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ORIGINAL ARTICLE

Monitoring tumor progression by mapping skin microcirculation with laser Doppler flowmetry

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Abstract

Laser Doppler flow meter is a safe, useful, and noninvasive tool to monitor microvascular blood flow. It measures the changes in flow either over time or differences in flow over an area of skin or other exposed tissue. The primary function of LDF is to produce blood perfusion output signal that is proportional to the red blood cell perfusion (or flux). A simple analog-based laser Doppler flow meter is used to record the biosignals arising from the microvascular network of a surface lesion in the face of a human being. An innovative technique is developed to overcome one of the major technical limitations that involved removing the continuous artifacts or noise generated by the movement of the cable, the probe as well as the operator or the patient. A color heat map in MATLAB is created to facilitate its easy analysis, interpretation, and understanding of the blood flow changes over a period of time across a cross-sectional area in an image format processed after acquiring the biosignals (flux) using LDF by the clinicians or by the technician who is performing the vascular assessment of the lesion.

Keywords Laser Doppler blood flow meter · MATLAB · Artifacts · Color heat map · Flux

Introduction

Blood flow study is important to understand basic physiological processes and also one of the most difficult to measure accurately [1]. Blood flow and changes in blood volume are usually correlated with concentration of nutrients and gaseous and other components in the blood. The instruments for measuring the flow through blood vessels within the body have to meet certain stringent specifications, e.g., sensitivity and stability requirements depend upon the magnitude of flow, location, and the diameter of the individual vessels [1]. Blood flow measurement is thus a difficult engineering and clinical problem [1]. There are various methods to measure blood blow. We used laser Doppler blood flow meter to measure the capillary blood flow in the superficial lesion area. Laser Doppler is a standard technique for the noninvasive blood flow monitoring and measurement of blood flow in the microcirculation. The strength of the technique is in picking up the changes in the blood flow either over time or differences in flow over an area of skin or other exposed tissue [2]. The principle of this method is to measure the Doppler shift—the frequency change that light undergoes when reflected by moving objects, such as red blood cells. The primary function of laser Doppler flowmetry (LDF) is to produce blood perfusion output signal that is proportional to the red blood cell perfusion (flux). This represents the transport of blood cells through microvasculature and is defined as

Microvascular perfusion red blood cell flux = Number of red blood cells moving in the tissue sampling volume × Mean velocity of these cells.

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Fig. 1 LDF Moor's machine

Microvascular blood perfusion is, therefore, the product of mean blood cell velocity and mean blood cell number concentration present in the small measuring volume of tissue under illumination from the probe. Laser Doppler blood flow meter has a wide range of applications from basic physiology teaching to research studies. Applications are often noninvasive and may involve tests of microvascular function stimulated by changes in posture, vasoactive drug delivery by iontophoresis, heating and pressure, and tracking changes in flow with time or comparing flows from different measurement sites. Routine clinical uses are increasing and take advantage of the wide dynamic range to capture the cardiac cycle, vasomotor effects, or trends over hours, days, or weeks. Assessments include postoperative flap monitoring, prediction of amputation levels, tooth pulp vitality testing, endothelial function/dysfunction, toe pressure assessment, pulse volume recording, skin perfusion pressure, cerebral perfusion following injury, and postocclusive reactive hyperemia [2]. Research applications of LDF include cerebral monitoring (stroke, injury), transplantation surgery (skin grafting, free flaps), vital organ monitoring (organ viability), tumor vascular research (angiogenesis), and peripheral vascular disease (diabetes). The LDF technique

Fig. 2 Laser probe

Fig. 3 A laser operating in a single mood

offers substantial advantages over other methods (e.g., microbeads) in allowing continuous measurement of microvascular blood perfusion. LDF is both highly sensitive and responsive to local blood perfusion and is also versatile and easy to use for continuous monitoring.

A simple laser Doppler flow meter is used to record analog biosignals arising from the microvascular network of a surface lesion in the face of a human being. In this study, some of its main technical limitations as well as the innovative methods we developed to overcome those limitations are discussed. It involved removing the continuous artifacts generated by the movement of the cable as well as the probe. Noise is also caused due to the movement of the operator or the patient. The second part of the study included creating a color heat map in MATLAB to facilitate its easy interpretation in an image format processed after acquiring the biosignals (flux value) using LDF by the clinicians or by the technician who is

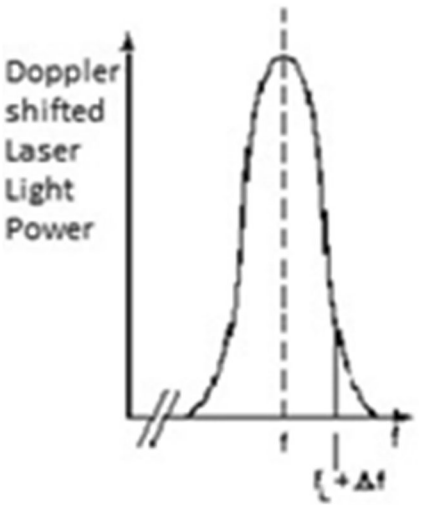


Fig. 4 Principle of LDF machine

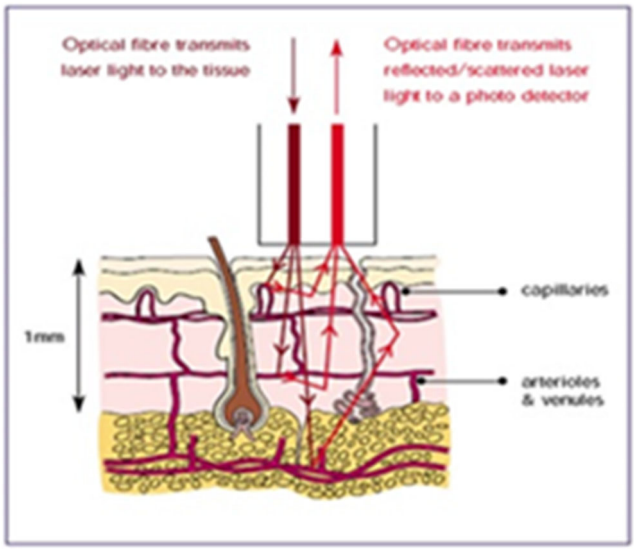


Fig. 5 Δf: Doppler shift frequency for well-perfused region for microcirculation

performing the vascular assessment of the lesion. It helped in better understanding and analysis of the changes in the blood flow either over time or differences in flow over an area of skin which can be stored, analyzed, and compared.

Materials and methods

Instrument and software used

Moor's laser Doppler blood flow meter is used for experiments which is a single-channel equipment working on AC mains, 100–230 V, 50–60 Hz, 30VA having dimensions of 235 × 60 × 200 mm ($W \times H \times D$) shown in Fig. 1 with skin surface laser probe shown in Fig. 2. Moor's laser Doppler blood flow meter has inbuilt moorLab software running on PC for real-time recording of signals and also to export the data to other formats if needed. The signal from Moor's software is exported in MATLAB in excel format. MATLAB R2013a is used for signal and image processing [Figs. 1 and 2].

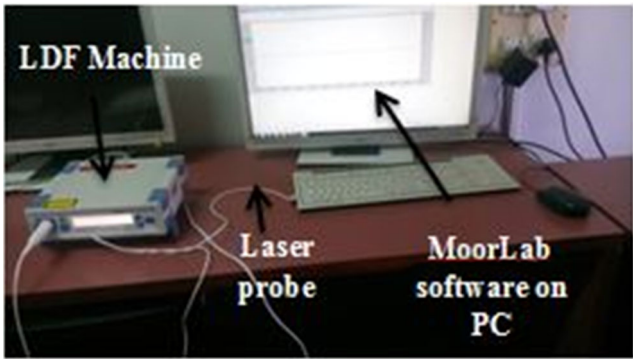


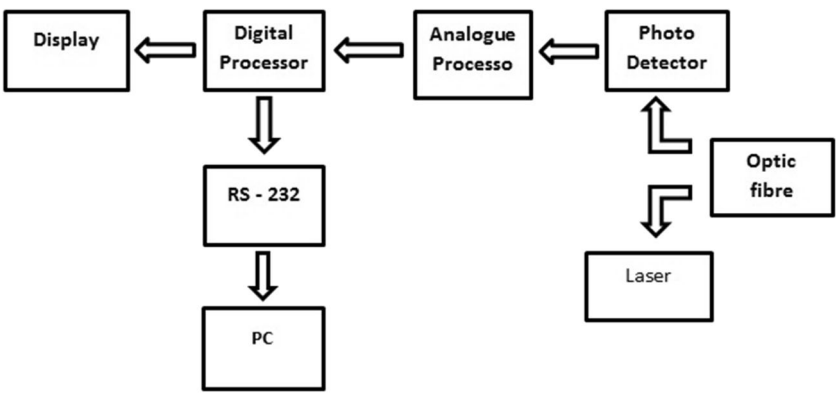
Fig. 7 Initial experimental setup

Principle

The laser Doppler technique measures blood flow in very small blood vessels of the microvasculature, such as the low-speed flows associated with nutritional blood flow in capillaries close to the skin surface and flow in the underlying arterioles and venules involved in the regulation of skin temperature. The tissue thickness sampled is typically 1 mm, the capillary diameters are 10 μm, and the velocity spectrum measurement is typically 0.01 to 10 mm/s.

The technique depends on the Doppler principle whereby low power light from monochromatic stable lasers as shown in Figs. 3 and 4 incident on tissue is scattered by moving red blood cells and, as a consequence, is frequency broadened as shown in Fig. 5.

Fig. 6 LDF working of LDF



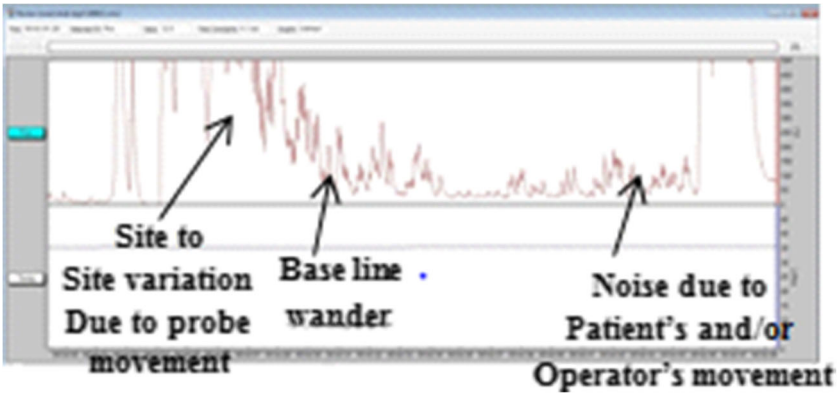


Fig. 8 Artifacts observed with the initial experimental setup

The frequency-broadened light, together with laser light scattered from static tissue, is photodetected, and the resulting photocurrent processed to provide a blood flow measurement. The backscattered light collected by the probe contains a component that has not undergone any frequency shifting and a component that is frequency shifted [3].

The frequency difference of these two light components is detected on the surface of a photodetector by a phenomenon called optical beating. The resulting photocurrent undergoes analog and digital signal processing (DSP) to produce flux and conc parameters relating to the movements and concentration of the blood cells. Laser light is scattered for tissue with a low red blood cell concentration, and the average Doppler frequency shift is proportional to the average speed of red blood cells. Laser light can be directed to the tissue surface via an optic fiber as shown in Fig. 5. The optic fiber terminates in an optic probe which can be attached to the tissue surface. One or more light-collecting fibers also terminate in the probe head, and these fibers transmit a proportion of the scattered light to a photodetector and the signal processing electronics [3].

Construction and working

The moorLAB uses solid-state semiconductor laser diodes (AlGaAs/GaAs) as the laser light source at about 785 ± 10 nm wavelength having max output power of 2.5 mW, emitting diverging beam at 26° from a probe type. Two 200-μm silica glass fibers are used, mounted in a probe head, one to transmit the laser light to illuminate the tissue and the other fiber to collect the reflected light. The separation distance between these two fibers is about 0.5 mm. Surface skin probes are used having adhesive pads for probe holder which are combined optic and temperature probe. The probe head height is 12.5 mm and the probe tip diameter is 8 mm. The reflected light from the optic fiber is then photodetected. The signal from the photodetector is amplified and processed by an analog processor. The signal is then sampled and further processed by a digital processor. The digital processor also

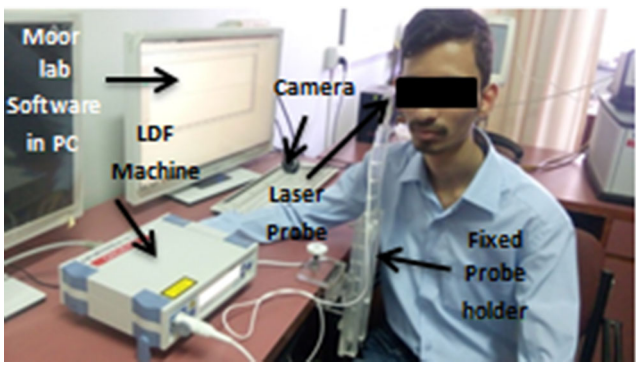


Fig. 9 Modified experimental setup with fixed probe holder

Fig. 10 Modified experimental setup with handy probe holder

Fig. 11 Artifacts observed in the modified experimental setup

performs all the user interface and display functions. The mean blood cell flux (flux), number of concentration of moving blood cells (conc), and mean speed of the blood (speed) of each moorLAB channel are output to a PC in real time via an RS232 serial link [4] as shown in Fig. 6.

Experimental setup

Figure 7 shows the experimental setup. MoorLAB running on a PC is used to collect, store, display, analyze, and convert information (recorded signal) into moorLAB format or text format.

The algorithms used to compute flux cone and speed are as follows:

$$\text{flux} = \frac{\text{k1}\int_{\omega 1}^{\omega 2}\omega P(\omega)\text{d}\omega - (\text{dark} + \text{short noise})}{\text{dc}^2}$$

$$\text{conc} = \frac{\text{k2}\int_{\omega 1}^{\omega 2}\omega P(\omega)\text{d}\omega - (\text{dark} + \text{short noise})}{\text{dc}^2}$$

$$\text{speed} = \text{k3} \times \frac{\text{flux}}{\text{conc}}$$

where ω is the frequency of Doppler shift ($\omega = 2\pi f$, f is the frequency in Hz). $P(\omega)$ is the power of signal at frequency ω. dc is the intensity of all detected light. ω_1 is the low cutoff frequency ($\omega_1 = 2\pi f_1$, f_1 is the frequency in Hz). ω_2 is the low cutoff frequency ($\omega_2 = 2\pi f_2$, f_2 is the frequency in Hz). k1, k2, and k3 are scaling constants.The optical units are arbitrary. The values assigned are determined by standardizing the

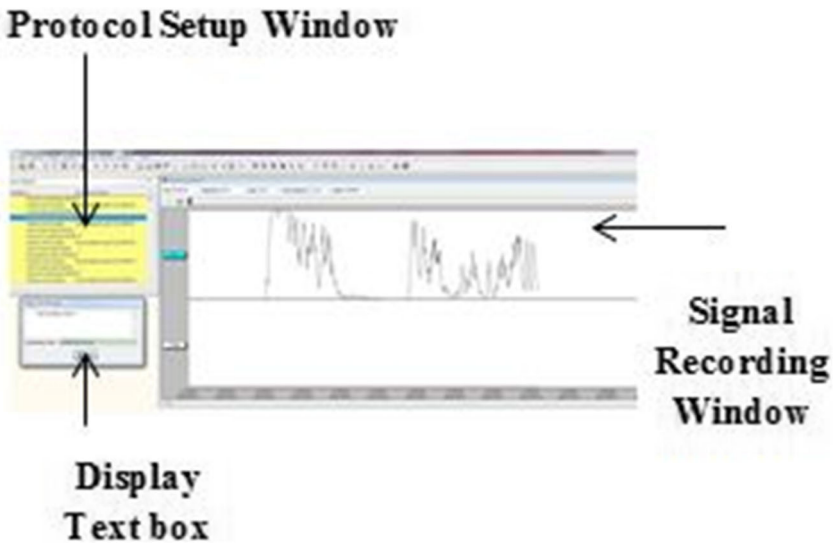


Fig. 12 MoorLAB software protocol setup

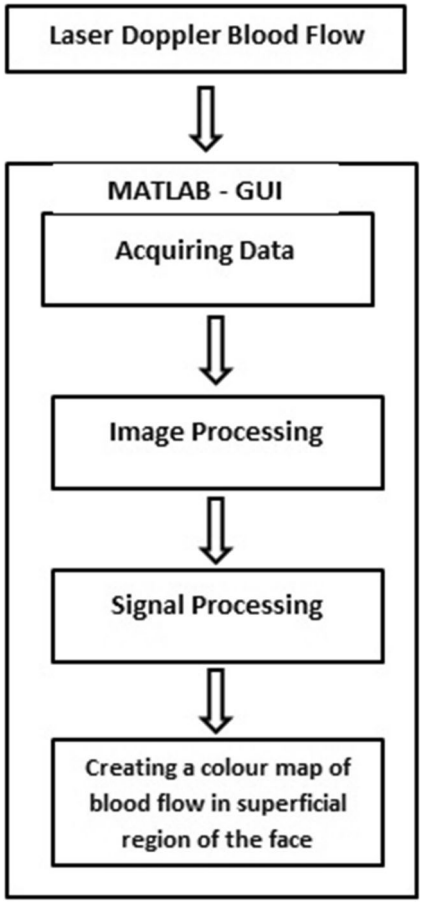


Fig. 13 Signal flowchart from input to output

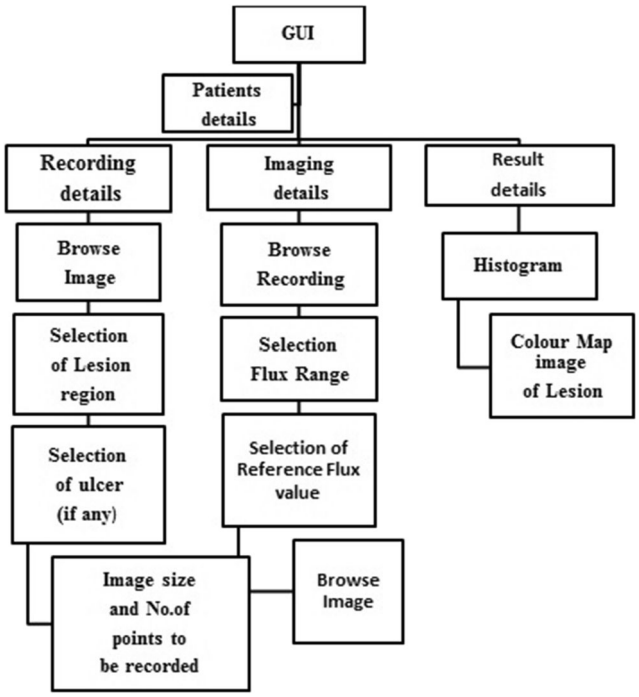


Fig. 14 Block diagram of GUI imaging software

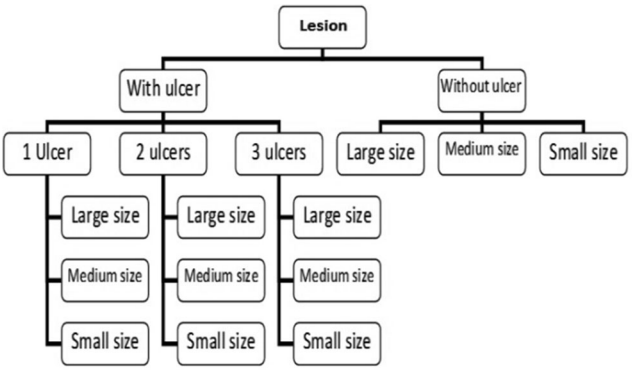


Fig. 15 Classification of lesion for imaging

moorLAB parameter of flux (blood flow), etc., using a physical standard, e.g., the thermal (Brownian) motion of polystyrene microspheres (submicron diameter sphere) in water [5].

It is observed that the signal consists of a lot of noise as shown in Fig. 8.

Modified experimental setup

To minimize the artifacts, the experimental setup was modified. An acrylic fixed probe holder was designed to avoid noise caused due to the operator's movement or cable movement as shown in Fig. 9. The experimental setup was further modified by designing an acrylic handy probe holder as shown in Fig. 10 for proper contact between the probe and patient and also to give the stability to the operator while holding the probe, thus reducing artifacts by minimal cable, operator, and patient movement.

The modified experimental setup reduced noise to a great extent as shown in Fig. 11, but still noise due to site to site variation in probe movement could not be eliminated.

Data analysis

A protocol was designed in moorLAB software [6] which can help to reduce noise due to site to site variation of probe movement by keeping recording of signal OFF for a fixed amount of time (10 s) while shifting from one point of recording to another point of recording in the targeted region. Also, markers were displayed with the display message "recording OFF" and "recording ON" so that the operator can know when to switch from one point to another while taking reading and this will also help to record the signal for the same amount of time for every point. The recording for every point was kept ON for 30 s. The recording of signals kept ON and OFF will continue for 25 points as the largest size consist of 25 points (refer to section "Acquiring Data"). The recording can be stopped at

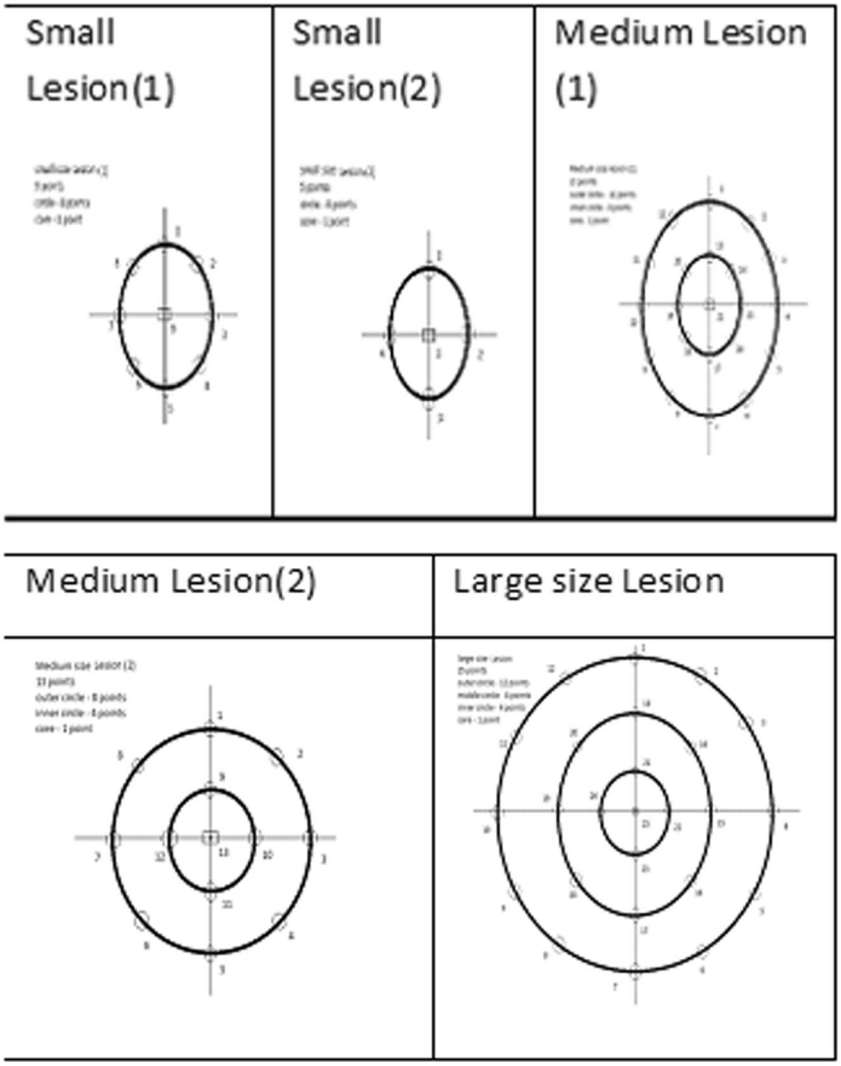


Fig. 16 Protocol to take readings from a lesion depending on different sizes

any point of time using the stop icon in the toolbar for the smaller targeted region. The moorLAB software protocol setup is shown in Fig. 12. The recorded signal from the moorLAB software is then converted into Excel sheet format which is further used in MATLAB GUI.

MATLAB GUI

The name MATLAB stands for matrix laboratory. It is a high-performance language for technical computing [7, 8]. It integrates computation, visualization, and programming in an easy-to-use environment where problems and solutions are expressed in familiar mathematical notation. Typical uses include math and computation; algorithm development, modeling, simulation, and prototyping; data analysis, exploration, and visualization; scientific and engineering graphics; and application development, including graphical user interface building.

MATLAB is used for building graphical user interface for laser Doppler blood flow meter imaging. The signal flowchart from input to output is shown in Fig. 13. A block diagram of GUI imaging software is shown in Fig. 14.

Acquiring data

In order to acquire data from all parts of a lesion of higher vascularity of different shapes and sizes, it was classified into lesion with ulcer and lesion with nonulcer as shown in Fig. 15, and also a protocol was designed to take readings in some standard sequence as shown in Figs. 16 and 17a–d defining the number of points for all sizes of lesion. The signal recorded in Moor's software is exported in Excel and then browsed in MATLAB for signal processing [9].

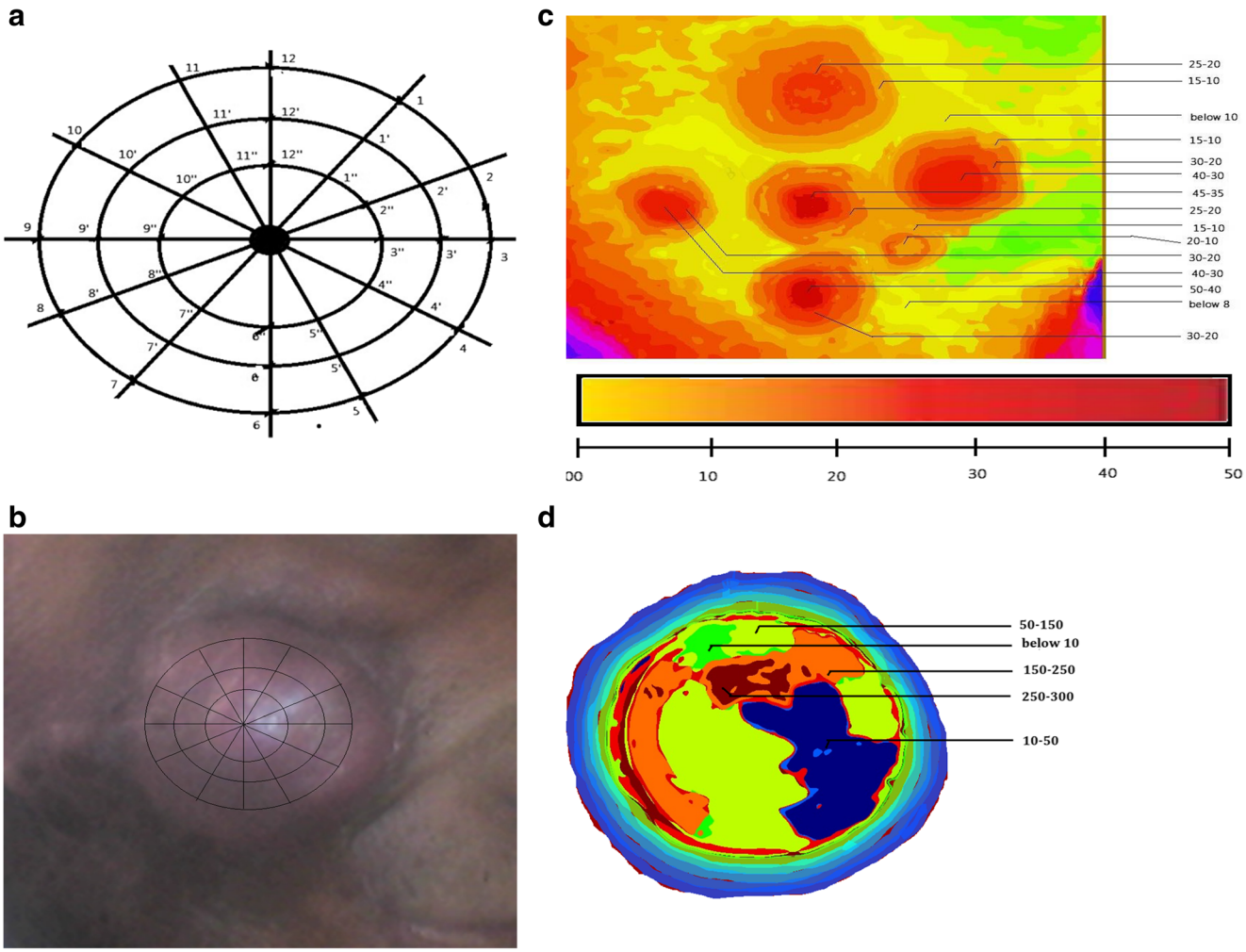


Fig. 17 **a** Points to be marked in a given circle to acquire the data with respect to the size of the lesion and area of interest. **b** Points marked in a given circle to acquire the data from over a site of a tumor with inflammation. **c** Heat map showing the flux intensity vis-à-vis color coding chosen for our analysis. **d** Color heat map showing the flux intensities derived after signal processing of the data acquired from the lesion

Image processing

The image of the lesion region of the subject is taken from an iBall C8.0 Face2Face web camera and then browsed into MATLAB GUI. The size of the image has to be 1450 × 1064 px for GUI to except the image. On the selection of the lesion region, GUI will calculate the area of the lesion in square centimeters and will tell the operator of its size and the number of recording points needed as shown in Fig. 18. For lesion with ulcers, GUI will assign zero flux (NaN) value to the points under ulcer, once the ulcer region is selected by the user.

Signal processing

The Excel sheet of the recorded signal is browsed in MATLAB GUI. Figure 18 shows the original signal from LDF. Depending on the number of points recorded, the entire signal is divided into the same number of wavelets. Each wavelet is filtered by using band pass filter (the higher and lower cutoff range is taken from the user as it is different for every individual) and then smoothened by using Gaussian filter as shown in Fig. 18. The mean of every single wavelet is calculated which would represent the flux value recorded for the respective point as shown in Fig. 18.

Color mapping imaging of the lesion

Taking *x*-*y* coordinates of every point of recording from the image and taking the corresponding flux value obtained from signal processing, surf function is used in MATLAB to build a color map depicting blood flow in the lesion region as shown in Fig. 18 (Appendix).

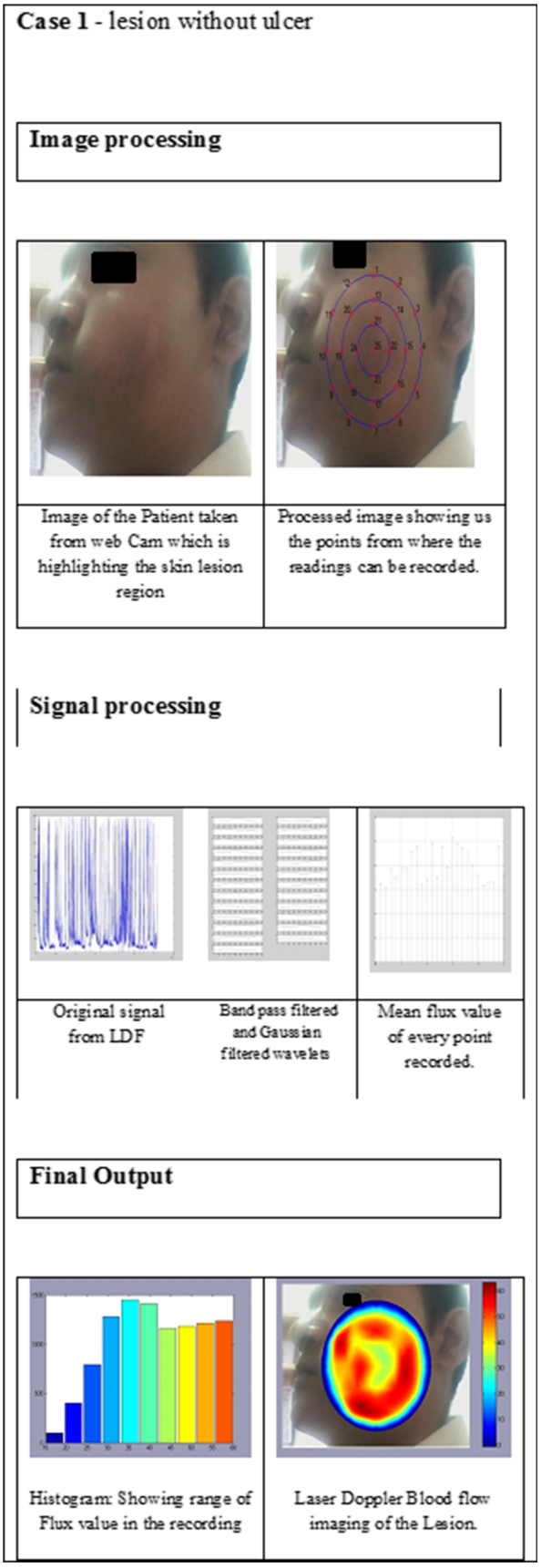


Fig. 18 MATLAB output for lesion without ulcer

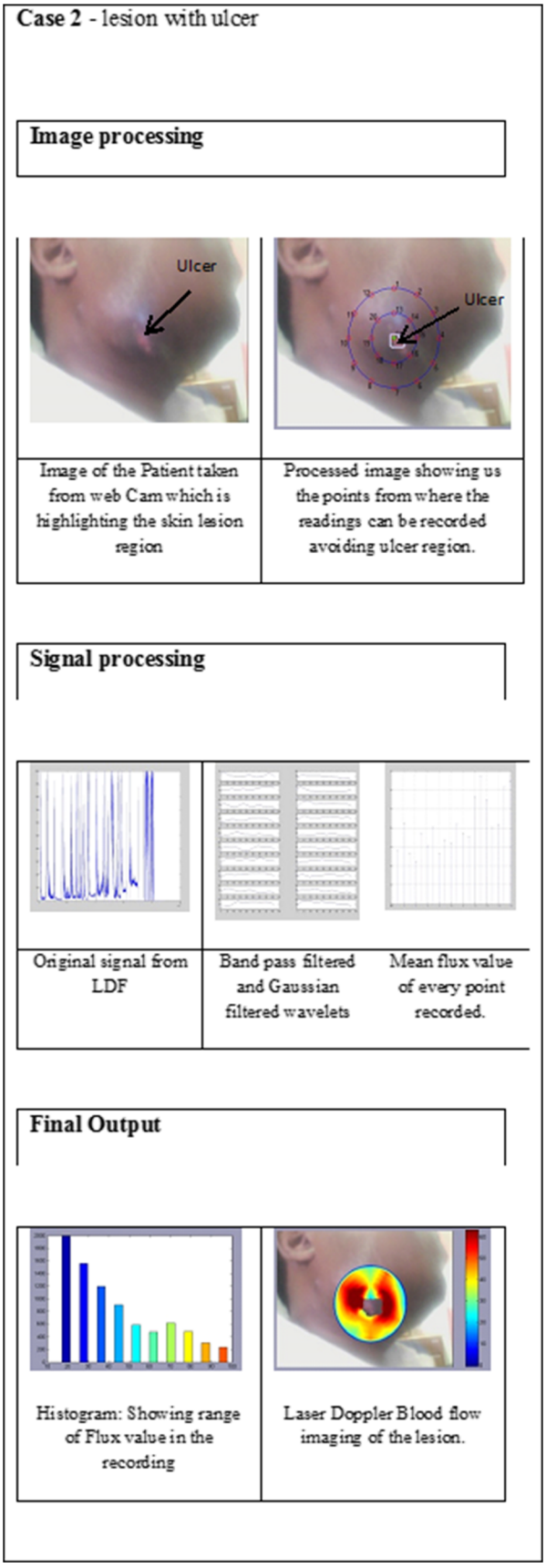


Fig. 19 MATLAB output for lesion with ulcer>

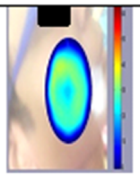
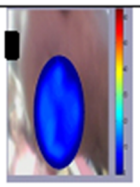
Sr. No.	Manual Readings Noted (flux value)	Flux Value obtained by software	MATLAB software Output
1. (Medium Size Total – 13 points)	Pt 1 - 20 to 10 Pt 2 - 20 to 15 Pt 4 - 20 to 15 Pt 7 - 30 to 20 Pt 10 - 30 to 20 Pt 11 - 40 to20 Pt 13 - 20 to 10	Pt 1- 18.15 Pt 2 - 20.29 Pt 4 - 23.43 Pt 7 - 27.95 Pt 10 - 28.44 Pt 11 - 26.05 Pt 13 – 20.81	
2. (Medium size Total – 21 points)	Pt 3- 15 to 5 Pt 6 - 15 to 5 Pt 9 - 15 to 5 Pt 12 - 15 to 5 Pt 16 - 15 to 5 Pt 20 - 15 to 5 Pt 21 – 15 to 10	Pt 3 - 10.6 Pt 6 - 9.94 Pt 9 - 11.81 Pt 12 - 10.4 Pt 16 - 12.1 Pt 20 - 12.76 Pt 21 - 11.02	

Fig. 20 Tabular representation of manual readings noted (flux value) and flux value obtained by software

Result and discussion

The result can be summarized in three parts. The first part consists of the output of GUI imaging software at every stage, i.e., image processing, signal processing, and color mapping imaging of the lesion. Figure 18 shows case 1—lesion without ulcer, and Fig. 19 shows case 2—lesion with ulcer. The mapping of flux value to color image gives a better understanding for analysis rather than analyzing the flux values measured at each point on the lesion by LDF.

Day 0

Flux intensity	High	Inter	Low	Normal (10pu)
location max flow rate (PU)		All Over 33.27		
Size	Medium size			
Area (cm^2)	309.86			
Average flux	Outer 24.9	Middle	Inner 21.97	Core 26.33
	Median flow – 22.93			

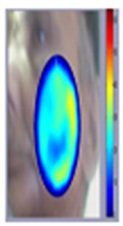


Fig. 21 Laser Doppler blood flow meter imaging for day 0, case 1

Day 180

Flux intensity	High	Inter All over	Low	Normal (10pu)
location max flow rate (PU)		27.95		
Size	Small size			
Area (cm^2)	157.07			
Average flux	Outer 24.125	Middle	Inner	Core 20.1
	Median flow – 23.29			

Fig. 22 Laser Doppler blood flow meter imaging for day 180, case 1

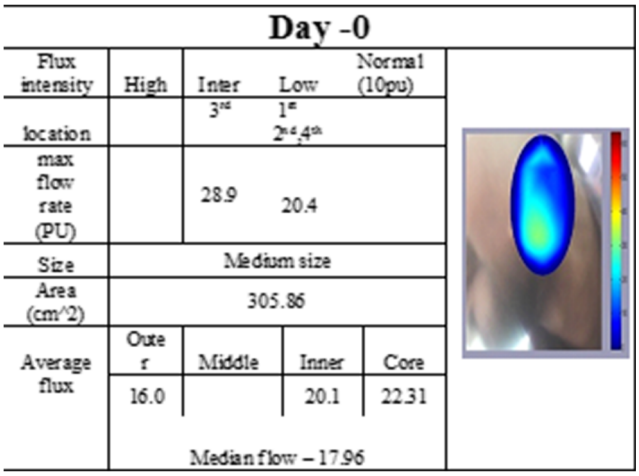


Fig. 23 Laser Doppler blood flow meter imaging for day 0, case 2

The second part consists of validation of the software output with manual reading noted. Two examples are shown in Fig. 20. It can be verified that the manual values noted are nearly equal to the values processed by the software in MATLAB at a particular location in the lesion.

The third part consists of how the above modification in hardware with the use of software built in MATLAB can be used in clinical research to monitor the changes in microvascularity over the same area over a period of time. Two case studies have been discussed. In case 1, patient's lesion has been analyzed on day 0 shown in Fig. 21 and day 180 as shown in Fig. 22. In case 2, patient's lesion has been analyzed on day 0 shown in Fig. 23 and day 30 shown in Fig. 24. It can be seen that vascularity of the lesion has changed over a period of time. We observed that by developing an innovative algorithm, any standard instrumentation can be used and scaled up as per the need of the user in clinical practice. The laser Doppler has been used with quite precision in monitoring skin microvascular circulation in different pathological conditions [10, 11].

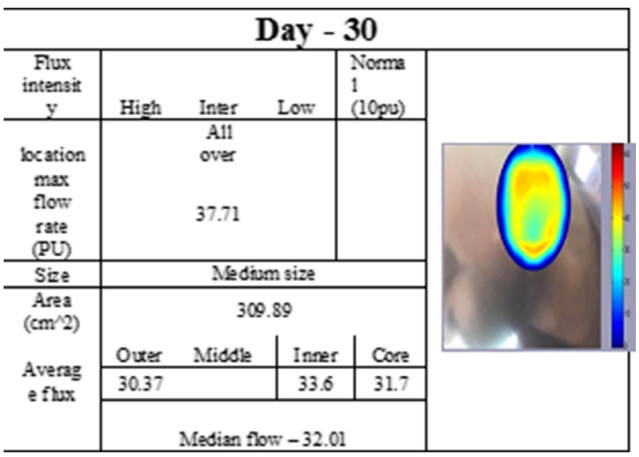


Fig. 24 Laser Doppler blood flow meter imaging for day 30, case 2

Clinical application and interpretations The data from 27 out of 100 patients having surface buccal tumor who fulfilled the criteria for undergoing LDF studies were analyzed. The inclusion criteria were developed after conducting preliminary studies in normal and comorbid conditions to standardize the protocol. Patients who presented with a bulging localized lesion with a circumscribed edge or margin and accessible from all directions with or without ulcer, abscess, inflammation, cavitation, fistula, and skin diseases were included. During the treatment follow-up, some tumors grew in size and/or developed superimposed infection, ulceration, cavitation, fistula, or overt inflammation and edema, while some tumor regressed and disappeared completely in some of the patients. Over the period of 1 year, we monitored change in the capillary blood volume flow (flux) or capillary density within the tumor as well as in comorbid conditions such as infection, edema, angioma, normal skin, ulceration, and healing process. Therefore, it has not only covered the limited pathological conditions currently being monitored but also expanded its application to include solid tumors in the armamentarium. Additionally, we developed a noble analytical method to map the flow redistribution as explained in detail in this paper. These two basic standard parameters will remain the foundation of clinical interpretation in all pathophysiological conditions where laser Doppler can be effectively used.

Precaution was taken to avoid applying any pressure by the probe over the data acquisition site because that may stop the flow velocity completely or generate huge noise leading to distortions and wrong interpretations. The data were acquired under normalized condition at a given temperature after the patient was acclimatized. No sudden change in the environmental temperature should be allowed during the data acquisition as it affects the microvascular flow due to autonomic NS response. The change from the baseline suggested change in the flow, either increased or decreased or no flow. It depended upon the experimental condition set for the purpose or the disease condition that was to be monitored and evaluated over a period or point of time.

LDF has been used widely to understand microvascular blood flow dynamics in experimental small animal studies. Though not routinely, but it has been used in human beings mostly as a research diagnostic tool in inflammatory conditions such arthritis, ulcer or wound, autonomic vascular and thermosensory disorders, etc. The value of

the flux intensity directly reflects immune or inflammatory or neovascularization or angiogenesis and angiostatic status and underlying process. The fundamentals of flux and heat map (redistribution of flux) interpretations in different clinical conditions are explained below:

1. Tumors undergoing treatment showed various patterns and trends (microvascular adjustments or flow redistribution) depending upon the response to the therapy. (a) In 6 patients who responded well to treatment over a period of 1 year, in three (3) patents, we observed marked reduction in the flux along with the reduction in the size of the tumor, suggesting good therapeutic response (anti-inflammatory and cytotoxic) and cessation of the process of neovascularization. (b) Higher baseline flux value and a marked increase in the flux and flow redistribution ranging from diffusion, area expansion, and shift in the area of localization/interest were observed suggesting neovascularization and/or flow abnormalities, possibly due to unstable/immature capillary and microvessel formation or flow turbulences. Such changes were also seen in inflammatory conditions independently of the tumor response or progression.
2. Ulcer, cavitation, and fistula—No flow was detected from the margins or surface of the lesions, while flux varied in the neighborhood or the surrounding areas depending upon the severity of the inflammation and the anti-inflammatory process and repair mechanism.
3. Wound-healing process—A change in the flux was observed over a period of time, i.e., increased where the wound was healing and vice versa.
4. Inflammation—Flux and heat map localization (flow redistribution) depended upon the severity and the area of extension, whenever inflammation was progressing, rising and/or not responding to treatment, and flux increased and vice versa. There was diffusion or shift in the position of the heat map area in a number of cases where there was spread of the inflammatory process.
5. Change in the environmental temperature or any disturbance or obstruction (partial or complete) due to tightness of clothes, etc.
6. Influence of drugs acting upon the blood vessels, i.e., central or peripheral acting such as antihypertensives, vasodilators, aspirin, etc. had corresponding short-tem effects, while once the flow stability develops, no change/shift was observed from the baseline.

The LDF instrumentation and the noble and simple yet sensitive method proposed in this study can be used as a portable, point-of-care diagnostic tool in outpatient setup or at bedside or at any other setup to monitor various pathophysiological conditions. Clinical interpretation will be based upon two parameters, i.e., flux intensity and heat map. They can be read as one of the following: (a) "increased and decreased flux and no flow"; (b) flow redistribution in terms of area and site "diffusion, localization, and shift in the site of max flux"; or (c) various combinations. The clinical judgment made by the clinician depends upon their expectation in terms of treatment outcome and response. The decision may be taken accordingly: either add or delete or change the drug regimen. We believe that the analytical method and heat mapping technique developed by us using a basic laser Doppler instrumentation will expand its application horizon from its limited use in research lab to cover a wide range of clinical conditions. It will enable the clinicians to monitor the microvascular flow alterations or modulations in any clinical setup without putting much economic burden on the patients.

Conclusion

The technical limitations of the simple analog-based laser Doppler blood flow meter in accessing the microvascularity of any surface lesion were studied. Ways to overcome some of its limitations by modifying the experimental setup in order to reduce the artifacts while at the same time developing GUI in MATLAB were suggested. The GUI helped in creating visual results in a pictorial format of the signals (flux value) obtained from LDF in the form of a color map for better understanding and analysis of changes in the blood flow either over time or differences in flow over an area of skin which can be stored and analyzed and compared. We believe that the analytical method and heat mapping technique developed in this paper will not only enable the clinicians to easily monitor and interpret the microvascular flow modulations but also expand its application horizon from its limited use in research lab to cover a wide range of clinical conditions as explained above.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical guidelines The study was approved by the TMC-ACTREC institutional ethics committee number 94 dated. 06–06-2013 "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

Informed consent Informed consent was obtained from all individual participants included in the study.

Appendix

MATLAB function representing the biosignal processing and conversion into a heat map or image along with superimposition on the photographic image of a surface lesion recorded using a webcam

The entire code is very big, and therefore, only the main part of the program is given below.

```
h = imfreehand;              %select the lesion region
BW1 = createMask(h);
BW = getPosition(h);
CC = bwconncomp(BW1);
area_in_pixels = cellfun(@length,CC.PixelIdxList);
%calculate the area of selected lesion region
pixperinch = get(0,'ScreenPixelsPerInch');
area_in_inch = area_in_pixels/pixperinch;
area_in_squarecm = round(area_in_inch*2.54);
stats = regionprops(BW1,'Centroid');
STATS1 = regionprops(BW1,'MajorAxisLength');
STATS2 = regionprops(BW1,'MinorAxisLength');
centers = stats.Centroid; % get the center of the selected area
x = round(centers(1));
y = round(centers(2));
diameters = mean([STATS1.MajorAxisLength STATS2.MinorAxisLength],2);
R1 = round(diameters/2);   % get the radius of the selected area
( note :- only example of large lesion is discussed below)
if (R1 > 320);
delete(hPlotAxes);
    hPlotAxes1 = axes(...    % Axes for plotting the selected plot
              'Parent',hsp1, ...
              'Units', 'normalized', ...
              'box','on',...
              'HandleVisibility','callback', ...
              'Position',[0.325 0.13 0.35 0.67]);
delete(t2);
if  (340 >= R1 ) && ( R1 > 320);   % To fix the radius.
 R2 = 340;
elseif (380 >= R1 ) && ( R1 > 340);
R2 = 380;
else
   if R1> 380;
   R2 = 380;
   end
end
if R2 == 340;
      r = R2;
      r1 = round(r - 113.33);       % To fix other radius and core
      r2 = round(r1 - 113.33);
      r3 = r + 80;
      r4 = r3 + 5;
else R2 = 380;
       r = R2;
      r1 = round(r - 126.67);
      r2 = round(r1 - 126.67);
      r3 = r + 80;
      r4 = r3 + 5;
end
%image processing – software will tell us the no. of points to be recorded on an image.
imshow(a)
hold on
th = 0:pi/50:2*pi;
xunit = r * cos(th) + x;
yunit = r * sin(th) + y;
plot(xunit, yunit);
hold on
th = 0:pi/6:2*pi;
xmarker = r * cos(th) + x;
ymarker= r * sin(th) + y;
plot(xmarker,ymarker,'ro')
a1 = round(xmarker);
b1 = round(ymarker);
Text1 = text(a1(10)+10,b1(10)-20,'1','FontSize',10);
Text2 = text(a1(11)+10,b1(11)-20,'2','FontSize',10);
Text3 = text(a1(12)+10,b1(12)-20,'3','FontSize',10);
Text4 = text(a1(1)+10,b1(1)-20,'4','FontSize',10);
Text5 = text(a1(2)+10,b1(2)+30,'5','FontSize',10);
Text6 = text(a1(3)+10,b1(3)+30,'6','FontSize',10);
Text7 = text(a1(4)+10,b1(4)+30,'7','FontSize',10);
Text8 = text(a1(5)-20,b1(5)+30,'8','FontSize',10);
Text9 = text(a1(6)-20,b1(6)+30,'9','FontSize',10);
Text10 = text(a1(7)-50,b1(7)+30,'10','FontSize',10);
Text11 = text(a1(8)-50,b1(8)+10,'11','FontSize',10);
Text12 = text(a1(9)-50,b1(9)-10,'12','FontSize',10);
```

```
th = 0:pi/50:2*pi;
xunit = r1 * cos(th) + x;
yunit = r1 * sin(th) + y;
plot(xunit, yunit);
hold on
th = 0:pi/4:2*pi;
xmarker = r1 * cos(th) + x;
ymarker= r1 * sin(th) + y;
plot(xmarker,ymarker,'ro')
a2 = round(xmarker);
b2 = round(ymarker);
Text13 = text(a2(7)+10,b2(7)-20,'13','FontSize',10);
Text14 = text(a2(8)+10,b2(8)-20,'14','FontSize',10);
Text15 = text(a2(1)+10,b2(1)-20,'15','FontSize',10);
Text16 = text(a2(2)+10,b2(2)-10,'16','FontSize',10);
Text17 = text(a2(3)+10,b2(3)+20,'17','FontSize',10);
Text18 = text(a2(4)-10,b2(4)+30,'18','FontSize',10);
Text19 = text(a2(5)-50,b2(5)+30,'19','FontSize',10);
Text20 = text(a2(6)-50,b2(6)-20,'20','FontSize',10);
th = 0:pi/50:2*pi;
xunit = r2 * cos(th) + x;
yunit = r2 * sin(th) + y;
plot(xunit, yunit);
hold on
th = 0:pi/2:2*pi;
xmarker = r2 * cos(th) + x;
ymarker= r2 * sin(th) + y;
plot(xmarker,ymarker,'ro')
a3 = round(xmarker);
b3 = round(ymarker);
Text21 = text(a3(4)+10,b3(4)-20,'21','FontSize',10);
Text22 = text(a3(1)+10,b3(1)-20,'22','FontSize',10);
Text23 = text(a3(2)+10,b3(2)+20,'23','FontSize',10);
Text24 = text(a3(3)-50,b3(3)-10,'24','FontSize',10);
xmarker = x;
ymarker= y;
plot(xmarker,ymarker,'ro')
a4 = round(xmarker);
b4 = round(ymarker);
Text25 = text(a4(1)+10,b4(1)-20,'25','FontSize',10);
if R2 == 340;
th = 0:pi/50:2*pi;
xunit = r3 * cos(th) + x;
yunit = r3 * sin(th) + y;
hold on
th = 0:pi/6:2*pi;
xmarker = r3 * cos(th) + x;
ymarker= r3 * sin(th) + y;
a5 = round(xmarker);
b5 = round(ymarker);
th = 0:pi/50:2*pi;
xunit = r4 * cos(th) + x;
yunit = r4 * sin(th) + y;
hold on
th = 0:pi/60:2*pi;
xmarker = r4 * cos(th) + x;
ymarker= r4 * sin(th) + y;
a6 = round(xmarker);
b6 = round(ymarker);
else
th = 0:pi/50:2*pi;
xunit = r3 * cos(th) + x;
yunit = r3 * sin(th) + y;
hold on
th = 0:pi/6:2*pi;
xmarker = r3 * cos(th) + x;
ymarker= r3 * sin(th) + y;
a5 = round(xmarker);
b5 = round(ymarker);
th = 0:pi/50:2*pi;
xunit = r4 * cos(th) + x;
yunit = r4 * sin(th) + y;
hold on
th = 0:pi/60:2*pi;
xmarker = r4 * cos(th) + x;
```

```
ymarker= r4 * sin(th) + y;
a6 = round(xmarker);
b6 = round(ymarker);


end
t = 25;       % total no of points to be recorded for non-ulcer lesion

A1 = [a1(10) a1(11) a1(12) a1(1) a1(2) a1(3) a1(4) a1(5) a1(6) a1(7) a1(8) a1(9) a2(7) a2(8) a2(1) a2(2) a2(3) a2(4) a2(5) a2(6) a3(4)
a3(1) a3(2) a3(3) a4(1)];
% matrix A gives x co-ordinates of the points to be recorded used for colour heat mapping later.
B1 = [b1(10) b1(11) b1(12) b1(1) b1(2) b1(3) b1(4) b1(5) b1(6) b1(7) b1(8) b1(9) b2(7) b2(8) b2(1) b2(2) b2(3) b2(4) b2(5) b2(6) b3(4)
b3(1) b3(2) b3(3) b4(1)];
% matrix B gives y co-ordinates of the points to be recorded used for colour heat mapping later.


% R is the row signal from LDF and t is the no. of points which are  recorded.
 P = round(numel(R)/t);   % dividing the raw signal into wavelets
 for i = 1:1:t-1;
xi = R(1+((i-1)*P):i*P);
idx = xi > upperlimitoflux;         % filtering upper limit of flux taking from user
xi(idx) = [];
idx = xi < lowerlimitoflux;          % filtering lower limit of flux taking from user
xi(idx) = [];
g = gausswin(100);       % applying gausswin filter to smoothen the wavelet
g = g/sum(g);
ci = conv(xi,g,'same');    % calculating mean for every wavelet
z(i) = mean(round(ci));
 end
 xt = R(1+(t-1)*P:end);
 idx = xt > tanvi1;
xt(idx) = [];
idx = xt < rohin;
xt(idx) = [];
g = gausswin(100);
g = g/sum(g);
ct = conv(xt,g,'same');
zt = mean(round(ct));

(Note :- for lesion without ulcer)

if tanvi == 2;
   x1 = A1;
    y1 = B1;
    z1 = z(:);
    z = z1.';
    z(end+1) = zt;
    disp(z)
(Note :- for lesion with ulcer)
else


 z1 = z(:);
    z = z1.';
    z(end+1) = zt;
     if numel(A1) ~= numel(z);
  k = numel(A1) - numel(z);
  z(end+k) = 0;
     end
zdef = 0;
 for s = 1:1:numel(A1);
   if (c1(s)~=0) && (d12(s)~=0);
       z(s) = z(s);
   else
       for s1 = numel(A1):-1:s+1;
            z(s1) = z(s1-1);
       end
          z(s) = zdef;
   end


 end
 disp(z)
```

(**Note**: - matrix A and matrix B are similar to matrix A1 and matrix B1 shown above but having higher elements than 25 for large tumour in this case to apply condition of NaN. For lesion with ulcer Matrix c and Matrix d2 are first initialized as equal to matrix A and matrix B respectively and later elements under ulcer region selected are made 0)

```
for s = 1:1:numel(A);    % condition to apply NaN to  Matrix z
      if  c(s) == 0 && d2(s) == 0;
        Z1(s) = NaN;
      else
         Z1(s) ;
      end
end
x1 = A;
y1 = B;
z = Z1;
end
```

(Note :- common part for both ulcer and non ulcer lesion)

```
gridincr=3;
rangeX=floor(min(x1))-gridincr:.2:ceil(max(x1)+gridincr);
rangeY=floor(min(y1))-gridincr:.2:ceil(max(y1)+gridincr);
 [X,Y] = meshgrid(rangeX,rangeY);
Z = griddata(x1,y1,z,X,Y,'cubic');
delete(hsp2);
hsp3 = uipanel('Parent',hp,...
            'Title','Results',...
            'fontweight','bold',...
            'highlightcolor','black',...
            'foregroundcolor','black',...
            'borderwidth',2,...
            'bordertype','line',...
            'fontname','calibri',...
            'BackgroundColor',[0.6 0.6 0.7],...
            'FontSize',14,...
            'Position',[.02 .03 .96 .85]);


hPlotAxes3 = axes(...    % Axes for plotting the selected plot
             'Parent', hsp3, ...
             'Units','normalized', ...
             'box','on',...
             'HandleVisibility','callback', ...
             'Position',[0.55 0.13 0.35 0.67]);
image(b)
axis off
 hold on
 h = surf(X,Y,Z);   % creating heat map to depict blood flow in lesion area
view([0 90]);
set(gca, 'CLim', [5, 55]);
colorbar
shading interp
set(h,'LineStyle','none')
tt1 = uicontrol('Parent',hsp3,...
            'Units','normalized',...
            'Style','text',...
            'String','Laser Doppler Blood Flow Imaging of the tumor.',...
            'fontsize',12.5,...
            'fontname','calibri',...
            'fontweight','bold',...
            'horizontalalignment','center',...
            'backgroundcolor',[ 0 1 1],...
            'Position',[0.55 0.90 0.35 0.04]);


 R = A12;
 idx = R> tanvi1;
R(idx) = [];
idx = R < rohin;
R(idx) = [];
ax = axes(...    % Axes for plotting the selected plot
            'Parent',hsp3, ...
            'Units', 'normalized', ...
            'box','on',...
            'fontweight','bold',...
            'HandleVisibility','callback', ...
            'Position',[0.09 0.13 0.35 0.67]);
 hist(R)        % to create histogram
 [counts,centers] = hist(R);
  barColorMap = jet(numel(counts));
 for b = 1 : numel(counts)
```

```
handleToThisBarSeries(b) = bar(centers(b), counts(b), 'BarWidth', 4);
set(handleToThisBarSeries(b),'FaceColor', barColorMap(b,:));
hold on;
end
 tt2 = uicontrol('Parent',hsp3,...
            'Units','normalized',...
            'Style','text',...
            'String','Histogram: Showing the frequency of Flux value in successive numerical intervals of equal size in the recording.',...
            'fontsize',12.5,...
            'fontname','calibri',...
            'fontweight','bold',...
            'horizontalalignment','center',...
            'backgroundcolor',[ 0 1 1],...
            'Position',[0.09 0.90 0.35 0.06]);
 end
```

References

1. RS Khandpur (2014) Blood flow meter, laser Doppler blood flow meter. Handbook of biomedical instrumentation, third edition. McGraw Hill Education (India) Private Limited, pp 323-343
2. Tissue blood flow and temparature monitoring with moorvMS-LDF, manual version (3.0 english): 19/09/2012.
3. Basic theory and operating principles of laser Doppler blood flow monitoring and imaging (LDF & LDI), issue 1. https://us.moor.co.uk/ckfinder/userfiles/.../Moor_Laser_doppler_theory_Issue_1.pdf
4. Moor Instruments Limited, “Moorsoft for Windows for moorLAB V1.3”, (12th Nov, 2001)
5. moorVMS-LDF, vascular monitoring system, user manual. Manual version—moorVMS-LDF user manual (version 3.0 English). Revision date: 19/09/2012
6. moorVMS-PC, moorVMS recording and analysis system. Software manual manual version: moorVMS-PC user manual (version 3.1 English). Revision date: 18/11/2011
7. http://in.mathworks.com
8. Matlab—help documentation
9. Nilsson GE (1984) Signal processor for laser Doppler tissue flowmeters. Med Biol Eng Comput, 22 (4):343–348
10. Briers JD (2001) Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging. Physiol Meas 22:R35–R66
11. Mirdell R, Iredahl F, Sjoberg F et al (2016) Microvascular blood flow in scalds in children and its relation to duration of wound healing: a study using laser speckle contrast imaging. Burns 42: 648–654