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A Survey on a Novel Entropic Thresholding Technique for Diagnostic Analysis of Microcirculatory Image

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Abstract: ***Understanding the functionality of microcirculation is a input feature in the analysis of blood circulatory system. The blood pour supply changes, based on the physiological things of disorders. This study present a method for analysis of microcirculation videos captured from lingual surface of 10 animal subjects. The practice applies sophisticated image processing methods to lessen videos, segment 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: preprocessing, entropic-based adaptive local thresholding segmentation and post-processing. The key objective is to quantitatively examine the changes that occur in microcirculation over handling periods for diseases as well as for the recovery process. The intended system will help physicians and medical researchers in diagnostic and therapeutic decision making to determine the sufficiency of recovery process and the effect of drug eating in patients. In particular, the system focuses on minimizing user dealings while improving the accuracy of the analysis. Visual evaluation of the results by medical experts indicates that the technique is capable of identifying 95% of active capillaries and blood vessels in videos.***

Keywords: Microcirculation, Image processing, multi-resolution, entropic thresholding, Adaptive local thresholding.

I. INTRODUCTION

1.1 Definition of the Microcirculation

The microcirculation is essential to many functions of the organism. In addition to delivering nutrients and removing waste products essential for moment to moment function, the microcirculation plays an essential role in fluid exchange between blood and tissue, delivery of hormones from endocrine glands to target organs, bulk delivery between organs for storage or synthesis and providing a line of defense against pathogens. To execute these functions satisfactorily, certain features are necessary in the microcirculation. In the description that follows we provide an overview of these features, based in large part on skeletal muscle, which constitutes 50% of body mass and has perhaps the largest capability of any organ for altering blood flow according to need. On a practical level, it is also more accessible for microcirculatory studies than most other organs.

As a first approximation, the microcirculation consists of those blood vessels too small to be seen with the naked eye. This limitation of visual acuity required Harvey in 1628 to postulate the existence of invisible ―pores of the flesh‖ to support his hypothesis that blood passes through microscopic channels in circulating from artery to vein. However, Harvey‘s critics suggested that such porosities did not exist but rather that blood moved through the tissue by a general seepage. Development of the first single lens microscope enabled Malpighi in 1661 to observe discrete capillaries connecting arteries and veins in the tortoise lung. Van Leeuwenhoek in 1674 was able to provide quantitative information on the size and spatial density of microcirculatory vessels in the tail fin of the eel as well as measure the velocity of red cells in these vessels [4].Studies of the

regulation of blood flow by the microcirculatory vessels have also benefited considerably from determination of flow and vascular resistance in individual organs and in the whole organism.

1.2 Function of Microcirculation

While the heart provides the potential energy that drives the blood through the peripheral circulation, blood flow in individual organs is determined by primarily by changes in the luminal diameter of the arterioles. Firm establishment of the contribution of the arterioles to flow regulation was hampered for many years by technical limitations that precluded direct measurements of pressure and flow in these vessels. For example in skeletal muscle the arterioles are responsible for 50–60% of the total pressure drop, and therefore of resistance, in the vascular bed [4].While considering the blood circulation of the system Microcirculation is a key factor. With the microcirculation the distribution of blood to every small and big vessel throughout the body is taken place. And this distribution is based on physiological effects of disorders. Information about healthy distribution and circulation of blood in capillaries and small blood vessels is always a key feature of human physiological health. Knowledge about microcirculation has shown potential value in treatment and diagnosis of diseases such as sepsis, sickle cell, chronic ulcers, diabetes mellitus, and hypertension. Currently with the available system we can just analyze structure of capillaries and quality of blood flow in the capillaries. And for this also we have to work on large part of the body instead of only defective part. So here with the proposed system by introducing an adoptive entropic thresholding technique we can not only just analyze but also automatically extracts capillaries and small blood vessels. And for this we also used two parameters they are Functional Capillary Density (FCD) and Perfuse Vessel Density (PVD).

Thresholding is one of the most common approaches to image segmentation. It often provides sufficient accuracy and high processing speed. A problem to be solved in a specific application is automated threshold selection. Generally speaking, we can make a choice between algorithms that find the threshold globally (i.e., for the whole image) and those that find it locally (i.e., for each pixel separately).

The output of the thresholding operation is a binary image whose gray level of 0 (black) will indicate a pixel belonging to a print, legend, drawing, or target and a gray level of 1 (white) will indicate the background. Depending on the application, the foreground can be represented by gray-level 0, that is, black as for text, and the background by the highest luminance for document paper that is 255 in 8-bit images or conversely the foreground by white and the background by black. Various factors, such as no stationary and correlated noise, ambient illumination, busyness of gray levels within the object and its background, inadequate contrast, and object size not commensurate with the scene, complicate the thresholding operation. There are numerous applications where we can used this proposed system like for chronic analysis, analysis of tumor stages, analysis of lymph vessel for effect of drugs in Sevier diabetes, detection of Varicose veins and Spider veins, early detection of ulcer, analysis of hydrostatic pressure on walls of blood vessels and many more.

1.3 Why Vessels Detection Is Important?

Vascular-related diseases are among the most important public health problems nowadays. Heart and cerebrovascular diseases are respectively the first and third cause of death in 2006 in the U.S.A. Malignant tumors are the second cause of death, and their growth is directly associated with vessel recruitment and angiogenesis .Besides, vascular diseases are one of the principal causes of death and disability in people with diabetes . These facts are justify the research efforts providing a better understanding of the structure of the vascular system and related processes and diseases, and leading to any improvement of diagnostic and intervention procedures.

The vessel structure of the blood circulatory system is one of the most complex structures of the body. Blood vessel anatomy has been studied from castings and in vivo examinations in order to build models that provide valuable insight into the normal and variant circulatory anatomy, helping to understand the causes, evolution and outcome of several vascular-related

diseases. However, many answers to simple questions about vascular morphology and angiogenesis remain open Recent advances in medical imaging technology provide high resolution images of the vessel structures, so that the generation of accurate patient-specific geometric in-vivo vessel models and related quantitative measurements has become feasible.

II. Related Work

2.1 Microcirculatory Techniques

2.1.1 Imaging Methods

Studies of the microcirculation have employed primarily bright field and fluorescence microscopy and formation of an image of a small portion of the field or of an individual vessel.

2.1.2 Bright Field Microscopy

Bright field microscopy is most often used in microcirculatory studies as it provides a convenient and relatively inexpensive means of obtaining information on vessel dimensions, movement of formed elements and, with addition of specialized sensors, spectrophotometric variables. For vessels near the surface, an advantage of bright field over fluorescence microscopy is that some of the details of the vascular wall structure may be more visible.

2.1.3 Fluorescence Microscopy

Fluorescence microscopy is also used to study several aspects of microcirculatory structure and function. To examine the micro vascular network, a fluorescent dye attached to albumin or other macromolecule such as Dextran 500 is injected into the circulation to delineate the lumen of the micro vessels. A fluorescent marker has the advantage that generally the vessels in which the overlying tissue does not fully absorb the fluorescence will be seen whereas with bright field illumination the overlying tissue more readily obscures the view. Sahoo ET al.22 surveyed nine thresholding algorithms and illustrated comparatively their performance. Glasbey23 pointed out the relationships and performance differences between 11 histogram-based algorithms based on an extensive statistical study. This survey and evaluation, on the one hand, represents a timely effort, in that about 60% of the methods discussed and referenced are dating after the last surveys in this area.19.In many applications of image processing, the gray levels of pixels belonging to the object are substantially different from the gray levels of the pixels belonging to the background. Thresholding then becomes a simple but effective tool to separate objects from the background. Examples of thresholding applications are document image analysis, where the goal is to extract printed characters, logos, graphical content, or musical scores: map processing, where lines, legends, and characters are to be found: scene processing, where a target is to be detected: and quality inspection of materials, where defective parts must be delineated [10].

The output of the thresholding operation is a binary image whose one state will indicate the foreground objects, that is, printed text, a legend, a target, defective part of a material, etc., while the complementary state will correspond to the background. Depending on the application, the foreground can be represented by gray-level 0, that is, black as for text, and the background by the highest luminance for document paper that is 255 in 8-bit images or conversely the foreground by white and the background by black. Various factors, such as nonstationary and correlated noise, ambient illumination, busyness of gray levels within the object and its background, inadequate contrast, and object size not commensurate with the scene, complicate the thresholding operation.

T. S. Koh*, L. H. Cheong, Z. Hou, and Y. C. Soh present a multiple compartment, mammillary distributed-parameter model for capillary–tissue exchange, which can be implemented with dynamic contrast-enhanced imaging to study kinetic heterogeneity in tumors. The proposed –compartment model consists of a vascular distributed-parameter compartment in direct exchange with a number (1) of interstitial compartments. It is applied to a prostate tumor case study to illustrate the possible co-existence of two kinetically distinct compartments in the tumor, and the estimation of useful physiological parameters (such as

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perfusion, mean transit time, fractional volumes, and transfer and rate constants) associated with tissue microcirculation. The present model exhibits the convenient property of a separable impulse residue response function in time domain, which can be used to provide further insights and understanding on the physiological basis of tissue enhancement parameters commonly used for correlation studies with tumor histological diagnosis[12].

Chih-Kuang Yeh, Sheng-Yi Lu, and Yung-Sheng Chen presents in Microcirculation volumetric Flow Assessment Using High-Resolution, Contrast-Assisted Images a method to improve the resolution of contrast-assisted imaging systems, we previously developed a 25-MHz micro bubbles-destruction/replenishment imaging system with a spatial resolution of 160*160m. The goal of the present study was to propose a new approach for functionally evaluating the micro vascular volumetric blood flow based on this high-frequency, ultrasound imaging system. The approach includes locating the perfusion area and estimating the blood flow velocity therein. Because the correlation changes between before and after micro bubble destruction in two adjacent images, a correlated-based approach was introduced to detect the blood perfusion area. We also have derived a new sigmoid-based model for characterizing the micro bubbles replenishment process. Two parameters derived from the sigmoid-based model—the rate constant and inflection time—were adopted to evaluate the blood flow velocity. The results indicate that the actual flow velocity is highly correlated with the estimates of the rate constant and the reciprocal of the inflection time. B-mode imaging experiments for mean flow velocities ranging from 0.4 to 2.1 mm/s were used to assess the volumetric flow in the microcirculation. The results indicated the high correlation between the actual volumetric flow rate and the product of the estimated perfusion area and rate constant, and the reciprocal of the inflection time. The perfusion area can be located, and the corresponding flow velocity can be estimated simultaneously in a one-stage, micro bubble destruction/ replenishment process, which makes the assessment of the volumetric blood flow in the microcirculation feasible using a real-time, high-frequency ultrasound system[13].

Eran Eden*, Dan Waisman, Michael Rudzsky*, Haim Bitterman, Vera Brod, and Ehud Rivlin in an Automated Method for Analysis of Flow Characteristics of Circulating Particles From In vivo Video Microscopy presents the behavior of white and red blood cells, platelets, and circulating injected particles is one of the most studied areas of physiology. Most methods used to analyze the circulatory patterns of cells are time consuming. We describe a system named Cell Track, designed for fully automated tracking of circulating cells and micro-particles and retrieval of their behavioral characteristics. The task of automated blood cell tracking in vessels from in vivo video is particularly challenging because of the blood cells‘ no rigid shapes, the instability inherent in vivo videos, the abundance of moving objects and their frequent superposition. To tackle this, the Cell Track system operates on two levels: first, a global processing module extracts vessel borders and center lines based on color and temporal patterns. This enables the computation of the approximate direction of the blood flow in each vessel. Second, a local processing module extracts the locations and velocities of circulating cells. This is performed by artificial neural network classifiers that are designed to detect specific types of blood cells and micro-particles. The motion correspondence problem is then resolved by a novel algorithm that incorporates both the local and the global information. The system has been tested on a series of in vivo color video recordings of rat mesentery. Our results show that the synergy between the global and local information enables Cell Track to overcome many of the difficulties inherent in tracking methods that rely solely on local information. A comparison was made between manual measurements and the automatically extracted measurements of leukocytes and fluorescent microspheres circulatory velocities [14].

III. PROPOSED ALGORITHM

The proposed methodology is an extension of Key modifications was applied to improve the segmentation part. Furthermore, the algorithm was examined on more data samples to evaluate the capabilities of the technique in this project. The microcirculation images used to validate the results of the proposed research study are based on SDF imaging technique, captured by Micro Scan hardware Capillaries become visible in the presence of Red Blood Cells (RBCs).A major challenge in

the image processing of microcirculation images are their low resolution and local contrast that complicates the distinction between objects of interest (capillaries and small blood vessels) and frame background. An instance of an original microcirculation frame is provided. Another challenge is the inconsistency of the gray level intensity in background and blood vessels from one sample to another. The effect of uneven lighting due to the movement of camera and/or subjects results indifferent levels of intensity in different frames. These challenges can be overcome with the help of various image processing techniques like pre-processing, segmentation and post processing.

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 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).Different steps of the algorithm are provided in Figure 1 [3].

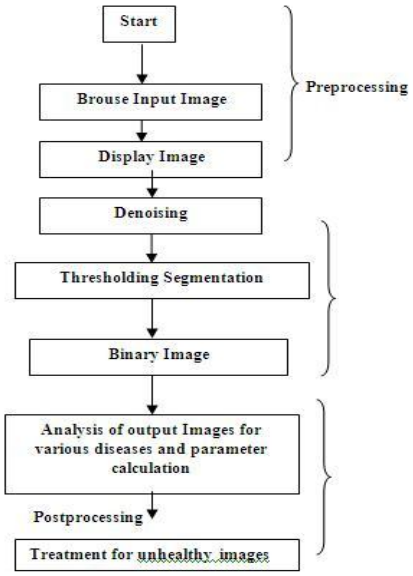

Fig 1 Flow Diagram of Algorithm [1, 2, 15]

3.1 Pre-Processing

Analysis of Output image for various diseases and parameter calculation. 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 [1]. 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

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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 [3, 5, and 10]. 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.

- **Denoising**

Removal of noise is an important step in the image restoration process. Denoising is used to remove the noise from corrupted image, while retaining the edges and other detailed features as much as possible. This noise gets introduced during acquisition, transmission & reception and storage & retrieval processes. Medical images are analyzed for the diagnosis of various diseases like cancer, tumor and fracture etc. But, they are susceptible to different types of noises called as Gaussian noise, Speckle noise, Uniform noise, Impulse noise, etc. Therefore it is an important task to remove the noise from medical images especially in Microcirculation, MRI, CT, PET, SPECT, Digital Mammogram and Ultrasound images. Selection of appropriate filter is a tough task. We propose a technique that uses Wavelet Transform and Curvelet Transform for denoising the medical images based on the Histogram equalization.

3.2 Segmentation with Thresholding

Image segmentation is performed to partition image into its comprising components. The objective of image segmentation is to separate background from blood vessels and capillaries using greyscale values. Such separations make the analysis of the image an easier task. The outcome of segmentation is a binary image whose background is shown with white pixels and objects of interest with black pixels. One main class of techniques that is incorporated for image segmentation is 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 greyscale 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. Following are the different stages which come under the adoptive entropic thresholding.

Modeling of vessels

Detection of Vessels

Segmentation

3.2.1 What Is Thresholding?

In image processing, thresholding is used to split an image into smaller segments, or junks, using at least one color or gray scale value to define their boundary. Thresholding is used to segment an image by setting all pixels whose intensity values are above a threshold to a foreground value and all the remaining pixels to a background value. Segmentation refers to the operation of partitioning an image into component parts, or into separate objects. Thresholding method is a simple concept for image segmentation that yields all the pixels and assumes the algorithm in two cases; darkness and brightness. The output might be two label-object || or-background || which due to its dichotomous nature, can be represented as a Boolean variable-1|| or-0||.

The output from the test condition could be based by some other properties, rather than simple brightness. Edge finding method is a technique that finds pixel belongs to the border of the object of closed contours around the objects of interest. It can be generated in the Laplacian of an image like the appropriate smoothing filter which uses both properties in the frequency

domain and the spatial domain. In this project segmentation algorithm using maximum entropy-based thresholding approach is introduced. Thresholding is a vital part of image segmentation, where we wish to isolate objects from the background. It is also an important component of robot vision. Thresholding can be done very simply in Matlab.

3.2.2 Thresholding Types

Thresholding is the simplest method of image segmentation. From a gray scale image, thresholding can be used to create binary images. Sezgin and Sankur (2004) categorize thresholding methods into the following six groups based on the information the algorithm manipulates (Sezgin et al., 2004):

- Histogram shape-based methods, where, for example, the peaks, valleys and curvatures of the smoothed histogram are analyzed. This group of thresholding methods is based on the form and shape properties of image histograms. Rosenfeld and de la Torre and Lee et al. used histogram concavity analysis to derive the optimal threshold value for a given image. In their paper, a convex hull of the image histogram is calculated and the deepest concavity points are selected as candidates to be the threshold value. In Seazan, the histogram function is convolved with a smoothing kernel. The gray levels where the peaks start, end and attain maxima are estimated.
- To add some data reduction, the algorithm sets gray-level thresholds between the peaks, and the gray levels at which the peaks attain a maximum are chosen as quantization levels. In Chang et al., a multi-modal histogram thresholding method is proposed based on a combination of regularization and statistical approaches. The original histogram is decomposed in several non-overlapping distributions by modeling it with a mixture of Gaussian density. The only caveat in this paper is the heuristic nature of the smoothness factor, which would generate different thresholding values depending on the particular election.
- There is opportunity for future work regarding smoothness factors for different gray-level images. Ramesh et al. were among the first who used a simple two-step approximation function to the normalized histogram. Thus, the sum of squares between the function and the histogram is minimized and the optimal threshold value can be obtained by performing an iterative search. Prewitt and Mendelsohn, in their analysis of cell images, proposed a method that iteratively smoothed the histogram using a running average of size 3, until there is two local maxima, j and k. The final threshold t is computed as the average, (j + k) =2.
- Clustering-based methods, where the gray-level samples are clustered in two parts as background and foreground (object), or alternately are modeled as a mixture of two Gaussians. These methods rely on generating clusters from gray-level information. Because the end result is a binary image, there are only two classes, or clusters. Each cluster corresponds to a lobe of the histogram. There are several clustering approaches which can be further sub classified as Iterative-based methods, Clustering thresholding, Minimum error thresholding, Fuzzy clustering thresholding.
- Entropy-based methods result in algorithms that use the entropy of the foreground and background regions, the cross-entropy between the original and binarized image, etc. In this category we can enclose methods that use the entropy of the distribution of gray levels in the picture. The entropy is a concept that comes from the second law of Thermodynamics and measures spontaneous dispersal of energy. It was later introduced to communications theory by Shannon as a measure of the efficiency in data transmission over a noisy channel. High entropy is indicative of a great information transfer. The opposite consideration, preservation of information, can be achieved by minimizing cross-entropy between the input, gray level image and the output, threshold image.
- Object Attribute-based methods search a measure of similarity between the gray-level and the binarized images, such as fuzzy shape similarity, edge coincidence, etc. These methods extract a threshold value based on similarity between the original image and the binarized one using some attribute quality or similarity measure. Some of those measures are gray-level moments and fuzzy measures.

- Spatial methods [that] use higher-order probability distribution and/or correlation between pixels.
- Local methods adapt the threshold value on each pixel to the local image characteristics.

3.2.3 How Thresholding Works?

Thresholding is used to segment an image by setting all pixels whose intensity values are above a threshold to a foreground value and all the remaining pixels to a background value. Whereas the conventional thresholding operator uses a global threshold for all pixels, adaptive thresholding changes the threshold dynamically over the image. This more sophisticated version of thresholding can accommodate changing lighting conditions in the image, e.g. those occurring as a result of a strong illumination gradient or shadows. Adaptive thresholding typically takes a greyscale or color image as input and, in the simplest implementation, outputs a binary image representing the segmentation. For each pixel in the image, a threshold has to be calculated. If the pixel value is below the threshold it set to the background value, otherwise it assumes the foreground value. There are two main approaches to finding the threshold: (i) the Chow and Kaneko approach and (ii) local thresholding. The assumption behind both methods is that smaller image regions are more likely to have approximately uniform illumination, thus being more suitable for thresholding. Chow and Kaneko divide an image into an array of overlapping sub images and then find the optimum threshold for each sub image by investigating its histogram. The threshold for each single pixel is found by interpolating the results of the sub images. The drawback of this method is that it is computational expensive and, therefore, is not appropriate for real-time applications. An alternative approach to finding the local threshold is to statistically examine the intensity values of the local neighbourhood of each pixel. The statistic which is most appropriate depends largely on the input image. Simple and fast functions include the mean of the local intensity distribution, T= Mean The median value, T=Median Or the mean of the minimum and maximum values, T= (max + min)/2 The size of the neighbourhood has to be large enough to cover sufficient foreground and background pixels, otherwise a poor threshold is chosen. On the other hand, choosing regions which are too large can violate the assumption of approximately uniform illumination. This method is less computationally intensive than the Chow and Kaneko approach and produces good results for some applications.

3.3 Segmentation

In the last decade many methods were developed to facilitate the segmentation task, over even take over the task completely. Live wire methods for interactive contour delineation, automated identification of the calked plaque in the sclerosis, and segmentation of the thrombus and the inner lumen in aortic aneurisms by one click are just a few examples. However, the segmentation of a dissected aorta including the membrane and the two Lumina has never been tackled before. Different segmentation methods are as follows:

Intensity-based Methods A. Thresholding

Thresholding is one of the simplest techniques and was earlier the basis of manual or semi-automatic segmentations. In this approach, part with an intensity value below or above a dened threshold is regarded as part of the object whereas remaining part is interpreted as background. Ney et al. in [introduced for example an editing tool for 2D medical imaging based on simple Thresholding. Suri et al. in defined a threshold value to extract the vessel structure from MRA dataset. Shiman et al. used multilevel thresholding calculated by a neural network in order to segment aortic aneurysm from CTA images. One serious shortcoming of these approaches is the usage of global thresholding, which performs poorly if the vessel intensity varies significantly throughout the image. Although Fiebich et al. in refined the technique by combining the threshold value with local gradient information, the concept still has limitations, e.g. merging different tissues with similar intensity.

B. Region Growing

Region growing methods rely on the principle of allowing seed points to grow into a region within the image as long as the addition of new points to the region does not violate predefined criteria. Two criteria appear always in the literature, namely intensity similarity and spatial proximity. In other words, if adjacent pixels have the same characteristics and they are close enough to each other, then the pixels belong probably to the same region. The main limitation of region growing methods for vessel segmentation is that they often result in holes or leakage into other tissues due to the variations in image intensity and noise.

C. Watershed Transformation

The watershed transformation introduced by Beucher et al. is derived from mathematical morphology and often used in medical image segmentation. The main idea of the method is to interpret the grayscale image as altitude surface in which high-intensity pixels correspond to ridge points, and low-intensity pixels correspond to valley points. By successive separation of the surface from valley points (local minima), so called watersheds are constructed to avoid merging adjacent catchment basins. If the separation process is performed on the gradient map of the image, the watersheds form along the edges.

- **Vessel Enhancement Methods**

A. Matched Filter

The matched filter principle is a broadly used feature detection approach in image analysis, which has also been used for vessel enhancement by relying on the response to an appropriately constructed filter bank. On the enhanced image Thresholding or connected component analysis is then performed in order to get the final vessel contours.

- **Model-based Methods**

The above discussed approaches use the assumption that segmentation can be performed based on image features such as intensity, gradient or curvature. To arrive at a reasonable image interpretation, a priori knowledge about the object is incorporated into the model-based algorithms.

A. Rigid Templates

Early investigations in model-based analysis focused mainly on rigid templates where the matched shapes were obtained by applying simple transformations (e.g. translation, rotation, scaling, and transformation) to the model template.

B. Free-Form Deformable Models

It is also called as active contours in 2D or active surfaces in 3D). It can be treated as parametric curves that are allowed to move towards desired features (e.g. vessel boundary) under the influence of internal-, image- and external energies. Internal energy enforces the smoothness of the surface while the surface is pulled towards the desired feature by external and image forces.

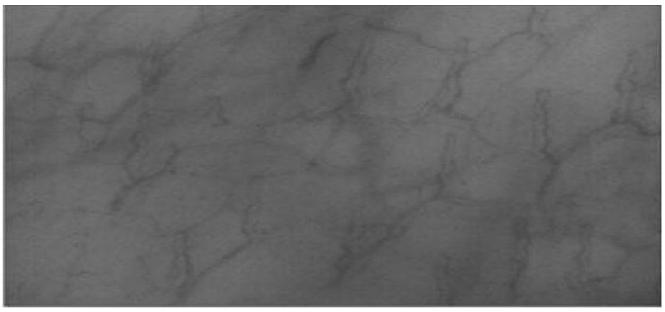

FIG 2: ORIGINAL IMAGE

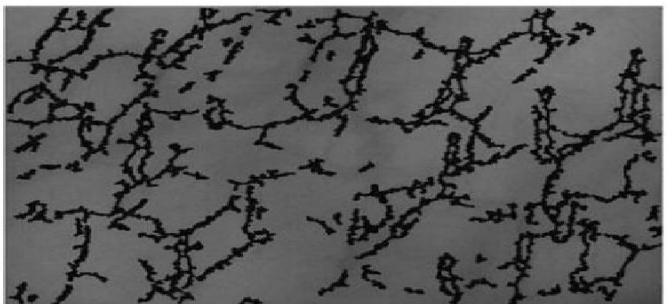

FIG 3: SEGMENTED IMAGE

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3.4 Post-Processing

3.4.1 Parameter Calculation

A. Functional Capillary Density and Perfused Vessel Density

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 image 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 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 image screen. 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 venues). 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).

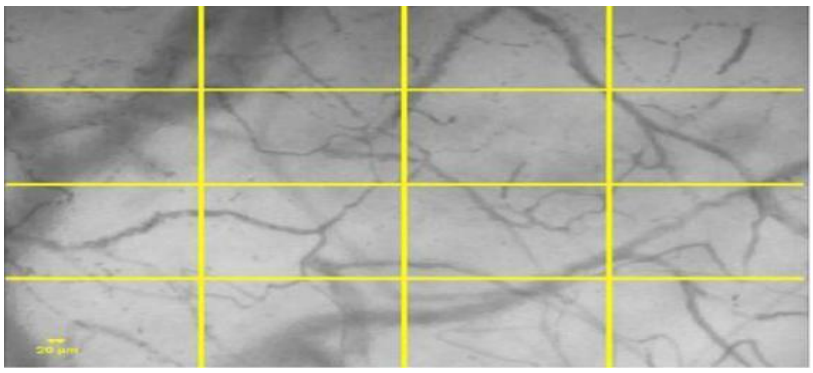

FIG 4. PERFUSED VESSEL DENSITY (PVD)

B. FCD Calculation

Segmentation processes described up until this stage detect all blood vessels at each frame of the video recording. To provide quantitative information on blood flow, the vessels through which blood is flowing must be identified. To that end, the difference of consecutive segmented frames is calculated pixel by pixel. If the summation of difference for twenty segmented

frames is higher than a threshold value, the pixel is assigned as an active blood vessel. FCD is currently one of the main parameters used to evaluate the microcirculation. FCD can be calculated using two different approaches: one is completely manual by gridding the frame and counting the number of vessels crossing the grid lines; the second approach calculates the ratio of perfused vessels to the total surface using a software tool. FCD is calculated automatically in this study by dividing the area of active vessels to the total area of interest. It is much easier to form the skeleton of the network of active capillaries and calculate the length of this skeleton to form the density measure. However, since the width/thickness of capillaries along this network would be inconsistent (on the actual sublingual surface, the captured video, and in the processed image), the density measure calculated on this length would be the least reliable measure, as it does not incorporate the changes in the thickness of the capillary and therefore the true extent of circulation inside the capillary. The area-based density measure, on the other hand, since it incorporates the thickness of the capillaries into the calculation, is not susceptible to this issue. We have also included the length-based FCD calculation in this paper for comparison with the output from AVA.

IV. CONCLUSION

Perfused vessel density gives the total numbers of vessels present in the input image while functional capillary density gives us total number of vessels which are activate or running functionally. This can leads 95% accurate result of gaining unhealthy vessels in microcirculation. In addition to this, system can give the advice to take the treatment according to healthy and unhealthy images.

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