
\]

where \(A\) is the number of points in \(N\). 12 different matched filters at every 15\(^\circ\) are applied to detect vessels in different directions. Because vessels have different sizes, a range of kernel sizes, \(\sigma\), are applied. Here \(\sigma\) is given from 1 to 5 pixels. At each pixel for the final filtered image, only the maximum of their response in the direction and the kernel size is retained. Larger vessels can be enhanced with bigger kernel sizes. However, the matched filter not only enhances vessels but also step edges. Thus, some noises are introduced in the filtered image.

Analyzing eigenvalues of the Hessian matrix of the image intensity at each scale, \(\sigma\), can help suppress non vessel structures and further highlight vessels. Let \(\lambda_1, \lambda_2\) be the eigenvalues corresponding to two principal directions of the Hessian matrix of the image intensity, and their magnitudes are sorted in an ascending order, \(|\lambda_1| \leq |\lambda_2|\). One eigenvector direction is along the centerline of the vessel. Intensity value has minimum variance along the centerline.
Thus, the smaller eigenvalue $|\lambda_1|$ corresponds to the eigenvector along the vessel. Intensity value undergoes the largest change along the direction orthogonal to the centerline direction. The larger eigenvalue $|\lambda_2|$ corresponds to maximum principal curve of the Hessian matrix reflecting vesselness. $|\lambda_2|$ has larger values along centerlines of tubular structures, and low values near the boundary. Because vessel sizes have big ranges, different scales are applied to highlight different sizes of vessels. The final maximum eigenvalue image is obtained by obtaining maximum largest eigenvalues across different scales. However, because the fact that local maximum response tends to be higher for larger blood vessels, largest eigenvalue $|\lambda_2|$ is normalized with their corresponding scales, $max_s[|\lambda_2|(s)]$ [116].

Morphological bottom hat filter highlights dark vessels in bright background by performing morphological closing and then subtracting the original intensity image. The size of the structuring element should be higher than the largest vessel size in order to enhance all vessels. Figure 4.6 shows the maximum projected image obtained by the matched filter, the largest eigenvalue image, and the bottom hat filtered image.

![Figure 4.6: Filtered images](image)

2) Texture-based features: Statistical measures of texture are calculated including first, second moments, contrast, uniformity, and entropy. The $n$-th statistical moment of gray levels $g$ is defined as:

$$\mu_n(g) = \sum_{i=0}^{L-1} (g_i - m)^n p(g_i)$$

where $L$ is the number of bins for gray levels $g$, $p(g_i)$ is the histogram for each gray level bin, and $m$ is the average gray level, $m = \sum_{i=0}^{L-1} g_i p(g_i)$ [117].
Contrast is defined as
\[ C = 1 - \frac{1}{1 + \sigma^2(g)} \]
where \( \sigma^2(g) \) is the second moment (variance). For regions with constant intensity, variance is low and contrast is around 0. While for regions with fluctuating intensity, contrast approaches 1.

Uniformity is defined as
\[ U = \sum_{i=0}^{L-1} p^2(g_i) \]
If intensities in an image are the same, uniformity reaches maximum value.

Entropy is defined as
\[ H = - \sum_{i=0}^{L-1} p(g_i) \log_2 p(z_i) \]
Entropy measures the randomness and is maximum when intensities are uniformly distribute.

These statistical measures are calculated for every pixel centered image in the bottom hat filtered image, and the window size is \( 7 \times 7 \).

### 4.2.2 Feature selection

Final features are then composed of three gray level features and 5 statistical texture features. Each feature is normalized to zero mean and unit variance. Some of these features may be redundant and some may be not even relevant with classification variables. In order to make classification process more computational efficient and more accurate, Minimum Redundancy Maximum Relevance (mRMR) method [118] is used to select relevant features and remove redundant features. Correlation between two features and correlation between class and feature can both be measured using mutual information. Minimizing redundancy for discrete variables can be interpreted as
\[ \min_{D,D} = \frac{1}{S^2} \sum_{i,j \in S} I(i,j) \]
where $S$ is the set of features, and $I(i, j)$ is mutual information between feature $i$ and $j$.

Maximizing relevance for discrete variables can be interpreted as

$$max L, L = \frac{1}{S} \sum_{i \in S} I(c, i)$$

where $c$ is class, and $I(c, i)$ is mutual information between class and feature.

Combining minimizing redundancy and maximizing relevance, the form becomes $max(L - D)$. The matched filtered image, contrast, and uniformity are selected as most relevant features and are used to train the classifier.

### 4.2.3 Classification

Linear discriminant analysis (LDA) is a method that projects data $x$ from a higher dimension onto a lower dimension through a linear transform $y = W \cdot x$ where the data can be best separated in a least square sense [88].

Figure 4.7 explains the problem solved by LDA. Three three-dimensional distributions are projected onto two-dimensional subspaces. $W_1$ and $W_2$ are the projection matrix for the two subspaces, respectively. LDA method seeks the optimum such subspace where the distributions have the greatest separation after the projection, here as associated with $W_1$. $W$ can be found by maximizing the ratio of the between-class scatter to the within-class scatter

$$J(W) = \frac{|W^T S_B W|}{|W^T S_W W|}$$

The between-class scatter is defined as $S_B = \sum_{i=1}^{c} n_i (m_i - m)(m_i - m)^T$. Where $c$ is the number of classes, $n_i$ is the number of data points in class $i$, $m_i$ is the mean vector for class $i$, $m$ is the total mean vector $m = \frac{1}{n} \sum_{i=1}^{c} n_i m_i$. The within-class scatter is defined as $S_W = \sum_{i=1}^{c} S_i = \sum_{i=1}^{c} \sum_{x \in D_i} (x - m_i)(x - m_i)^T$, where $D_i$ is the subset for class $i$.

All pixels are then classified by LDA as either vessel pixel or non vessel pixel. Morphological processing is then applied to refine the segmentation results. Small and disconnected regions
with areas below 100 pixel square are removed. Unconnected vessel areas are bridged by a dilation followed by a close and an erosion. Figure 4.9 show the segmented image by LDA and the human labelled vessels. For this particular segmentation, the detection rate is 0.8093, the sensitivity is 0.8936, and the specificity is 0.6671. Sensitivity is calculated by dividing the number of true positive by the sum of the true positive and false negative. Specificity is calculated by dividing the number of true negative by the sum of the false positive and false negative. Detection rate is calculated by dividing the sum of true positive and true negative by the total number of pixels in the image.

### 4.2.4 Skeletonization

Foreground vessel pixels are then skeletonized and divided into vessel segments based on the bifurcation points. Each vessel segment is ordered by using one of the end points as the starting point, starting at the starting point and finding the closest point to the previous
one until the other end point is reached. Space-time images will then generated from each individual vessel branch. Figure 4.9 demonstrates the results for vessel skeletonization and division.

(a) The vessel skeleton  
(b) The vessel segments

FIGURE 4.9: Vessel skeletonization and division
Chapter 5

Velocity Estimation

5.1 Principal Curve based Velocity Estimation Method

A novel application of a principal curve tracing algorithm was presented to automatically track RBCs across video frames and estimate their velocity based on the displacements of RBCs between two consecutive frames. Figure 5.1 shows the flowchart of the proposed framework. Our methodology is composed of four steps. The first step is preprocessing. The

Figure 5.1: Flowchart of the proposed framework for RBC velocity estimation.

sublingual surface video sequences are noisy and have low contrast between the vessels and
the background. Furthermore, tissue appear transparent and RBCs dark in the SDF images. It is only possible to identify vessels that contain blood. The video sequences are first pre-processed to remove noise and enhance the contrast. The second step is video stabilization. Blood flow is estimated by looking at the displacements of RBC groups at consecutive frames. However, the videos are not stable due to camera or subject movement during video capture. Before tracking of RBCs, videos must be stabilized robustly. The third step is background subtraction. In order to ensure more accurate vessel tracing, a background image is subtracted from each frame in video sequences. Remaining objects in video sequences will be only moving RBCs. Finally, a tracking step is executed in the RBC video sequences and velocity indices of RBCs are estimated based on the tracking results.

5.1.1 Background Subtraction

Background subtraction from a sequence of video frames is helpful for identifying the foreground objects of interest. Tracing of RBCs in the RBC only videos is more robust without the effect of the background, especially the background with the low contrast to the vessels. Robust Principal Component Analysis (RPCA) developed by Candes, Wright, Ma et al [119] is applied for background subtraction.

RPCA can recover a low rank matrix $A \in \mathbb{R}^{m \times n}$ and a sparse matrix $E \in \mathbb{R}^{m \times n}$ from an observed matrix $D \in \mathbb{R}^{m \times n}$, $D = A + E$. Low rank matrix $A$ represents the low-dimensional data while spare matrix $E$ represents the outliers. Therefore, RPCA can estimate the low-dimensional subspace from the high-dimensional observed data corrupted by large errors. RPCA can be applied to model the background from video sequences [119]. Background is stationary over all the frames, thereby highly redundant. Background can then be treated as the low rank matrix. Moving foreground objects are seen as sparse errors because only small portions of the images are occupied by moving objects. RBCs can be represented with the sparse matrix $E$. RPCA for background subtraction is also robust to illuminance changing background.
5.1.2 Principal Curve Tracing for Velocity Estimation

The tracking step is implemented in the background subtracted videos. Principal curve tracing [111] is a subspace constrained tracing method following the definition of locally defined principal curves [113]. A point is on the principal curves iff the local gradient of the point is orthogonal to the normal subspace estimated from the first and second order derivatives of the underlying density function. Given a seed point and an initial approximate direction, locally defined principal curve is estimated from the data points in the vicinity of the seed point and will be traced along the initial and later corrected direction through the data space. This tracing algorithm has been applied to trace the centerline of tubular structures in 2D and 3D, such as retinal vessels [109], and airways [120]. Since RBCs move inside the vessels, movement of a cloud of RBCs will form a tubular structure over time. It is not feasible to identify individual RBCs in sublingual microcirculation videos. We then assume that a group of neighboring RBCs at each vessel has similar motion pattern. Therefore, neighboring RBCs can be tracked across frames.

Stacking frames of background subtracted microcirculation videos into a 3D volume data with the third axis representing time instances, and let \( \{p_i\}_{i=1}^{N} \) be the voxel locations of this 3D data, where \( p_i \in \mathbb{R}^n \), \( N \) is the number of voxels in the data. The probability density of these voxels can be estimated using the Kernel Density Estimate (KDE). If a Gaussian kernel is used, the Gaussian kernel based KDE is defined as

\[
 f(p) = \sum_{i=1}^{N} w(p_i)k_{\Sigma_i}(p - p_i)
\]

where \( w(p_i) \) is the weight, it can be an equal value for each voxel, \( w(p_i) = 1/N \), or it can be the intensity value of each voxel; \( \Sigma_i \) is the covariance of the Gaussian kernel \( k_{\Sigma_i}(p) = C_{\Sigma_i}e^{-\frac{1}{2}p^T\Sigma_i^{-1}p} \). The gradient and the Hessian of the kernel interpolation are defined as

\[
 g(p) = -\sum_{i=1}^{N} w(p_i)k_{\Sigma_i}(p - p_i)u_i
\]

\[
 H(p) = \sum_{i=1}^{N} w(p_i)k_{\Sigma_i}(p - p_i)(\Sigma_i^{-1}(p - p_i)(p - p_i)^T\Sigma_i^{-1} - \Sigma_i^{-1})
\]
The local covariance inverse based on the gradient and Hessian is defined as

\[ \Sigma^{-1}(p) = -f(p)^{-1}H(p) + f(p)^{-2}g(p)g^T(p) \]

Eigendecomposing this local covariance inverse will obtain three eigenvalue-eigenvector pairs 
\( ((\lambda_i(p), v_i(p)))_{i=1}^3 \). If we sort the eigenvalues by increasing order, \( v_1(p) \) will be the eigenvector corresponding to the smallest eigenvalue. This eigenvector forms a tangent subspace along which the intensity has the minimum variation. The normal subspace is spanned by the remaining two eigenvectors. Following the normal subspace, \( p \) can be converged to the mode through Mean-shift updates, 
\[ p \leftarrow p + Q_\perp(p)Q_\perp^T(p)m(p), \]
where \( Q_\perp(p) \) is a matrix containing the eigenvectors of the Hessian that will be orthogonal to the ridge we wish to climb to.

Given a seed point inside the cloud of RBCs and an initial tangent direction, a ascent to the nearest local maximum returns a corrected seed point which is on the principal curve. The same cloud of RBCs will appear in the following frames moving along the vessel axis until it moves out of the field of view. The moving path of this cloud can be tracked through the tangent subspace formed by the minimum eigenvector of the local covariance inverse along the principal curve with proper directions and the specified step length. However, each step taken in the moving direction of this cloud of RBCs will cause trajectory to diverge from the principal curve. One therefore has to move the candidate point back to the principal curve iteratively in the constrained normal subspace. Once the point is close to the principal curve, the tracking will continue along the moving direction of this cloud of RBCs in the tangent subspace. This algorithm will return the coordinates of the tracked RBCs at each frame. Tracking will terminate if RBCs are out of image boundary, or the trajectory has been previously tracked. This iterative tracing algorithm combines the correction in the constrained normal subspace and propagation in the tangent subspace with proper directions.

Assuming that a cloud of RBCs is tracked over \( F \) frames, \((x_t, y_t)\) is the coordinate of the tracked RBCs at the \( t \)-th frame, \( t = 1, ..., F \). The velocity of this cloud of RBCs is defined as

\[ v = \frac{\sum_{t=2}^{F} \sqrt{(x_t - x_{t-1})^2 + (y_t - y_{t-1})^2}}{F - 1} \]
5.1.3 Experimental Results

We implemented the proposed framework on one sublingual microcirculation video. A total of 32 frames were processed. Figure 5.2 shows one of the original frames, and Figure 5.3 shows this original frame after pre-processing. Note that the image is less noisy, and vessels have more contrast against the background.

![Figure 5.2: One of the original frames.](image1)

After the video has been stabilized, RPCA is applied to separate the background and foreground images. The background image contains the non-moving objects being the same at each time instance if the video is well stabilized. The foreground image (Figure 5.4) contains the moving RBCs. The foreground image may also contain noise, however, this noise does not form tubular structures over time. Such random noise will not be tracked by our algorithm.

![Figure 5.3: The original frame after pre-processing.](image2)
Figures above a predefined threshold are chosen as the starting points for tracking. Figure 5.5 shows one tracked group of RBCs at four consecutive frames of the foreground RBC video. Figure 5.6 shows three groups of tracked RBCs projected into 2D and overlayed on the maximum image of the foreground RBC images. The white cross is the seed points of the tracking. The velocity of the red tracking is 36.5 pixel/frame or 1643 \( \mu \text{m/s} \); the velocities of the green and blue trackings are 12.2 pixel/frame or 540 \( \mu \text{m/s} \). These blood flow velocities correspond to continuous flow, in agreement with semi-qualitative assessment by an expert scorer.
Our preliminary results show that a principal curve tracing algorithm is able to track clouds of RBCs in time and estimate the velocity. Tracking along the principal curves is achieved through an iterative correction in the constrained normal subspace to the principal curves and propagation in the tangent subspace of the tubular structures formed by moving RBCs across frames. Principal curves are estimated from the gradient and Hessian of the image intensity of the local voxels having similar characteristics with the seed points. This tracing algorithm will return the trajectories of each group of RBCs at each frame. Velocity indices of RBCs can then be estimated by looking at the displacements at two consecutive frames.

One potential limitation of this framework is that principal curve tracing algorithm utilizes local information to estimate the tangent subspace and normal subspace. If the density of RBCs is low in one vessel, there will be not enough local voxels to calculate the gradient and Hessian of the image intensity. The same voxel will then be tracked, thereby stopping the tracking. A more sophisticated velocity estimation approach is proposed in the following section.
5.2 Entropy based Velocity Estimation Method

5.2.1 Renyi Entropy

Renyi entropy is defined as

\[ H_\alpha(X) = \frac{1}{1 - \alpha} \log \int p^\alpha(x) dx \]

where \( X \) is a random variable with probability density function (pdf) \( p(\cdot) \), and \( \alpha \) is the order \[121\]. Renyi entropy is a generalization of other defined entropies. For example, as \( \alpha \to 1 \), Renyi entropy converges to the Shannon entropy,

\[ H_1(X) = - \int p(x) \log p(x) dx \]

Among bounded support pdf’s, the uniform density maximizes Renyi’s entropy.

5.2.2 Renyi Entropy for Line Detection

Renyi entropy can be used to detect lines in images. Figure 5.7 demonstrates determination of location and direction of the line segment in a simulated image. The bright line in the image is 60° or 120° away from the horizontal direction. The centroid of the line is at the center of the image. A candidate line rotates from −90° to 90° with 1° steps in the image (Figure 5.7(a)). Let \( v = [\cos \theta; \sin \theta] \) be the line at angle \( \theta \). Every pixel in the image is then projected onto this rotating candidate line. Let \( q_i = [x_i; y_i] \) be the \( i \)-th point in the image. The scalar projection of the point \( q_i \) onto the candidate line is \( \bar{q}_i = q_i^T v \). The histogram, \( p^\theta \), of projected points weighted by pixel intensity values at each bin width is calculated. The bin width is calculated as the maximum projected point minus the minimum projected point divided by the specified number of bins. At this example, the number of bins is \( K = 100 \). The value of the histogram at the \( k \)-th bin, \( p^\theta_k \), is the summation of intensity values of projected points within the \( k \)-th bin, \( p^\theta_k = \sum_n c_{kn} \), where \( c_{kn} \) is intensity values of \( n \) number of projected points within the \( k \)-th bin. When the rotating line is parallel to the linear segment in the image (Figure 5.7 (a)), bright pixels on the line are uniformly distributed in most bins (Figure 5.7
Figure 5.7: Illustration of the algorithm in finding the line segment in a simulated image: (a) The red line denoting the parallel projection direction; (b) The histogram at the parallel projection direction. (c) Orthogonal projection direction; (d) The histogram at the orthogonal projection direction; (e) The estimated Renyi entropy of the projection to the candidate line rotates from $-90^\circ$ to $90^\circ$; The minimum value is attained at $-30^\circ$, which corresponds to the optimal projection; (f) Selected pixels with high line-likelihood within 3-pixel wide band centered at the detected line; (g) Histogram of selected pixels projected onto the detected line orientation with a threshold 0.0001 (green) superimposed; (h) The estimated line segment.
The calculated Renyi entropy will be high at this orientation. While the candidate line rotates toward the orthogonal direction of the line (Figure 5.7 (c)), bright pixels on the line are concentrated only in a few particular bins. Intensities of these bright pixels on the line will be summed at its corresponding bins producing a high value in the histogram (Figure 5.7 (d)). The histogram in this case is peaky and the least uniform compared to histograms obtained at other orientations. The Renyi entropy at this orientation will have low values. The location of the peak also indicates the location of the sought line. The maximum value of the histogram is at the 50-th bin reflecting the centroid position of the line in the image. A clear dip can be identified in the Renyi entropy plot (Figure 5.7(e)) corresponding to the optimal projection angle, $-30^\circ$. In order to visually validate the estimated orientation of the line segment, the length of the line segment can be determined, and the line segment can be overlayed on the original image. The length of the line segment is determined using local evidence obtained from the intensity information around the detected line. 3 pixel wide neighboring points of the detected line are projected onto the detected line (Figure 5.7 (f)). The weighted histogram of projected points is then calculated. Projected points with probability above a predefined threshold (green line in (Figure 5.7 (g))) determine the length of the line segment (Figure 5.7 (h)).

### 5.2.3 Renyi Entropy for RBC Velocity Estimation

Figure 5.8 shows the steps for RBC velocity estimation using Renyi entropy: (1) centerline points of each vessel segment are obtained; (2) centerline points are corrected to be equidistant; (3) corrected centerlines of each vessel segment are used to generate space-time images; (4) RBC or plasma gap traces are enhanced in space-time images; (4) global orientation of traces is estimated; (5) velocities of RBCs are then calculated based on the estimated orientation.

![Figure 5.8: Flowchart for RBC velocity estimation.](image)
Centerline points of each vessel segment are obtained first. However, these centerline points are randomly spaced. These points are thus first linearly interpolated according to the arc length of each vessel segment. Corrected centerline points become equidistant with distance between two neighboring points along the centerline at 1 \( \mu m \) intervals. Figure 5.9 shows the generation of a space-time image. Assuming RBCs flow parallel to the vessel axial direction,

![Space-time image](image)

**Figure 5.9:** A synthetic space-time image.

...the horizontal axis of space-time images is the time instances of a video, and the vertical axis is path length along the centerline of each vessel segment. Each column of a space-time image represents image intensities of centerline points at each video frame. Movement of RBCs generates tilted dark lines, while movement of plasma gaps generates tilted bright lines. Let \( \phi \) be the angle from the horizontal direction to RBC or plasma gap traces in space-time images. If RBCs or plasma gaps move with constant speeds, their traces will appear straight. The velocity can then be calculated as

\[
v = \frac{\Delta z}{\Delta t} = \frac{R_z}{R_t} \cdot \frac{h}{w} = \frac{R_z}{R_t} \tan \phi
\]
where $\Delta z$ is the displacement along the vessel centerline in $\mu m$ within $\Delta t$ s, $h$ is the height of a space-time image, $w$ is the width of a space-time image, $R_t = 1/30$ s and $R_z = 1 \mu m$ are the horizontal axis and vertical axis resolutions of space-time images for our SDF microcirculatory videos. The bigger the angle is, the more tilted traces are, thus, the lower the velocity is.

Linear or non-linear interpolation using neighboring points of centerline points can also represent each column of a space-time image. Here we use nonlinear bilateral filtering [122] of neighboring points to interpolate intensity values of corrected centerline points. This method assigns the weighted sum of intensity values of nearest $M$ pixels to each centerline point. Given $d(a, b)$ representing the distance between points $a$ and $b$, $N_b$ as the set of nearest $M$ pixels to the point $b$, and the intensity value at point $b$ is defined as

$$I(b) = \sum_{a \in N_b} w_a I(a)$$

where $w_a \propto w_a^{(l)} w_a^{(c)}$, with $w_a^{(l)} = \exp(-d^2(a, b)/(2\sigma_l^2))$ is the weight based on locations of neighboring pixels, and $w_a^{(c)} = \exp(-d^2(I(a), I(b))/(2\sigma_c^2))$ is the weight based on intensities of neighboring pixels. $d(a, b)$ and $d(I(a), I(b))$ are distances between coordinates and intensities, respectively. Weighting by both distance and intensity improves the effect of nearest intensities that resemble the nearest pixel of the current point to be interpolated.

Figure 5.10 demonstrates determination of location and direction of plasma gap traces in a space-time image. White strips represent traces of plasma gaps. A candidate line rotates from $-90^\circ$ to $90^\circ$ with $1^\circ$ steps and every pixel in this image is projected onto the rotating candidate line. When the candidate line is rotated at $-6^\circ$ which is orthogonal to bright bands in the image, the projection histogram is peaky, thus, the Renyi entropy has the minimum value. The orientation of plasma traces is orthogonal to the optimal candidate line orientation.

We assume the velocity of RBCs is approximately equivalent to the velocity of plasma gaps. This is always the case especially if there is leukocyte adhesion and rolling. Centerline points are ordered. Because traces are tilted from left to the right, plasma gaps flow in the same direction from the starting point to the end point in the centerline, otherwise, they flow in the opposite direction. The estimated orientation $-6^\circ$ by our entropy based method is orthogonal
(a) The space-time image

(b) The projection histogram

(c) The estimated Renyi entropy

(d) Selected pixels

(e) Histogram of selected pixels

(f) The line segment

**Figure 5.10:** Illustration of the algorithm in detecting traces in a space-time image: (a) Optimal solution of line detection search is illustrated with the red line segment denoting the orthogonal projection direction for which projection entropy is minimized; (b) The projection histogram at 1-pixel bin width shown for the optimal projection. Locations of peaks also indicate locations of sought lines; (c) The estimated Renyi entropy of the projection to the candidate line rotates from -90 to 90 degrees; The minimum value is attained at $-6^\circ$, which corresponds to the optimal projection; (d) Selected pixels with high line-likelihood within 3-pixel wide band centered at the detected line; (e) Histogram of selected pixels projected onto the detected line orientation with a threshold 0.0001 (green) superimposed; (f) The estimated line segment.
to RBC or plasma gap bands in the space-time image. Thus, the absolute velocity estimated from Figure 5.10 is 

\[ v = \left| \frac{R_z}{R_t} \cdot \frac{1}{\tan(\frac{-6\pi}{180})} \right| = 0.285 \text{mm/s}. \]

This space-time image has two clear strips because dense RBCs are separated by long plasma gaps flowing along the vessel in good focus. If long plasma gaps flow along vessels in poor focus or less dense RBCs are separated by short plasma gaps, space-time images are more noisy. In order to obtain better estimation results, enhancement as a preprocessing step is necessary. Chaudhuri designed a matched filter to detect and enhance blood vessels [54].

Intensity profiles of the cross section of a bright vessel along its length can be approximated as a kernel

\[ K_B(x, y) = \begin{cases} 
\exp(-x^2/2\sigma^2) & \text{if } |y| \leq L/2 \\
0 & \text{else} 
\end{cases} \]

where \( \sigma \) is the spread of the intensity profile, and \( L \) is the length of the segmented vessel determined empirically. This basic filter can be rotated to highlight objects at all directions. Let \( q = [x; y] \) be a point in the kernel. Assuming the center position of the kernel is at the origin \([0; 0]\), \( q = [x; y] \) can be rotated through an angle \( \theta \) by the rotation matrix 

\[ R = \begin{bmatrix} 
\cos \theta & -\sin \theta \\
\sin \theta & \cos \theta 
\end{bmatrix}. \]

Point \( q = [x; y] \) becomes \( \overline{q} = \begin{bmatrix} u \\
v \end{bmatrix} = Rq = \begin{bmatrix} \cos \theta & -\sin \theta \\
\sin \theta & \cos \theta \end{bmatrix} \begin{bmatrix} x \\
y \end{bmatrix} = \begin{bmatrix} x \cos \theta - y \sin \theta \\
x \sin \theta + y \cos \theta \end{bmatrix} \)

in the rotated coordinate system. The kernel is restricted within a neighborhood \( N \) such that \( N = \{(u; v)||u| \leq 3\sigma, |v| \leq L/2\} \). The kernel is then given by

\[ K_B(x, y) = \exp(-(x \cos \theta - y \sin \theta)^2/2\sigma^2) \quad \forall \overline{q} \in N \]

In order for the mean of the kernel to be zero, the mean is subtracted in the final matched filter,

\[ \overline{K}_B(x, y) = K_B(x, y) - \sum_{\overline{q} \in N} K_B(x, y) / A \]

where \( A \) is the number of points in \( N \). A negative sign will be given if the matched filter is used to approximate dark vessels \( \overline{K}_D(x, y) = -\overline{K}_B(x, y) \).

Bright plasma gap traces can be enhanced by \( \overline{K}_B \) and dark RBC traces can be enhanced by \( \overline{K}_D \). Let \( S \) be the original space-time image. \( S \) is filtered with \( \overline{K}_B \) at every 10° from 0° to 170°. There are 18 matched filters, \( \overline{K}_{Bi}, i = 1, \ldots, 18 \). The filtered image by the \( i \)-th matched
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filter is $\hat{\mathbf{S}}_{B_i} = \mathbf{S} \ast \mathbf{K}_{B_i}$. Let $\mathbf{S}_B$ be the final enhanced space-time. It can be determined as the maximum response across all $18 \hat{\mathbf{S}}_{B_i}$. However, pixels not belonging to plasma gap or RBC traces are highlighted as well, especially when there are horizontal artifacts. It has been noted that RBC traces or plasma traces in each space-time image have similar orientations. Instead of maximum projection across all $18 \hat{\mathbf{S}}_{B_i}$, we can select the $o$-th filtered image $\hat{\mathbf{S}}_{B_0}$ as the final enhanced image $\mathbf{S}_B$ in which only RBC or plasma gap traces are enhanced. $\hat{\mathbf{S}}_{B_0}$ can be determined if $\hat{\mathbf{S}}_{B_0}$ multiplies $\mathbf{S}$ has the maximum intensity summation.

$$\mathbf{S}_B = \hat{\mathbf{S}}_{B_0} = \arg\max \left( \sum_{z=1}^{h} \sum_{t=1}^{w} s_{zt} \hat{\mathbf{S}}_{B_i,zt} \right)$$

In this way, if enhanced plasma gap or RBC traces in $\mathbf{S}_B$ lie in similar directions of these bands in $\mathbf{S}$, the overall summation intensity will be at its maximum. Let $\mathbf{S}_D$ be the final enhanced space-time image by $\mathbf{K}_D$. $\mathbf{S}_D$ can be determined from

$$\mathbf{S}_D = \hat{\mathbf{S}}_{D_0} = \arg\max \left( \sum_{z=1}^{h} \sum_{t=1}^{w} s_{zt} \left( \max_{z} \max_{t} \hat{\mathbf{S}}_{D_i} - \hat{\mathbf{S}}_{Di,zt} \right) \right)$$

Figure 5.11 compares enhanced space-time images by maximum projection and maximum summation intensity methods. Figure 5.11(a) and 5.11(b) show the vessel branch and its corresponding space-time image. The bright horizontal artifact is mostly due to the bright intensity near the sharp turning of this vessel branch. This bright horizontal artifact is also enhanced in the maximum projected image by $\mathbf{K}_B$ (Figure 5.11(c)), but not in maximum summation intensity obtained image at angle $60^o$ (Figure 5.11(d)). There is no bright horizontal artifact at the maximum projected image by $\mathbf{K}_D$ (Figure 5.11(e)), but other noise are enhanced. The maximum summation intensity obtained image at angle $50^o$ by $\mathbf{K}_D$ is less noisy (Figure 5.11(f)). Figure 5.11(d) and (f) show the estimated line segment aligning with plasma gap traces and RBC traces.

If there are constant transitions between plasma gaps and RBCs within vessel segments, there will be obvious transitions of bright and dark traces in space-time images. However, if there are no constant transitions between plasma gaps and RBCs, there will not be clear plasma gap and RBC trace transitions in space-time images. In this case, we can only observed either plasma gap traces or RBC traces in space-time images instead of both. Therefore, we
Figure 5.11: Comparison of enhanced space-time images by maximum projection and maximum summation intensity methods: (a) A vessel segment overlayed on the minimum projected frame; (b) The original space-time image generated from this vessel segment; (c) The maximum projection of all filtered images by the bright matched filter; (d) The selected filtered image at angle 60° by the bright matched filter with the estimated line overlayed; (e) The maximum projection of all filtered images by the dark matched filter; (f) The selected filtered image at angle 50° by the dark matched filter with the estimated line overlayed.
use both $K_B$ and $K_D$ to the original space-time image in purpose of enhancing both plasma gap and RBC traces. Figure 5.12 shows the difference between $\bar{S}_B$ and $\bar{S}_D$ filtered by $K_B$ and $K_D$. The original space-time image is very noisy (Figure 5.12(b)), but a few plasma gap traces can be still identified. These tilted plasma gap traces are enhanced in $\bar{S}_B$ (Figure 5.12(c)). However, only vertical artifacts are highlighted $\bar{S}_D$ (Figure 5.12(e)). Comparing entropy shapes of two enhanced images (Figure 5.12(d) and (e)), the entropy shape for $\bar{S}_B$ has a clearer dip and the minimum entropy is lower than the one for $\bar{S}_D$. An image with constant orientation patterns is supposed to have lower entropy than a noisy image without clear orientation patterns. Thus, minimum entropy can used as the criterion to select the best estimated orientation.

It is still possible that the selected filtered image is from a different orientation than plasma gap or RBC trace orientations. In this case, the estimated orientation will not be the true orientation. The enhanced image, $\bar{S}_B$, (Figure 5.13(c)) does not reflect trace patterns in the original space-time image (Figure 5.13(b)). However, the enhanced image, $\bar{S}_D$, has obvious RBC traces (Figure 5.13(e)). Based on the minimum entropy criterion, the minimum entropy of $\bar{S}_D$ (Figure 5.13(f)) is lower than that of $\bar{S}_B$ (Figure 5.13(d)). Thus, velocity can be determined based the orientation estimated from $\bar{S}_D$.

In sum, two velocities are estimated from both enhanced space-time images $\bar{S}_B$ and $\bar{S}_D$. A few criteria are used to determine the final velocity for each space-time image: (1) if orientations estimated from both enhanced images are less than $10^\circ$ difference, and velocity difference is less than $1 \text{ mm/s}$, the velocity for that space-time image will be the average of both velocities. $10^\circ$ and $1 \text{ mm/s}$ are determined empirically; (2) in other cases, the one with the lower minimum entropy will represent the velocity for that space-time image.

For Figure 5.12, Figure 5.13, and the rest space-time images processed by the entropy based method, the orientation search ranges from $-120^\circ$ to $-119.5^\circ$ with $0.5^\circ$ step size. This is because local minimum entropy is used to determine the optimal orientation. When there are stopped flows, the optimal orientation is around $-90^\circ$ or $90^\circ$. If searching range is from $-90^\circ$ to $89.5^\circ$, there will not be local minimum around $-90^\circ$ or $90^\circ$. Thus, the searching range extends from $-120^\circ$ to $119.5^\circ$. 
Figure 5.12: Comparison of enhanced space-time images by the bright and dark matched filter in a space-time image without clear transitions of plasma gap and RBC traces. (a) A vessel branch overlayed on the minimum projected frame; (b) The original space-time image generated from this vessel branch. Only a few plasma gap bands can be identified; (c) The enhanced space-time image by the bright matched filter; (d) Calculated Renyi entropy at angles from $-120^\circ$ to $119.5^\circ$ with $0.5^\circ$ step size in the enhanced space-time image by the bright matched filter; (e) The enhanced space-time image by the dark matched filter; (f) Calculated Renyi entropy at angles from $-120^\circ$ to $119.5^\circ$ in the enhanced space-time image by the bright matched filter.
Figure 5.13: Comparison of enhanced space-time images by the bright and dark matched filter and illustration of orientation selection criterion based on the minimum entropy. (a) A vessel branch overlayed on the minimum projected frame; (b) The original space-time image generated from this vessel branch; (c) The enhanced space-time image by the bright matched filter; (d) Calculated Renyi entropy at angles from $-120^\circ$ to $119.5^\circ$ with $0.5^\circ$ step size in the enhanced space-time image by the bright matched filter; (e) The enhanced space-time image by the dark matched filter; (f) Calculated Renyi entropy at angles from $-120^\circ$ to $119.5^\circ$ in the enhanced space-time image by the bright matched filter.
The order of Renyi entropy, $\alpha$, throughout the whole study is 3. $\alpha$ is varied from 2 to 20 and tested in a few video sequences. The absolute velocity error and correlation coefficient between gold standard and velocities obtained under different $\alpha$ on these video sequences are calculated. When $\alpha = 3$, the absolute velocity errors are comparatively low and correlation coefficient are comparatively high for all these video sequences. Therefore, 3 is chosen as the order for the whole study.

There are some limitations with this entropy method for line detection. The histogram for calculating Renyi entropy is weighted by the brightness of each pixel. If there are some extremely bright pixels in the image, these bright pixels will produce far higher probability at their corresponding bin than that of other bins. The distribution becomes peaky and non uniform, thus, the final orientation of the line can then be estimated as the orientation conforming to these bright pixels instead of actual trace orientations. Figure 5.14(b) shows the enhanced space-time image with some bright pixels at the left bottom corner. These bright pixels are not along plasma gap trace direction like other less bright pixels. Even though there is obvious orientation pattern in this image, the angle corresponding to the minimum entropy (Figure 5.14(c)) is not the trace orientation but the bright pixel orientation (Figure 5.14(d)). Comparing the projection distribution at angle $0^\circ$ (Figure 5.14(e)) and angle $-8^\circ$ (Figure 5.14(f)), because of these bright pixels, there is a high probability around bin 7 at angle $0^\circ$. While at angle $-8^\circ$, the orthogonal angle to plasma gap traces, there are a few peaks in the distribution reflecting the location of each plasma gap trace. Distribution at this orientation is more uniform compared to the distribution at orientation $0^\circ$ with a single, higher probability value peak. Therefore, the calculated entropy at angle $0^\circ$ is lower than angle $-8^\circ$.

### 5.2.4 Hough Transform for RBC Velocity Estimation

Hough Transform converts points on the straight line, $y = ax + b$, in the image space to the parameter space with one dimension for the slope, $a$, and another dimension for the intercept, $b$ [123]. However, vertical lines correspond to unbounded values of parameters. Therefore, instead of parameter $a$ and $b$, polar parameters, $\rho$ and $\theta$, are typically used to replace the slope and the intercept, $y = (-\frac{\cos \theta}{\sin \theta}) x + (\frac{\rho}{\sin \theta})$ [88], where $\rho$ is the distance between the origin and the line, and $\theta$ is the angle from the horizontal line passing through the origin to the normal
Figure 5.14: Falsely estimated orientation by the proposed entropy method if bright pixels do not conform to trace orientation.
vector to the line. $\rho$ can be written as $\rho = x\cos\theta + y\sin\theta$. Thus, a number of lines at different angles going through each point in the image space form a sinusoidal curve in the parameter space. Points on the same lines at the image space will intersect at the Hough Transform image, called the accumulator cell. The more points are on the same line, the brighter the cell at the specified orientation. Lines can then be extracted by looking for the local maxima in the accumulator space which can be determined by thresholding cell values.

Figure 5.15 shows the application of Hough Transform for estimating plasma gap or RBC trace orientation. Edges are detected in $\overline{S}_B$ first (Figure 5.15(a) and (b)). For each edge pixel, lines with $\theta$ from $-90^\circ$ to $89.5^\circ$ with step size $0.5^\circ$ pass through it. Corresponding $\rho$ for each angle can be calculated through the equation above. $\theta$ and $\rho$ form a sinusoidal curve in the accumulator cell. This process is repeated for all edge pixels in the image. Edge pixels on the same line fall into the same bin in the accumulator, thus incrementing values for that bin. Hough Transform of the edge map is shown in Figure 5.15(c), the axes are $\theta$ and $\rho$, respectively. The red box highlights the bin with the highest value in the accumulator cell. The corresponding horizontal axis is the optimal orientation of the dominant lines in the edge map.

Hough Transform based on edge maps works well on clean images. However, if images are noisy, and edges of noisy objects are detected, these noisy edges can predominate trace edges. Even though plasma gap and RBC traces are enhanced, enhanced images can be still noisy. Traces can be falsely detected using Hough Transform method. Figure 5.16 (a) and (b) shows edge maps of enhanced space-time images and falsely detected traces. Hough transform also fails to find correct orientation of non horizontal traces if the width of space-time images is way longer than the height. This is mostly because if the width of the space time is too long, the number of pixels along an orientation close to the horizontal direction can be more than the number of pixels along the short plasma gap or RBC traces. Figure 5.16 (c) and (d) shows falsely detected lines in these kind of space-time images.
5.2.5 Radon Transform for RBC Velocity Estimation

2D Radon Transform of an object $f(x, y)$ is the line integral of the object at each angle. Each line integral has parameters $(l, \theta)$, where $l$ is the lateral position of the line, and $\theta$ is the angle of a unit normal to the line. The line integral can then be defined as $R_\theta(l) = \int_{\theta,l} f(x, y) ds$. Similar to Hough Transform, let $l = x\cos\theta + y\sin\theta$. Using a delta function, Radon Transform is then defined as

$$g(l, \theta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) \delta(x\cos\theta + y\sin\theta - l) dx dy$$

Radon Transform is mostly used in tomographic imaging. The source sends out parallel rays or fan beam rays. These rays pass through an object and project to detectors at the other side of the object. What are received at detectors are the object intensity integral at each specific
angle. A sinogram will be formed when the source is rotated at a certain angle range. The horizontal axis of the sinogram is the rotating angle, and the vertical axis is the detector bins. Applying Radon Transform to line orientation detection, parallel rays pass through space-time images at different angles. If these parallel rays are in the same orientation as plasma gap or RBC traces, intensities of trace pixels will less likely be summed with intensities of non trace pixels. Therefore, projection values will be high at some detector bins and low at other detector bins. While rays are in other orientations, projection values will be comparably more uniform at all detector bins. Theoretically, the angle with the maximum variance along bins reflects the orientation of plasma gap or RBC traces. Figure 5.17 shows the application of Radon Transform for line orientation estimation. Parallel rays are rotated from $-90^\circ$ to $89.5^\circ$ with $0.5^\circ$ step size. Figure 5.17(b) shows the Radon Transformed image of $\overline{S}_B$. Bright dots
in this sinogram represent high integral values at particular angles and detector radius. These bright dots are all corresponding to one angle. Other pixels at each angle have similar values. Thus, the angle where bright dots align should have the maximum variance along detector radius. Figure 5.17(c) shows the variance along detector radius for each angle. The maximum variance lies at angle 4. Figure 5.17(d) displays a line along the estimated orientation. When the width of the space-time image is much longer than the height, the number of pixels along an orientation close to the horizontal direction can be more than the number of pixels along the short plasma gap or RBC traces. Therefore, summed intensities are very high at these angles at some detector radius and low at other radius. Figure 5.18(b) shows the Radon Transformed
image of the enhanced space-time image of Figure 5.18(a). Figure 5.18(c) shows that variance goes higher when parallel rays rotate towards the horizontal direction. However, real trace orientation is actually orthogonal orientation to $37^\circ$ where the peak is located at. Thus, instead of global maximum variance, local maximum variance is used as the criterion to select the optimal angle. If there are multiple local maxima, the angle with the largest local maximum variance will be selected as the optimal angle. Figure 5.18(d) shows the estimated orientation of traces. When there are stopped flows, there will be horizontal plasma gap or RBC traces.

**FIGURE 5.18:** Line orientation estimation in the space-time image with the width way longer than the height by Radon Transform.

in space time images. If looking at local maximum, the true orientation will not be selected. This is because the rotating angle goes from $-90^\circ$ to $89.5^\circ$. Variance will go up towards $-90^\circ$.
or 89.5° and reach maximum at −90° and 89.5°. There will not be local maximum detected at angle −90° and 89.5°. A searching range from −120° to 119.5° can be used. However, when the width of space-time images is much longer than the height of space-time images, there will be local maximum around −90° or 90°. Thus, in order to estimate orientation in situations with long width space-time images and stopped flows, the orientation searching ranges from −90° to 89.5° with conditions that when local maximum is near the minimum variance value, the optimal angle will be the one corresponding to the global maximum instead of local maximum.

Because of varieties of space-time images, this criterion may not work for every single space-time image. Figure 5.19) shows one of failures of Radon Transform for line orientation detection in a space-time image. Radon Transformed image is based on the summation of pixel intensities along each projection ray. Bright pixels at different traces happen to align vertically falling in the same detector bins around angle 0.5. This causes two bright spots around 0.5° (Figure 5.19 (b)). The variance is then higher at 0.5° degree than the real trace orientation, −12.5°. In this case, orientation can be falsely estimated.

5.2.6 Experimental Results

5.2.6.1 Gold Standard Velocity Generation

In order to validate estimated velocity by the entropy based method and compare it with Hough and Radon Transform methods, centerlines of all vessels for each video are manually labeled by a human expert. Space-time images can be generated for each vessel segment. The human expert manually draws a line or a few lines to match orientation of plasma gap or RBC traces on space-time images. Velocities based on the manually drawn lines are calculated. If there are multiple lines, standard deviation velocities are calculated. Velocities estimated in this way are used as gold standard.
5.2.6.2 Optimal Velocity Selection Validation

The proposed method is applied to 556 vessel segments extracted from 13 microcirculatory videos including those videos taken from healthy and sick subjects. The entropy method, Hough Transform method, and Radon Transform method have orientation search from \(-120^\circ\) to \(120^\circ\), \(-90^\circ\) to \(89.5^\circ\), \(-90^\circ\) to \(-89.5^\circ\) with 0.5\(^\circ\) step size, respectively for this whole study. The order, \(\alpha\), for Renyi entropy is 3. Two velocities are calculated for each vessel segment from both \(\overline{S}_B\) and \(\overline{S}_D\). In order to evaluate the velocity selection criterion based on the minimum entropy, optimal velocities are manually picked by humans from either the velocity estimated from \(\overline{S}_B\) or \(\overline{S}_D\) or the average of both velocities, whichever has least difference.
to the average gold standard velocity. Figure 5.20 shows the correlation between manually selected optimal velocities and automatically selected optimal velocities for all 556 vessel segments. The fitted line has slope 0.9846 and intercept 0.0152. Table 5.1 compares $R^2$ and correlation coefficient (CC) between velocities selected manually and automatically for each video sequence and all together. Based on visual and quantitative comparison, most automatically selected optimal velocities correlate strongly with manually selected velocities.
TABLE 5.1: Statistical comparison between manually and automatically selected velocities

<table>
<thead>
<tr>
<th>Video Sequence</th>
<th>( R^2 )</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (36 vessels)</td>
<td>0.9575</td>
<td>0.9785</td>
</tr>
<tr>
<td>2 (84 vessels)</td>
<td>0.9661</td>
<td>0.9829</td>
</tr>
<tr>
<td>3 (103 vessels)</td>
<td>0.841</td>
<td>0.9296</td>
</tr>
<tr>
<td>4 (83 vessels)</td>
<td>0.9825</td>
<td>0.9912</td>
</tr>
<tr>
<td>5 (23 vessels)</td>
<td>0.9935</td>
<td>0.9968</td>
</tr>
<tr>
<td>6 (22 vessels)</td>
<td>0.9615</td>
<td>0.9805</td>
</tr>
<tr>
<td>7 (22 vessels)</td>
<td>0.9725</td>
<td>0.9862</td>
</tr>
<tr>
<td>8 (61 vessels)</td>
<td>0.9866</td>
<td>0.9933</td>
</tr>
<tr>
<td>9 (24 vessels)</td>
<td>0.9221</td>
<td>0.9603</td>
</tr>
<tr>
<td>10 (40 vessels)</td>
<td>0.9494</td>
<td>0.9743</td>
</tr>
<tr>
<td>11 (18 vessels)</td>
<td>0.98</td>
<td>0.9899</td>
</tr>
<tr>
<td>12 (11 vessels)</td>
<td>0.9743</td>
<td>0.9870</td>
</tr>
<tr>
<td>13 (29 vessels)</td>
<td>0.8423</td>
<td>0.9177</td>
</tr>
<tr>
<td>All (556 vessels)</td>
<td>0.9474</td>
<td>0.9743</td>
</tr>
</tbody>
</table>

5.2.6.3 Velocity Validation and Comparison

Velocities estimated by the proposed method is evaluated with gold standard and compared with Hough Transform and Radon Transform methods. Figure 5.21 shows how velocities obtained by each method are compared with gold standard. For each vessel segment, the bright and dark matched filters, \( K_B \) and \( K_D \), are applied to generate two enhanced space-time images, \( S_B \) and \( S_D \). Each method is then applied to both enhanced space-time images. Consequently, two velocities are calculated for one vessel segment with each method. In order to validate each method for velocity estimation with gold standard, one final optimal velocity out of two velocities for each method has to be determined. For the entropy method, the optimal velocity can be selected by the selection criterion mentioned in section 5.2.3. However, no velocity selection criterion have been devised for Hough and Radon Transform methods. Thus, two velocities for each method are presented to the human expert in order to choose the clinically more accurate one. Because the maximum measurable blood flow rate is 2 mm/s for our imaging device, if both estimated velocities are below 2 mm/s, the human expert will pick either velocity or the average velocity which has the closest value.
Figure 5.21: Flowchart of how velocities obtained by each method are compared with gold standard.

To the gold standard. If one estimated velocity is below 2 mm/s, and the other is above 2 mm/s, if the one which is below 2 mm/s has a close value to the gold standard, this velocity will be picked by the human expert. If both velocities are above 2 mm/s, corresponding vessel segments will be excluded from final analysis. Figure 5.22 shows fractions of vessels excluded from final velocity comparison in each method for each video sequence. If space-time images are noisy and vertical artifacts are dominating real plasma gap or RBC traces, if the orientation estimation method is more sensitive to noise, vertical orientation instead of real orientation will most probably be obtained, and more number of vessels will be excluded. Hough Transform is the one most sensitive to noise and our entropy based method is the one least sensitive to noise. For all video sequences, entropy based method excludes the least number of vessels, while Hough Transform method excludes the most.

Final optimal velocities automatically selected for the entropy method, and manually selected
for the entropy, Hough Transform, and Radon Transform methods are then compared with gold standard. Figure 5.23 shows correlations between velocities estimated by each method with gold standard for all 556 vessel segments. The magenta line represents the ideal fitting. The maximum correlation is obtained if points on the scatter plot are on this reference line. Most velocities measured by all these methods correspond with gold standard. This plot also shows that some velocities estimated by these methods have very high values even though gold standard velocities are low. High velocities are obtained if orientations estimated are near vertical. This is most likely due to vertical artifacts dominating space-time images.
Figure 5.23: Comparison of scatter plot between gold standard and optimal velocities estimated by each method. The magenta line represents the gold standard reference based on expert’s manually generated velocity data.

In order to quantitatively compare velocities estimated by the proposed method with the ground truth and other methods, absolute relative error is calculated. It is the ratio of absolute differences between estimated velocities and human labeled gold standard velocities to human labeled gold standard velocities, \( \frac{|v_{\text{estimate}} - v_{\text{human}}|}{v_{\text{human}}} \). Figure 5.24 shows median absolute relative errors for each video sequence. Because some low velocities are falsely estimated as high velocities, median instead of mean is calculated to avoid bias by outliers.

Table 5.2 quantitatively compares median absolute relative errors and percent of vessels excluded for all these methods. Hough Transform method has low median absolute relative
errors in a few video sequences. However, Hough Transform method excludes a large proportion of vessels from absolute relative error calculation. For video sequence 6, 10, 11 and 12, both entropy based method and Radon Transform method have the same number of vessels excluded from absolute relative error calculation. For video sequence 12, the entropy based method obtains similar performance compared to Radon Transform method. For other three video sequences, the entropy based method produces lower median absolute relative

Figure 5.24: Comparison of median absolute relative errors for the proposed entropy method with optimal velocities manually selected, the proposed entropy method with optimal velocities automatically selected, Hough Transform method with optimal velocities manually selected, and Radon Transform method with optimal velocities manually selected.
error compared to Radon Transform method. Radon Transform method obtains lower absolute relative error in video sequence 8 compared to the entropy based method. However, the fraction of exclusion in Radon Transform method is twice more than the fraction of exclusion in entropy based method. For the rest videos, the entropy based method with manual velocity selection has similar or better performance compared to Radon Transform method with much fewer number of vessels excluded from error calculation. If a more optimal automatic velocity selection criterion can be devised, the entropy based method with automatic velocity selection has the potential to produce similar performance as the entropy based method with manual velocity selection. Overall, for these 556 vessels together, the entropy based method obtains the least absolute relative error with the least number of vessels excluded from error calculation, while Hough Transform method still produces the highest absolute relative error even though a large number of vessels already excluded from error calculation.

<table>
<thead>
<tr>
<th>Video</th>
<th>Entropy(auto)</th>
<th>Entropy</th>
<th>Hough</th>
<th>Rodon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.19/5.56</td>
<td>0.14/5.56</td>
<td>0.14/22.22</td>
<td>0.15/13.89</td>
</tr>
<tr>
<td>2</td>
<td>0.21/3.57</td>
<td>0.16/3.57</td>
<td>0.2/30.95</td>
<td>0.16/7.14</td>
</tr>
<tr>
<td>3</td>
<td>0.35/2.91</td>
<td>0.18/2.91</td>
<td>0.21/22.33</td>
<td>0.16/9.71</td>
</tr>
<tr>
<td>4</td>
<td>0.24/8.43</td>
<td>0.18/8.43</td>
<td>0.84/10.84</td>
<td>0.21/12.05</td>
</tr>
<tr>
<td>5</td>
<td>0.19/4.35</td>
<td>0.19/4.35</td>
<td>0.14/21.74</td>
<td>0.16/8.7</td>
</tr>
<tr>
<td>6</td>
<td>0.13/0</td>
<td>0.11/0</td>
<td>0.17/18.18</td>
<td>0.17.3/0</td>
</tr>
<tr>
<td>7</td>
<td>0.20/9.09</td>
<td>0.14/9.09</td>
<td>0.1/45.45</td>
<td>0.12/27.27</td>
</tr>
<tr>
<td>8</td>
<td>0.30/8.2</td>
<td>0.29/8.2</td>
<td>0.15/29.51</td>
<td>0.2/18.03</td>
</tr>
<tr>
<td>9</td>
<td>0.31/4.17</td>
<td>0.19/4.17</td>
<td>0.4/33.33</td>
<td>0.3/16.67</td>
</tr>
<tr>
<td>10</td>
<td>0.11/7.5</td>
<td>0.07/7.5</td>
<td>0.14/22.50</td>
<td>0.14/7.5</td>
</tr>
<tr>
<td>11</td>
<td>0.21/0</td>
<td>0.16/0</td>
<td>0.13/5.56</td>
<td>0.22/0</td>
</tr>
<tr>
<td>12</td>
<td>0.21/0</td>
<td>0.14/0</td>
<td>0.07/9.09</td>
<td>0.14/0</td>
</tr>
<tr>
<td>13</td>
<td>0.22/6.9</td>
<td>0.12/6.9</td>
<td>0.14/44.83</td>
<td>0.18/13.79</td>
</tr>
<tr>
<td>All</td>
<td>0.24/5.22</td>
<td>0.17/5.22</td>
<td>0.19/24.28</td>
<td>0.18/10.97</td>
</tr>
</tbody>
</table>
Chapter 6

Classification of Pathological Conditions

6.1 Microvascular Measurement Assessment

Figure 6.1 shows the steps toward assessment of sublingual microcirculation. Vessels are extracted and velocities of RBCs are estimated. Microvascular measurements can then be calculated automatically from extracted vessels and estimated velocities. These quantitative microcirculatory measurements are then used to assess sublingual microcirculation in healthy subjects and infected patients.

Based on extracted vessels and estimated RBC velocities, a few potential measurements are automatically calculated to characterize sublingual microcirculation.

1) Vessel density \((mm/mm^2)\): 
\[
VD = \frac{\text{the total length of vessels}}{\text{the total area of the video frame}}
\]
2) Mean velocity \((mm/s)\): average velocity of all vessels in each video, 
\[ \overline{v} = \frac{\sum_{i=1}^{N} v_i}{N}, \]
where \(N\) is the number of vessels in the video.

3) Standard deviation inverse of trace orientation histogram: \(1/\sigma\). Orientations of RBC or plasma gap traces in space-time images are obtained for each vessel segment. Velocities are calculated based on estimated orientations. Orientation is inversely proportional to velocity. Figure 6.2 shows histograms of orientation of RBC or plasma gap traces in a healthy subject and an infected patient. There is a hypothesis that fast flow corresponds to healthy condition and slow flow corresponds to sick condition. The spread, \(\sigma\), of the histogram is larger in slow flow case than in fast flow case. The orientation histogram is fitted with Gaussian Mixture Model (GMM). The GMM is defined as a weighted sum of \(K\) component multivariate normal distribution [124]
\[ p(x|\theta) = \sum_{k=1}^{K} w_k g(x|\mu_k, \Sigma_k) \]
where \(x\) is a \(D\)-dimensional random variables, \(w_k\) is mixture weights, \(\sum_{k=1}^{K} w_k = 1\). The \(k\)-th component is represented by normal distributions with mean \(\mu_k\) and covariance matrix \(\Sigma_k\),
\[ g(x|\mu_k, \Sigma_k) = \frac{1}{(2\pi)^{D/2}|\Sigma_k|^{1/2}} e^{-\frac{1}{2}(x-\mu_k)'\Sigma_k^{-1}(x-\mu_k)} \]
Weights \(w_k\), means \(\mu_k\) and covariance matrices \(\Sigma_k\) can be estimated using expectation maximization algorithm (EM) [125]. For one-dimensional data, covariance matrix becomes standard deviation, \(\sigma\). After removing absolute orientation bigger than 50°, one component Gaussian distribution is fitted to the trace orientation histogram. Red lines in Figure 6.2 are fitted Gaussian mixture densities. Estimated \(\mu\) in Figure 6.2 are near 0. Estimated \(\sigma\) for Figure 6.2 (a) is 13.9619, and for Figure 6.2 is 83.6598. The spread of the trace orientation histogram can be a potential measurement to distinguish different pathological conditions. In this study, the inverse of the spread is calculated for characterization.

4) Flux of RBCs \((n/s)\): the number of RBCs exists in a vessel per second. In this study, only small vessels (diameter smaller than 20 \(\mu\)m) are analyzed. We assume that there is only one single RBC along the cross section of small vessels. Space-time images reflect all RBCs passing through a vessel at the whole video time. The number of RBCs exists in a vessel can be estimated from space-time images. In our entropy based velocity estimation method, peaks of projection histogram indicate locations of each RBC trace in pace-time images. We then define flux here as the number of peaks of
Chapter 6. Classification of Pathological Conditions

Figure 6.2: Histograms of trace orientation and fitted Gaussian densities (red).

Projection histogram divided by the recording time of the video, \( l = \frac{\# \text{ peaks of projection histogram}}{t(s)} \).

Figure 6.3 (a) shows an enhanced space-time image by the dark matched filter. These highlighted strips are RBC traces. Figure 6.3 (b) show the projection histogram of all pixels in the image projected onto the orthogonal orientation of RBC traces. A peak is declared if differences between the peak and its surrounding points are above a predefined threshold. In this study, the threshold is 0.001.

Flux is calculated for all small vessels in each video. It is likely that healthy subjects have higher flux compared to infected patients. A few flux related measurements can be extracted. Let \( N \) be the number of vessels in the video and \( l_i \) is the flux value in the \( i \)-th vessel. Mean flux can be calculated as \( \bar{l} = \frac{\sum_{i=1}^{N} l_i}{N} \). Median flux, \( \tilde{l} \), is the middle value of flux in all vessels. Histogram of flux for each video is also investigated in this study. Kernel Density Estimate (KDE) can be used to estimate probability density of flux in all vessels for each video. If a Gaussian kernel is used, the Gaussian kernel based weighted KDE is defined as

\[
f(l) = \sum_{i=1}^{N} w(l_i) p_{\Sigma_i}(l - l_i)
\]
Chapter 6. *Classification of Pathological Conditions* 85

(a) An enhanced space-time image

(b) Projection histogram

**Figure 6.3:** Detected peaks (red stars) of a projection histogram.

where $w(l_i)$ is the weight; $\Sigma_i$ is the covariance of the Gaussian kernel $p_{\Sigma_i}(l) = C_{\Sigma_i} e^{-\frac{1}{2}l^T \Sigma_i^{-1} l}$. Figure 6.4 compares the histogram and KDE of the flux from a healthy subject and an infected patient. The flux corresponding to both peak of histogram and KDE for the healthy subject is larger than the one for the infected patient. Therefore, the flux corresponding to the peak of flux histogram, $l_H = \arg\max_h(l)$, where $h$ is the histogram, and the flux corresponding to the peak of flux KDE, $l_K = \arg\max_f(l)$, can be considered as potential indicators for characterizing sublingual microcirculation.
6.2 Correlations among quantitative and semi-quantitative microcirculatory measurements

MFI characterizes flow pattern of RBCs. Velocity for each individual vessel is automatically calculated. It is highly likely that there is a correlation between MFI and RBC velocities. PPV characterize perfusion of vessels. Flux of RBCs provides an information about how perfused each vessel is. It is likely that there is a correlation between PPV and flux related measurements. Because PVD is calculated by the multiplication of TVD and PPV, it is likely that there is a correlation between PVD and the multiplication of VD and flux related measurements. Besides correlations between quantitative and semi-quantitative microvascular parameters, it is also likely different quantitative and different semi-quantitative measurements are dependent. Figure 6.5 shows a correlation coefficient image calculated from pairs of microvascular measurements. The measurement has correlation coefficient value 1 with
itself. Elements in the diagonal of the image are all 1. While at redish blocks at the upper left

\[ \begin{array}{cccccccc}
\text{DB} & \text{TVD} & \text{PVD} & \text{PPV} & \text{MFI} & \overline{\nu} & \frac{1}{\sigma} & \tilde{l} & l_h & l_k & \text{VD} & \text{VD} \times \overline{\nu} & \text{VD} \times \frac{1}{\sigma} & \text{VD} \times \tilde{l} & \text{VD} \times l_h & \text{VD} \times l_k \\
\hline
\text{DB} & & & & & & & & & & & & & & \\
\text{TVD} & & & & & & & & & & & & & & \\
\text{PVD} & & & & & & & & & & & & & & \\
\text{PPV} & & & & & & & & & & & & & & \\
\text{MFI} & & & & & & & & & & & & & & \\
\overline{\nu} & & & & & & & & & & & & & & \\
\frac{1}{\sigma} & & & & & & & & & & & & & & \\
\tilde{l} & & & & & & & & & & & & & & \\
l_h & & & & & & & & & & & & & & \\
l_k & & & & & & & & & & & & & & \\
\text{VD} & & & & & & & & & & & & & & \\
\text{VD} \times \overline{\nu} & & & & & & & & & & & & & & \\
\text{VD} \times \frac{1}{\sigma} & & & & & & & & & & & & & & \\
\text{VD} \times \tilde{l} & & & & & & & & & & & & & & \\
\text{VD} \times l_h & & & & & & & & & & & & & & \\
\text{VD} \times l_k & & & & & & & & & & & & & & \\
\end{array} \]

Figure 6.5: Correlation coefficient between each microvascular measurement

area, semi-quantitative measurement, De Backer score and TVD, are highly correlated. Similarly, semi-quantitative measurements PPV and MFI are highly correlated. While at redish blocks the lower right areas, it is not surprised that quantitative flux related measurements are all correlated with each other. Quantitative measurement, \( \overline{\nu} \), is highly correlated with \( \frac{1}{\sigma} \) and \( \tilde{l}, \tilde{l}, l_h, l_k \). This image confirms our hypothesis that there is a high correlation between semi-quantitative measurement, PPV, and quantitative flux related measurements, \( \tilde{l}, \tilde{l}, l_h, l_k \). Semi-quantitative measurements, MFI and PVD, are also somehow correlated with \( \tilde{l}, \tilde{l}, l_h, l_k \), but less significant compared to PPV. Among semi-quantitative measurements, MFI has the highest correlation coefficients with quantitative measurements, \( \overline{\nu} \) and \( \frac{1}{\sigma} \). Likewise, PVD has higher correlation coefficients with quantitative measurements, \( \text{VD} \times \tilde{l}, \text{VD} \times \overline{\nu}, \text{VD} \times l_h, \) and \( \text{VD} \times l_k \).

Figure 6.6 shows scatter plots of MFI versus \( \overline{\nu} \) and \( \frac{1}{\sigma} \). \( R^2 \) value for fitting MFI from mean velocity is 0.541 and from standard deviation inverse of orientation histogram is 0.527. These
Figure 6.6: Scatter plots of MFI versus $\bar{v}$ and $\frac{1}{\sigma}$. Black lines represent fitted MFI from $\bar{v}$ and $\frac{1}{\sigma}$. Pink markers represent healthy groups. Blue markers represent infected groups.

Scatter plots show correlations between semi-quantitative measurement, MFI, and quantitative measurements, $\bar{v}$ and $\frac{1}{\sigma}$. Furthermore, most healthy subjects are clustered together in the upper right side and infected patients are clustered together in the lower left side.

Figure 6.7 shows scatter plots of PPV versus $l_K$ and PVD versus $VD \times l_K$. $R^2$ values fitting PPV from $\bar{l}$, $\tilde{l}$, $l_H$, and $l_K$ are 0.626, 0.631, 0.659, and 0.652, respectively. It has been seen that there is a correlation between PPV and flux related measurements. $R^2$ values for fitting PVD from the multiplication of VD and $\bar{l}$, $\tilde{l}$, $l_h$, and $l_k$ are 0.551 0.527, 0.5498, and 0.519, respectively. For the scatter plot of PPV versus $l_k$, most healthy subjects are clustered
Figure 6.7: Scatter plots of PPV versus $l_K$ and PVD versus $VD \times l_K$. Black lines represent fitted PPV from $l_K$ and fitted PVD from $VD \times l_K$. Pink markers represent healthy groups. Blue markers represent infected groups.

together in the upper right side and infected patients are clustered together in the lower left side. However, for the scatter plot of PVD versus $VD \times l_K$, three pink markers and one blue marker seem to fall into the clusters of the opposite groups.
6.3 Quantitative and semi-quantitative microvascular measurements in assessing pathological conditions

There is a hypothesis that semi-quantitative microcirculatory measurements, PVD, PPV, and MFI, are able to characterize sublingual microcirculation in healthy subjects and infected patients. Because there are correlations between these semi-quantitative microcirculatory measurements and quantitative microcirculatory measurements, it is likely that these quantitative microcirculatory measurements have similar performance in assessing sublingual microcirculation. Figure 6.8 shows mutual information of each measurement and pathological condition labels. Mutual information here provides an information about how much pathological conditions are dependent on these microcirculatory measurements individually. Semi-quantitative microvascular parameter, PPV, has the largest mutual information compared to the rest parameters. MFI is the second important parameter in characterizing different pathological conditions. PVD is less significant compared to PPV and MFI. Among quantitative

![Figure 6.8: Mutual information](image)

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**Figure 6.8:** Mutual information
microvascular parameters, flux related measurements, $l_h$ and $l_k$, are the two most important measurements in assessing sublingual microcirculation. $\frac{1}{\sigma}$ is also significant. $\overline{v}$, $\overline{l}$, and $\overline{\tilde{l}}$ are less significant compared to $l_h$, $l_k$, and $\frac{1}{\sigma}$. Density related measurements, De Backer score, TVD, and VD show no significance in characterizing sublingual microcirculation.

Figure 6.9 shows mean and standard deviation values of each semi-quantitative and quantitative microcirculatory parameter in each pathological condition. Because of correlation among flux related measurements, we only show $l_k$ and $VD \times l_k$ in the figure. Table 6.1 shows P-values of them. P-values are calculated using Student’s t-test at 5% significance level.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DeBacker</td>
<td>0.048</td>
</tr>
<tr>
<td>TVD</td>
<td>0.043</td>
</tr>
<tr>
<td>PVD</td>
<td>$3.63 \times 10^{-5}$</td>
</tr>
<tr>
<td>PPV</td>
<td>$8.4 \times 10^{-6}$</td>
</tr>
<tr>
<td>MFI</td>
<td>$7.09 \times 10^{-6}$</td>
</tr>
<tr>
<td>VD</td>
<td>0.14</td>
</tr>
<tr>
<td>$\overline{v}$</td>
<td>$2.82 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\frac{1}{\sigma}$</td>
<td>$2.38 \times 10^{-3}$</td>
</tr>
<tr>
<td>$\overline{l}$</td>
<td>$4.74 \times 10^{-5}$</td>
</tr>
<tr>
<td>$l$</td>
<td>$5.69 \times 10^{-5}$</td>
</tr>
<tr>
<td>$l_h$</td>
<td>$2.91 \times 10^{-5}$</td>
</tr>
<tr>
<td>$l_k$</td>
<td>$2.25 \times 10^{-5}$</td>
</tr>
<tr>
<td>$VD \times \overline{l}$</td>
<td>$2.96 \times 10^{-4}$</td>
</tr>
<tr>
<td>$VD \times l$</td>
<td>$3.95 \times 10^{-4}$</td>
</tr>
<tr>
<td>$VD \times l_h$</td>
<td>$2.94 \times 10^{-4}$</td>
</tr>
<tr>
<td>$VD \times l_K$</td>
<td>$2.93 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Infected patients show decreased density and flow compared with healthy subjects. P-values are high in De Backer score, TVD, and automatically calculated VD. Differences between healthy subjects and infected patients in De Backer score, TVD, and VD have means approximately equal to zero. Rest microcirculatory parameters show significant differences between pathological conditions.
Figure 6.9: Average and standard deviation of semi-quantitative and quantitative microvascular measurements in different pathological conditions.
Chapter 7

Conclusion and Future Work

Velocities of RBCs are calculated based on orientation of traces estimated from space-time images. The proposed technique is capable of estimating orientation of RBC or plasma gap traces in space-time images. Successful line or curve detection would be attained even at higher noise levels as indicated by the success of the image enhancement step. Intersections of vessels cause horizontal lines, and periodic variations in illumination cause vertical lines in space-time images. The proposed method is still capable of determining the orientation of traces. Two enhanced space-time images are generated for each vessel segment by the bright and dark matched filter. Renyi entropy method is applied to estimate trace orientation in these enhanced space-time images. By comparing the minimum value of both local minimum entropy, the final velocity can be determined for that particular vessel segment. Final velocities determined manually are obtained by selecting one velocity or average velocity which is least difference to average gold standard velocities. Final velocities selected automatically correlate well with the ones selected manually. It achieves comparable performance after validating with ground truth velocities compared to final velocities selected using the manual methods. At high velocity, deviations of estimated RBC velocities from gold standard may be due to several factors including vessel length, cross-talk from collocated vessels, or multiple velocity components along the vessel. Current imaging devices only allow us to measure velocity up to 2 mm/s. Velocities estimated above 2 mm/s mostly due to noisy videos and vertical artifacts in space-time images. Furthermore, high velocity flow estimation is clinically less significant because microcirculatory dysfunction manifests
itself by RBC velocities below approximately 0.5 \( \text{mm/s} \). Different bin widths can produce different histograms. Selection criterion in the entropy based method can be tuned to obtain better performance. Optimization of all parameters based on extensive experimental data will be investigated for peak performances in the future.

In general, De Backer score, TVD, PPV, PVD, MFI, HetIndex, are measured semi-quantitatively from sublingual microcirculatory videos. It is a time consuming, labor intensive, and qualitative process. In this study, we present an automatic, computer-assisted tool which produces quantitative microcirculatory measurements for assessing sublingual microcirculation. Vessels are extracted first, and then velocities of RBCs are estimated automatically using our entropy based method for each vessel segment. Based on the extracted vessels and estimated velocities of RBCs, a few measurements are calculated quantitatively, such as vessel density, standard deviation inverse of orientation histogram, mean velocity, mean flux, median flux, flux corresponding to the peak of histogram, flux corresponding to the peak of KDE, and multiplication of vessel density and flux related measurements.

Infected patients show decreased flow, perfusion, and density compared to healthy subjects. TVD and De Backer score show no significant difference in different pathological conditions. PPV has the highest mutual information with pathological condition labels compared to MFI and PVD. HetIndex provides information about flow heterogeneity. It is demonstrated that there is increased heterogeneity in patients with septic shock [126]. HetIndex is not investigated in this study. In the future, microcirculatory videos will be collected in multiple sublingual sites in each subject to study flow heterogeneity. Quantitative microvascular measurements calculated based on automatically extracted vessels and estimated velocities are highly correlated with semi-quantitative microcirculatory measurements. They have similar performance in the assessment of sublingual microcirculation. Semi-quantitative microcirculatory parameters, MFI, PPV, PVD, and quantitative microvascular parameters, \( \overline{v}, \frac{1}{\sigma} \), flux related measurements, multiplication of VD and flux related measurements, all have low P-values and are demonstrated to be significant in characterizing sublingual microcirculation even in noisy and low quality videos. The proposed computer assisted tool makes the assessment of sublingual microcirculation automatic, quantitative, less labor intensive, and higher throughput.
Only 30 sublingual microcirculatory videos are analyzed in this study. We can only use one-dimensional features to perform some statistical significance calculations. In the future, we will label hundreds of more sublingual microcirculatory videos. We can then combine these one-dimensional features to high-dimensional features. A classifier can be designed based on these high-dimensional features to classify pathological conditions of each subject.
Bibliography


