Analysis Of Microcirculation Videos Based On Adaptive Thresholding Technique
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Abstract: The study of microcirculation is a key factor in the analysis of blood circulatory system. The blood flow distribution changes, based on the physiological effects of disorders. This study presents a method for analysis of microcirculation. The technique applies advanced image processing methods to the images. micro vessels (capillaries and small blood vessels), and estimate the average Functional Capillary Density on 20 consecutive frames for each subject. The algorithm consists of four main parts: pre-processing, averaging, adaptive local thresholding segmentation and calculation of parameters. The key objective is to analyse the malfunctioning of the microcirculation in various organs. The designed system will help physicians and medical researchers in diagnostic and decision making to determine the functionality of organ sufficiency of resuscitation process and the effect of drug consumption in patients. Calculation of two parameters capillary flow density , functional capillary density and mass flow index allows analysing severity of malfunctioning.

Keywords: Capillary flow density , functional capillary density, Image processing microcirculation , Thresholding

I. INTRODUCTION
The microcirculation is the part of the circulation where nutrients, water, gases, hormones, and waste products are exchanged between the blood and cells. The microcirculation minimizes diffusion distances, facilitating exchange, its most important function. Virtually every cell in the body is in close contact with a micro vessel. In fact, most cells are in direct contact with at least one micro vessel. As a consequence, there are tens of thousands of micro vessels per gram of tissue. The lens and cornea are exceptions because their nutrients are supplied by the fluids in the eye. A second major function of the microcirculation is to regulate vascular resistance and thereby interact with cardiac output to maintain the arterial blood [1,2,9]. Normally, all micro vessels, other than capillaries, are partially constricted by contraction of their vascular smooth muscle cells. If all micro vessels were to dilate fully because of relaxation of their smooth muscle cells, the arterial blood pressure would plummet. Cerebral blood flow in a standing individual would be inadequate, resulting in fainting, or syncope. Regulation of vascular resistance in the microcirculation is an important aspect of total health. There is a constant conflict between the regulation of vascular resistance to preserve the arterial pressure and simultaneously to allow each tissue to receive sufficient blood flow to sustain its metabolism. The compromise is to preserve the mean arterial pressure by increasing arterial resistance at the expense of reduced blood flow to most organs other than the heart and brain.

The organs survive this conflict by increasing their extraction of oxygen and nutrients from blood in the micro vessels as the blood flow is decreased. The microvasculature is considered to begin where the smallest arteries enter the organs and to end where the smallest veins, the venules, exit the organs. In between are microscopic arteries, the arterioles, and the capillaries. Depending on an animal’s size, the largest arterioles have an inner diameter of 100 to 400 µm, and the largest venules have a diameter of 200 to 800 µm. The arterioles divide into progressively smaller vessels to the extent that each section of the tissue has its own specific micro vessels [2,4,5].

Evidence suggests that information regarding the status of microcirculation plays a crucial role in treatment and diagnosis of several diseases such as sepsis, sickle cell, chronic ulcers, diabetes mellitus and hypertension. Research and clinical experience show that each of the mentioned diseases uniquely affects characteristics of microcirculation such as structure of capillaries and features of blood flow. Hence, investigation of microcirculatory changes has clinical significance in measurement and observation of the changes in response to treatment of micro vessels under clinical conditions. Timely detection of such changes potentially helps in taking proper actions which in turn improves the chances of treatment success. A technique to quantitatively assess and monitor these alterations is extremely valuable for further study of such pathological conditions [9-12]. Particularly, in trauma care, continuous monitoring of microcirculation and measurement of microcirculation indices while resuscitation process helps in determining when to start/stop resuscitation. The recent development of video microscopy technology has provided effective tools for detection and assessment of tissue perfusion and oxygenation through visualization of microvasculature. Quantitative analysis of microcirculation allows monitoring changes in micro vessels that occur due to diseases and other abnormalities. Both visual analysis and use of existing semi-automated video analysis tools are time-consuming and demanding, preventing real-time assessment of microcirculation [2,3,10,15].

II. METHODOLOGY
The images used for microcirculation analysis are SDF or OPS images. Side stream Dark Field (SDF) imaging, a stroboscopic LED ring based imaging modality, is introduced for clinical observation of the microcirculation. SDF imaging is validated by comparison to Orthogonal Polarization Spectral imaging. Nail fold capillary diameters and red blood cell velocities were measured using both techniques and equal quantitative results were obtained. An image quality system was developed to quantitatively compare the quality of sublingually-acquired microcirculatory images using OPS and SDF imaging. Venular contrast, sharpness, and quality were shown to be comparable for OPS and SDF imaging. However, capillary contrast and quality were shown to be...
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significantly higher using SDF imaging. Venular granularity, in addition was more clearly observable using SDF imaging.[10]

In OPS imaging, the tissue embedding the microcirculation is illuminated with polarized green light. Backscattered (and thus depolarized) light is projected onto a CCD camera after it passes an analyzer, i.e., a polarizer orthogonally-oriented with respect to the incident polarization. The light reflected by the tissue surface, which is depolarized, is blocked by this analyzer. By elimination of the reflected light and imaging of only the backscattered light, subsurface structures, such as the microcirculation, can be observed. The use of green light ensures sufficient optical absorption by the deoxyhemoglobin-containing red blood cells (RBCs) with respect to the lack of absorption by the tissue embedding the microcirculation, creating contrast (i.e., RBCs are visualized black and tissue is visualized white/greyish).[3,10,13,15]. Different steps of the algorithm are provided in Figure 1.

A. Pre-processing:
Pre-processing of microcirculatory images is essential considering the low local contrast of microcirculation images. Pre-processing usually comprise a series of operations to improve the quality of images in order to maximize the difference between image background and objects of interests. In microcirculation images, the intensity of capillaries and small blood vessels are exceptionally close to that of background and tissues. In order to process the images, the first main step is pre-processing.[6,9,11]

As the first step, adaptive histogram equalization is applied to the images to help enhance low local contrast of the images. The histogram of an image is a representation for the number of different pixel values in the image. Microcirculation images comprise of a narrow range of intensities. In adaptive histogram equalization, the histogram for various parts of the image is generated and interpolated. Bilinear interpolation eliminates the visibility of the boundary lines that were produced by local histograms. The result of adaptive histogram equalization is a modified image whose histogram is different from that of the original image.

To further reduce the effects of background noise, wavelet transformation is incorporated in this step. Wavelet transformation decomposes the image into its different frequency contents. Usually, high frequencies represent noise and low frequencies represent details in an image. The image is transformed to wavelet domain and decomposed with mother wavelet of aubechies 8, level 2. Following that, high frequencies present within the image are filtered. Then the image is reconstructed in the original domain. The noise in the resulting image is much lesser than the input image[7-9,11].

A microcirculation frame usually contains blood vessel in different levels of proximity to the surface of tongue. Representing images in multiple spatial frequency domains emphasizes the patterns of blood vessels in different scales that normally cannot be seen in the image. The significance of analyzing images at various resolutions is that the objects of different sizes are more visible in different resolution levels. Experiment shows that level 2 and level 3 of Gaussian pyramid separate blood vessels of different sizes, thus making the segmentation a more accurate process.

Matched filter is applied to the image to extract features. In this step, to enhance the edges of blood vessels, matched filter is applied to the image. Matched filter approximates the intensity profile of the image with Gaussian curves[9,13,15].

B. Image Averaging:

Since video contains more information compared to image. Despite the advantages of capturing videos for the study of microcirculation, the motion artefact due to the movement of the handheld camera and that of the subject are obstacles for effective analysis of microcirculation videos. To eliminate the effects of motion artefacts, video stabilization is performed at this step. The main aim of this step is to calculate the transformation between two consecutive frames in the sequence[8,9].

C. Segmentation:

Image segmentation is the process of partitioning a digital image into multiple segments. The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze[1] Image segmentation is typically used to locate objects and boundaries (lines, curves, etc.) in images. More precisely, image segmentation is the process of assigning a label to every pixel in an image such that pixels with the same label share certain visual characteristics[7,8].

Image segmentation is achieved through thresholding. The main classes of thresholding were mentioned in the introduction part. Depending on the application, one may use global thresholding or local thresholding. Uneven gray scale intensity of the background and the varieties in the intensity of the objects in microcirculation images make the global thresholding inefficient. However, local thresholding smoothly varies across the image and is capable to adapt the threshold value for different parts of the image. This study adaptively selects windows in the image based on Lorentz Information Measure (LIM). Following that, for the thresholding of each sub-image, the algorithm implements an extension of the entropic thresholding technique[6,8,9,14].

D. Calculation of Parameters:

Functional Capillary Density (FCD):
Functional capillary density (FCD) is one of the parameters obtained by intravital microscopy using epi-illumination of the tissue surface or trans-illumination of thin tissue layers. FCD, defined as the length of red cell-perfused capillaries per observation area (cm-1), has been used.
as an indicator of the quality of tissue perfusion in various animal models. Quantitative analysis of FCD in randomly selected regions of the tissue is performed by means of a computer-assisted video analysis system which allows calculation of the length of RBC-perfused capillaries. Basically, two different mathematical approaches can be employed: the first approach is based on the addition of the distances between two neighbouring points (pixels) on the video screen (Pythagorean principle). The second approach uses the superimposition of a grid system that allows estimation of the capillary length by counting the number of intersections between the capillaries and the grid lines (stereological approach). The immanent error has been calculated in our laboratory to be +/- 1% with the Pythagorean and +/- 5% with the stereological method. Beside these systematic errors of computerized measurement, the individual (user-dependent) errors occurring during recognition and redrawing of the capillaries on the video image with use of a digitizing tablet are in the range of +/- 10% (intra individual) and +/- 70% (inter individual) for the recognition and +/- 3% (inter individual) for the redrawing procedure. Our studies indicate that the errors resulting from the use of a computer-assisted calculation (Pythagorean or stereological approach) or the user-assisted redrawing of the capillaries are negligible when compared to the errors made during recognition of the capillaries on the video screen[2,8,9].

Vessel density is calculated as the number of vessels crossing the lines divided by the total length of the lines. Perfusion is then categorized by eye as present (continuous flow for at least 20 s), absent (no flow for at least 20 s) or intermittent (at least 50% of time with no flow). The proportion of perfused vessels (PPV [%]) and perfused vessel density (PVD) are then calculated. A 20 μm cut-off is used to separate small vessels (mostly capillaries) from large vessels (mostly venules).

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As shown in fig 2

![Fig 2 perfused vessel density (PVD)](image)

The micro vascular flow index (MFI) is commonly used to semi quantitatively characterize the velocity of microcirculatory perfusion as absent (0), intermittent (1), sluggish (2), or normal (3). There are three approaches to compute MFI: (1) the average of the predominant flow in each of the four quadrants (MFI(by quadrants)), (2) the direct assessment during the bedside video acquisition (MFI(point of care)), and (3) the mean value of the MFIs determined in each individual vessel (MFI(vessel by vessel))

The second score is the micro vascular flow index (MFI) score. This score is based on determination of the predominant type of flow in four quadrants . Flow is characterized as absent (0), intermittent (1), sluggish (2), or normal (3). The values of the four quadrants are averaged. The main advantage of this score is that it is relatively easy to measure[2,6,9]

![Fig 3 micro vascular flow index (MFI)](image)

**III. RESULTS**

The effectiveness of proposed algorithm can be verified by considering images of healthy and homomorph object. The width of capillaries is selected based on size of kernel which determines size of filter.

**IV. CONCLUSION**

Proposed method is fully automated process which will analyse the microcirculation videos. This can be used for detection of sepsis, arteriosclerosis and malfunction of the organs. The technique is capable of distinguishing between the healthy and haemorrhaged subjects.

**V. FUTURE WORK**

As future work, one can use multi-resolution concept with multi thresholding to improve the segmentation results. Other diagnostically useful measures such as, Proportion of Perfused vessels (PPV) and Micro vascular Flow Index (MFI) will be calculated using the proposed algorithm. A larger dataset will be acquired and the algorithm will be tested and validated on the new dataset. It can also be developed for early detection of other diseases like sepsis, Diabetes, and flow of various blood components depending on size.
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