Low Cost Laser Speckle Imaging System for Blood Flow Assessment

Sheena P. Philimon, Audrey K. C. Huong, Xavier T. I. Ngu

***Abstract*—This paper aims to demonstrate the feasibility of using a low cost laser speckle imaging built for the detection of changes of tissue blood flow with different experimental conditions. Images of anterior portion of the wrist of four healthy adult volunteers illuminated by a laser source of wavelength 650 nm were collected via a monochromatic charge-coupled device (CCD) imager. The mean and standard deviation (SD) of blood flow perfusion was predicted as 3.92 ± 1.47 and 2.90 ± 1.39, respectively, for measurements at rest condition and during blood flow occlusion. This work showed the ability of the developed system to detect changes in blood flow perfusion with differences in the experimental conditions. However, further works are required to further confirm the suitability of the system before it is used for different clinical applications such as monitoring of blood flow during diabetic foot ulcers healing following standard medical treatment.**

Keywords: Blood flow, Charge-coupled detector, Laser speckle, Multispectral imaging.

I. INTRODUCTION

Study of blood flow perfusion has gained significant attention in recent years especially in research concerning wound healing rate. Bedside clinical evaluation technique for wound healing assessment based on visual inspection is the most commonly used technique as it can be performed immediately without cost. This technique, however, lacked reliability and accuracy in its diagnosis. Among other available techniques, punch biopsy for bacterial load analysis is invasive in nature and time consuming, while the commonly practiced Doppler flowmetry technique is only able to provide information of blood flow perfusion measurement in tissue [1-3]. Meanwhile, modern clinical practice of diagnosing and monitoring the development of diseases involve the use of medical imaging techniques. Among the commonly used modalities at the present are magnetic resonance imaging (MRI), computerized tomography (CT) scan, positron emission tomography (PET) scan, and single photo emission computed tomography (SPECT) scan [4-6]. These cutting edge imaging techniques have been proven to be highly beneficial in detecting any abnormalities in the tissue structure based on two-dimensional (2D) or three-dimensional (3D) images that produce very detailed anatomic information of the scanned body. However, these machines are costly in price and require the use of contrast agents or radioactive tracers to be administered into the patient's body, hence, limiting the application of this approach on pregnant women in particular [7, 8].

In year 2013, a report by the World Health Organization (WHO) based on data sources collected from 219 countries and territories has stated that at least 381.8 million adults were diagnosed with diabetes mellitus [9]. This number was expected to increase up to 591.9 million by the year 2035. A majority of these diabetes cases were indentified among those living in low- and middle- income countries, with China having the highest number of population with diabetes followed by India. Social economics, living environment, and eating habits are among the factors often linked to the radical rise in diabetes cases worldwide [10]. Likewise, in Malaysia, a statistical study by the National Health and Morbidity Surveys (NHMS) predicted at least 21% of the population to be diagnosed with diabetes by the year 2020 [11]. Of this figure, it is estimated about 15% of this population suffered from diabetic foot ulcers are at risk of amputation. The prevalence of this disease is increasingly alarming as it has left deleterious effects on affected patients both emotionally and financially. In view of this matter, a reliable diagnostic tool to document changes in microcirculatory activities in diabetic foot ulcers is highly needed. Assessment of wound should include complete understanding of wound pathology and environmental factors that affect healing and intense study on tissue remodeling.

Various techniques were proposed to overcome the shortcomings of standard clinical routines. These recent techniques include laser Doppler flowmetry[1], laser speckle contrast imaging [12], photoacoustic imaging [13], and enhanced image processing algorithms [14], all of which were reported able to measure cutaneous blood flow and tissue perfusion. This is to identify the effectiveness of the standard medical treatment towards diabetic wound healing rate with minimal evaluation time, and hence reducing the feeling of anxiety amongst the affected patients. It was also suggested in previous works that one could possibly obtain the histology information and tissue blood flow information using a multispectral imaging system [15, 16]. Meanwhile, a study by Huong, Philimon, and Ngu[17] revealed a high association between blood perfusion and tissue oxygenation during the different stages of wound repair and regeneration. Tissue perfusion and local oxygenation parameters change simultaneously at different stages of wound healing namely

Revised Version Manuscript Received on August 19, 2019.

Sheena P. Philimon, Faculty of Electrical and Electronic Engineering, UniversitiTun Hussein Onn Malaysia, 86400 BatuPahat, Johor, Malaysia.

Audrey K. C. Huong, Faculty of Electrical and Electronic Engineering, UniversitiTun Hussein Onn Malaysia, 86400 BatuPahat, Johor, Malaysia.

Xavier T. I. Ngu, Faculty of Electrical and Electronic Engineering, UniversitiTun Hussein Onn Malaysia, 86400 BatuPahat, Johor, Malaysia.

inflammatory, proliferative and tissue remodeling phase. The inflammatory stage involves aggregation of platelets to initiate coagulation following an injury, proliferative phase stimulates microangiogenesis that promote development of new vessels while tissue remodeling phase involves differentiation of fibroblasts [18]. In cases of chronic wound such as that of diabetic foot ulcers, these processes may be disrupted by impairment of either tissue oxygenation, blood perfusion or both.

Laser speckle contrast imaging is a technique, which has advanced over the years, with its imaging application ranging from multimodal skin layers, cerebral cortex to retina using light of wavelength 633 nm [19, 20]. While there have been *in-vivo* wound healing studies on characterization of hemodynamic changes and microvascularremodeling using the corresponding technique, very few findings were reported on human subjects. Instead, most experimental investigations were performed on animals such as rat and porcine models [15, 21, 22]. Similarly, Wang, Cull, Piper, Burgoyne, and Fortune [23] attempted to investigate blood flow changes in the glaucomatous optic nerve head using nonhuman primate model that resembles the likes of human subjects. Recent report on diabetic retinopathy patients revealed a strong association between glycosylated hemoglobin and ocular blood flow based on a comparison study using laser speckle and Doppler flowmetry[24]. In general, laser speckle imaging and Doppler are the most commonly employed noninvasive approaches for monitoring of blood flow perfusion. Both methods are able to penetrate up to a superficial depth of ≤ 1 mm at a single measurement point. Although these techniques appeared to be almost similar, the differences lie in the method of measurement. The Doppler theory is based on the frequency shift triggered by the Doppler effect when light motion is interrupted through scattering or reflection, which was first reported by Johan Christian Doppler [25]. These effects take charge when relative movement is perceived between wave source and detector. Similarly, in laser speckle technique, measurement of the velocity of scattering particles is based on the illumination of fluid motion. This movement, in return, resulted in the fluctuation of speckle pattern. One of the factors that distinguished laser speckle and Doppler flowmetry technique is the filtering capability. The Doppler flowmetry technique holds an advantage over its counterpart with its ability to filter signals at high frequency (e.g. above 100 Hz) and reduce the effects of motion artefacts[26]. Besides, the laser speckle contrast technique is very much dependent on the exposure time, which determines the calculated speckle contrast value. Nevertheless, in terms of speckle argument, the laser speckle technique is theoretically simpler and faster in its calculations.

Laser speckle technique has been extensively applied in biomedical research to observe abnormal hemodynamic changes among diseased models and to understand the histological development of tumors, retinal diseases and diabetes. The fundamental of this concept is based on the interference pattern of reflected or scattered light from an illuminating surface, which in return produced a granular effect or known as the laser speckle [27]. The movement of speckle pattern is in correspondence with the movement of an object, prompting the idea of speckle fluctuation initiated by blood flow velocity during the mid 1970s[28]. This is in such a way that when blood flow is restricted, the speckle contrast appeared to be highly enhanced. On the contrary, the speckle pattern appeared to be more gradual and blurred out in regions of high blood flow. This occurrence can be explained by the diffraction of light into the diffraction halo for a high contrast speckle whereas in a region where speckles appeared to be vague, incident light is directed into the central maximum of a Fourier plane. Earlier work by Goodman [29] expressed laser speckle pattern as an arbitrary phenomenon which properties can be statistically analyzed. The associated work explained the diffusing surface roughness as a factor which could influence the resulting speckle pattern. The former can be obtained by means of asymptotic analysis using a Gaussian distribution function of surface height fluctuation. The observed reduction in speckle patterns contrast was concluded in the corresponding work as the resultant of a lower standard deviation compared to the mean intensity. This theory led to the implementation of laser speckle contrast analysis (LASCA) technique by Fercher and Briers [30] which has been commonly applied as a basis in most blood flow related work. During the earlier stage, this concept was applied as a non-invasive diagnostic tool to examine retinal blood flow in the field of ophthalmology. Various arguments on the reliability of the laser speckle technique were raised regarding the distribution of particle velocity of the blood medium in study where the speckle measurement were merely interpreted as the spatial distribution of speckle contrast [31]. This is attributed to the initially developed model that attempted to relate the speckle contrast to the correlation time but overlooked the presence of static scattering layers between intact and exposed skin layers. Recent works have tried to improve the correlation time value by manipulating the dependence of speckle contrast on the camera exposure time [32]. In view of the corresponding argument, the multi-exposure speckle imaging exposure (MESI) approach took into account the *nonergodic variance* for a robust estimation of flow variability in a static scattering layer.

Over years, the concept of laser speckle contrast imaging has been further extended and widely adopted in various biomedical applications. This is owing to the relative simplicity of the instrumentation setup which mainly consists of a laser light source and camera. Among the novel applications of laser speckle imaging are for quantitative measurement of cerebral blood flow for stroke studies [19] and post treatment of port wine stain laser therapy [33]. This work aims to demonstrate the feasibility of using a low cost laser speckle imaging system for *in-vivo* measurement of blood flow and to investigate if the system is able to produce results of equivalent standard. For this purpose, a 650 nm laser diode and a low cost imager were used. This is, to the authors' knowledge, the first laser speckle system developed to study one's blood flow using laser of wavelength 650 nm.

II. METHOD

A. Laser Speckle Imaging System Setup

A schematic of the laser speckle imaging system developed in this study for noninvasive and noncontact assessment of blood flow is shown in Fig.1. Instead of using a Helium-Neon laser, a 650 nm laser diode (Mybotic, 5 mW) was used in this work to illuminate the selected skin region. The selection of this light source was done owing to its high intensity and good stability in its output. The experiment was performed in reflection mode and the illuminating source was placed at 140 mm from skin surface. The laser source was located at approximately 45° from normal to reduce glare. The detection system consisted of a plano-convex lens with diameter, ϕ = 12.7 mm and focal length of 50.22 mm to focus light reflected from the targeted site onto a charge coupled detector (CCD) (BUC4−500C from BestScope) with a pixel size of 3.4 µm × 3.4 µm. This optical lens was placed at normal from skin surface in front of the CCD imager; the latter was positioned at 20 mm from the skin. The CCD was controlled using the ISCapture imaging software (Tucsen Photonics). The integration time of the CCD was set as 1.0 second and the field of view of the imager is given by 5 mm × 7 mm. A universal serial bus (USB) data transfer cable was used to transfer the 12-bit raw images to a personal computer for offline post processing. This entire system was mounted on an optical breadboard. The incorporated laser source was targeted at the selected skin site, and the detected phase shift was translated into tissue perfusion level.

The CCD and the optical lens were configured in tandem at a 1:1 ratio to achieve a magnification value, *M*, of 1.0. This arrangement was specifically chosen in order to give speckle size value of approximately one pixel using the following expression [34]:

$$1.22\lambda\, f/DM\ . \tag{1}$$

The *f*-number, $N = f/D$, is expressed as the ratio of effective focal length to the aperture diameter, *D*. Here, the *f*-number for the employed experimental setup was calculated as 4.55.

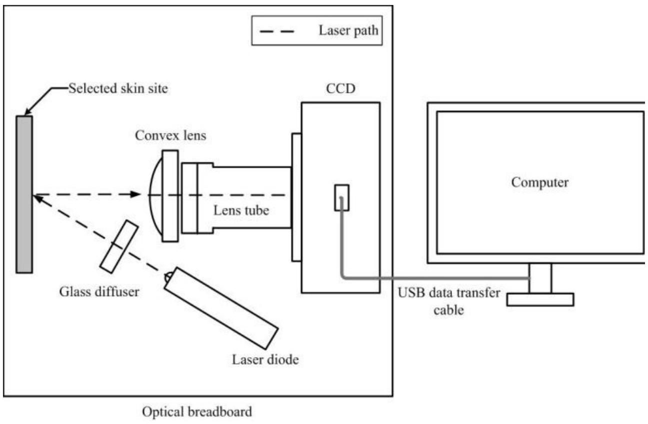


Fig.1 Optical arrangement of the developed laser speckle imaging system

B.In-vivo Experimental Procedure

A pilot experiment was conducted on the anterior region of the left wrist of four healthy Asian volunteers who declared to be nonsmokers in their informed consent statement prior to experiment. These volunteers were students from UniversitiTun Hussein Onn Malaysia (UTHM) aged between 25 – 27 years at the time of data collection. These volunteers declared to be healthy individuals free from underlying diseases such as cancer, heart diseases, and anemia. Images of skin samples were collected in a dark enclosed room. Volunteers were instructed to remain still and to breathe normally while measurements were obtained. Experiments were conducted under two conditions namely at rest and arterial blood flow occlusion. The blood flow occlusion experiment was by the inflation of a cuff around upper left arm of the recruits with a pressure of 140 mmHg for 60 seconds before the measurement. Measurements were taken from the same spot for both of the considered conditions to ensure consistency and for comparison purpose. These experimental settings were chosen to investigate if the developed system was able to detect changes in blood microcirculation under different circumstances.

C. Speckle Analysis

Images acquired using the CCD imager were saved in raw format for offline post processing. The analysis of the obtained data involved two main steps. The first step is to derive speckle contrast information from the raw image using a spatial sliding window, typically a 5 × 5 or a 7 × 7 sliding. window. A window size of 7 × 7 was employed in this study. This window size is considered an ideal size to determine the speckle contrast without reducing the spatial resolution [35].

Practically, the observed reduce in speckle patterns contrast is the resultant of a lower standard deviation compared to the mean intensity. Hence, the speckle contrast, *K*, is commonly defined as the ratio of standard deviation, σ, to the mean intensity, $\langle I \rangle$, given as:

$$\text{speckle contrast, } K = \frac{\sigma}{\langle I \rangle} \le 1\ . \tag{2}$$

The speckle contrast value, *k*, is described as the ratio of standard deviation to the mean of the measured intensity $\langle I \rangle$[36],

$$k = \frac{\sigma}{\langle I \rangle} = \frac{\sqrt{\dfrac{\sum_{i=1}^{N}\left(I_i - \langle I \rangle\right)}{N-1}}}{\langle I \rangle} \tag{3}$$

where*I* is the time-dependent intensity measurement and *N* is a positive integer. Since a 7 × 7 sliding window was performed across the raw data to compute for the *k* value, the *N* value is given by 49.

The second step is the conversion of the speckle contrast image to the correlation time, τ, via an iterative fitting procedure. The latter is normally taken as a quantitative measure of blood flow. The value of *k* is a function of correlation time, τ, as followed:

$$k(T,\tau)=\left(\beta\frac{\exp\left(-2(T/\tau)\right)-1+2(T/\tau)}{2(T/\tau)^2}\right)^{1/2} \qquad (4)$$

where parameter T is defined as the exposure time of the CCD imager and is given by T = 1 as discussed in section 0 (C). Parameter β is a constant that constitutes speckle averaging and is taken here as "1". Based on (4) and the k value calculated from (3), the unknown value of τ was determined using *fminsearch* nonlinear fitting function available in MATLAB (MathWorks, Inc.). The overall procedure involved seeking for the speckle contrast value using the *fminsearch* function; the iterative fitting process is summarized in Fig.2.

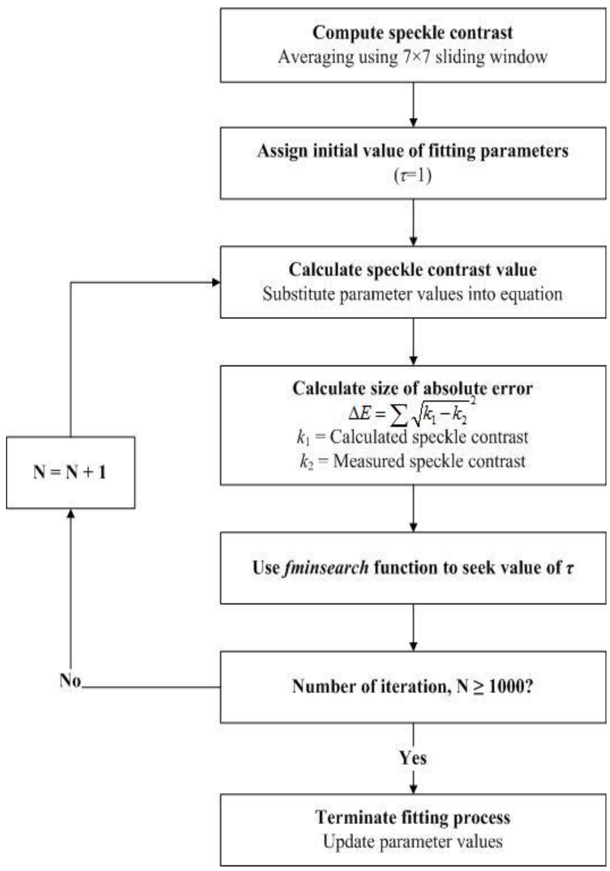
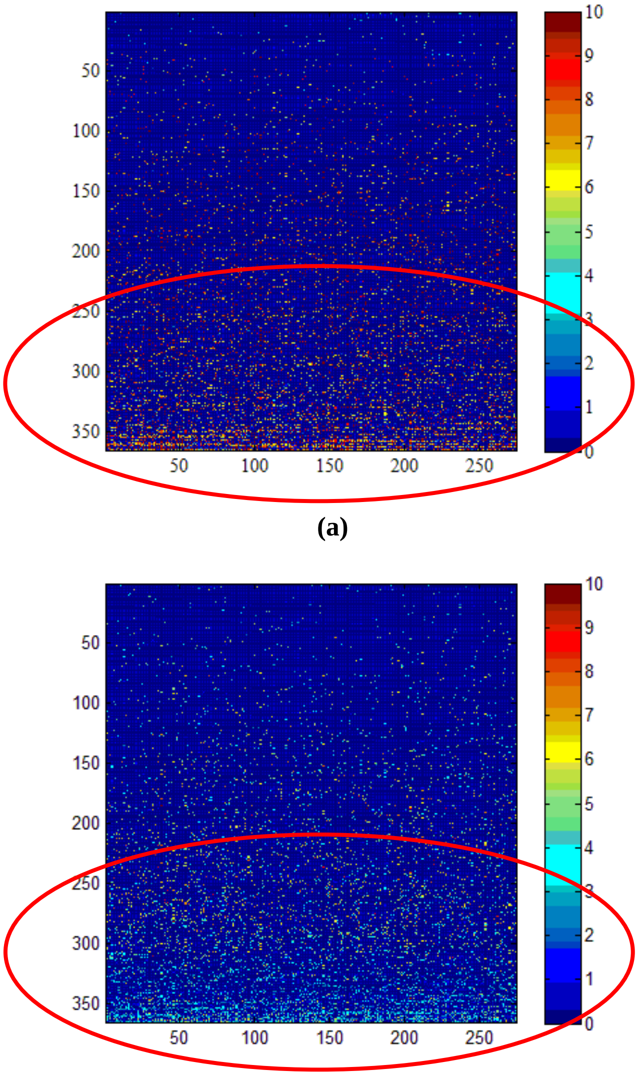


Fig.2 Iterative fitting procedure using MATLAB fminsearch function

III. RESULTS AND DISCUSSION

The speckle contrast images calculated for one the recruits (Volunteer A) using data obtained from the developed system are shown in Fig.3. Based on the acquired data, the CCD raw image with a resolution of 1920 × 2576 pixels was reduced to a 274 × 365 pixels speckle contrast image following the 7 × 7 sliding window operation in the calculation of the k value as discussed in section II (A). The decrease in the resolution of the pixel contrast value permits rapid mapping of motion estimation. These results also signified the importance of having speckle size of the same dimension as the pixel size, at the price of reduced field of view. It was reported that when speckle size is smaller than the pixel size (e.g. ≤ 0.5), this would degrade the imaging contrast due to the spatial averaging over uncorrelated speckles, a term known as spatial under sampling [37]. The speckle contrast images calculated for at rest and occlusion condition are shown, respectively, in the top and bottom window in Fig.3. Speckle pattern can be observed at regions where blood vessels are abundant as indicated by the red circle in Fig.3. The speckle pattern represents the movement of the scattering particles (i.e. blood flow). The color bar on the right of the figures indicated the speckle contrast value. The intensity of the speckle pattern appeared to be visibly high in regions of high blood, and vice versa, which is evident in Fig.3.



(a)

(b)

Fig.3 An example of speckle contrast image, *k*, calculated for at rest (a) and during blood flow occlusion (b)

Table I shows the mean correlation time value predicted for the four recruited subjects (referred to as Volunteers A, B, C and D). The correlation time is taken here as a unitless measurement of relative changes in blood flow with time. These results were compared for the recruited volunteers for at rest and during arterial blood flow occlusion. During ischemic condition, significant decrease in mean correlation time following simulated stress using cuff pressure occlusion method can be observed among these volunteers. Although these changes were not visually evident in the blood flow map, quantitative speckle contrast value determined via fitting indicated a constant reduction in flow. A higher mean ± standard deviation (SD) τ value of 3.92 ± 1.47 is calculated from Table I for volunteers at rest, this value dropped to 2.90 ± 1.39 when blood flow was obstructed. The mean correlation time values for different experimental settings was also analyzed and compared using a paired samples t-test in MaxStat statistical software (MaxStat Lite, Germany) with confidence level of 90%. The correlation test result revealed a significant value of ρ = 0.087.

Table I Correlation time value, τ, from the processed speckle contrast images

Volunteers	Correlation time value (τ)	
	At rest	During occlusion
A	5.05	2.85
B	2.04	1.29
C	3.45	2.79
D	5.11	4.68

The result in Table I shows continuous blood velocity (illustrated by higher speckle contrast values) when the muscle is relaxed. Meanwhile the simulated stress using cuff pressure occlusion method resulted in a decrease in speckle contrast value. This is further supported by the results shown in Table I that revealed a considerable decrease in the mean correlation time following the application of pressure on upper left arm of the recruits using the developed low cost laser speckle imaging system. These different experimental settings were decided following commonly used strategies in other works [27, 38] for the investigation of blood flow velocity and microcirculatory activities. In addition, a study of temporal variability of blood oxygen saturation conducted in our counterpart paper also showed that a pressure of 140 mmHg is able to shut the flow of arterial blood into the lower arm [39].

The trend in the calculated τ value shown in Table I agreed considerably well with that hypothesized in this study, wherein a drop in the mean from 3.92 for at rest experiment to 2.90 during blood flow occlusion is observed. This is supported by the ρ value obtained from MaxStat*t*-test (ρ = 0.087), which showed a positive association between the calculated correlation value and the experiment conditions. It is important to note the relatively consistent fluctuation in the τ value, which mean standard deviation value is given by 1.47 and 1.39, respectively, for at rest and during occlusion. This is likely to be contributed by the considerably consistency in the arterial vasomotion response system of each individual following external intervention.

The results presented in this work can only serve as a reference as these values may differ among individuals depending on physical health and skin pigmentation. The discrepancy in the predicted correlation time observed among the recruited volunteers (particularly for that observed in Volunteer D during occlusion experiment) may be contributed by several factors, which include differences in cardiac output and blood flow of each individual. The latter is often associated with gender differences, age, and other health conditions such as myocardial blood flow changes in postmenopausal women [40]. In addition, temporal differences in blood flow could also be influenced by hemodynamic factors such as muscle and cardiac contractions. Other factors that might affect the predicted correlation time value are the motion artifacts and presence of noise in the system. These effects, however, can be minimized by increasing the integration time of the detector system. It should also be noted that the location of blood vessel and skin thickness might affect the consistency of the measurement data, hence, the need to improve the developed strategy. Studies are still being carried out to ascertain these hypotheses.

The laser speckle system used in this work employed a market available 650 nm laser diode to replace the commonly used Helium-Neon (HeNe) 633 nm laser source for illumination of the selected skin site. It must also be mentioned that an advantage to this developed system is its capability to monitor changes in microvascular blood flow at the selected skin region without the need of a contrast agent. This work shows that a 650 nm laser diode was able to produce equally satisfying visible speckle patterns of blood flow as demonstrated in Fig.3 and Table I. Typically, a red laser diode has a power rating between 0.5 – 10 mW while a 633 nm HeNe laser can reach up to a maximum power rating of 75 mW, which in turn would give a better beam quality and coherence [41]. Nonetheless, results obtained from this work showed that the low power rating laser diode could be used as an alternative source for optical measurement of laser speckle patterns with agreeable efficiency and good beam quality. In addition to the low cost attribute, the laser diode has a relatively smaller size than the HeNe laser tube, which makes it suitable to be used and integrated into a field portable optical system shown in Fig.1.

It is to be noted, however, that this research is still in its initial stage. Although the results shown in this work featured preliminary characteristics, they reveal potential possibilities where the described technique can be further developed in various aspects. Future work would suggest recruiting more volunteers with diverse health backgrounds to further validate the reliability of the developed system. Practically, a commercially available Doppler flowmeter can be used as a comparison standard to confirm the findings of this study. Additional suggestions would include modifying the optical arrangement of the imaging system for an improved signal quality for analysis of blood flow velocity distribution by means of a speckle contrast measurement. Recent advances in spectroscopy imaging technique would suggest a frequency domain multispectral system that will simultaneously measure blood flow and tissue oxygenation. These aspects should take into account several factors such as exposure duration, signal to noise ratio, temporal coherence of the laser source and correlation time.

IV. CONCLUSION

This paper demonstrated the use of a low cost 650 nm laser source and a monochromatic CCD imager for *in-vivo* measurement of blood flow perfusion. Overall, laser speckle contrast analysis performed on the raw images collected in this work was able to reveal differences in results in response to blood microcirculatory and hemodynamic changes. Another highlight of this study is the ability of the employed system to map changes in blood flow without the requirement of a contrast agent. This work concluded that the blood flow quantification strategies explored in this work may be able to provide a better understanding of one's blood perfusion with revascularization. This, in return, could prove to be beneficial in evaluating the outcome of skin grafting especially during clinical assessment of diabetic foot ulcer recovery progress. This experimental work was supported by statistical analysis to confirm the repeatability of the obtained measurements.

V. ACKNOWLEDGMENT

This work is financially supported by the Ministry of Education Malaysia under FRGS grant no. 1581 and UniversitiTun Hussein Onn Malaysia under GPPS grant no. U935.

REFERENCES

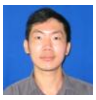
1. Humeau-Heurtier, A., et al., *Relevance of laser Doppler and laser speckle techniques for assessing vascular function: state of the art and future trends.* IEEE transactions on biomedical engineering, 2013. 60(3): p. 659-666.
2. Cracowski, J.L. and M. Roustit, *Current methods to assess human cutaneous blood flow: An updated focus on laser-based-techniques.* Microcirculation, 2016. 23(5): p. 337-344.
3. Sitohy, B., et al., *Early Actions of Anti–Vascular Endothelial Growth Factor/Vascular Endothelial Growth Factor Receptor Drugs on Angiogenic Blood Vessels.* The American journal of pathology, 2017. 187(10): p. 2337-2347.
4. Galldiks, N., K.-J. Langen, and W.B. Pope, *From the clinician's point of view-What is the status quo of positron emission tomography in patients with brain tumors?* Neuro-Oncology, 2015. 17(11): p. 1434-1444.
5. Bailey, D.L. and K.P. Willowson, *Quantitative SPECT/CT: SPECT joins PET as a quantitative imaging modality.* European journal of nuclear medicine and molecular imaging, 2014. 41(1): p. 17-25.
6. Flotats, A., et al., *Hybrid cardiac imaging: SPECT/CT and PET/CT. A joint position statement by the European Association of Nuclear Medicine (EANM), the European Society of Cardiac Radiology (ESCR) and the European Council of Nuclear Cardiology (ECNC).* European journal of nuclear medicine and molecular imaging, 2011. 38(1): p. 201-212.
7. Padole, A., et al., *CT radiation dose and iterative reconstruction techniques.* American Journal of Roentgenology, 2015. 204(4): p. W384-W392.
8. Desiderio, M.C., et al., *Contemporary patient radiation exposure from PET versus spect MPI.*Journal of the American College of Cardiology, 2018. 71(11): p. A1489.
9. Guariguata, L., et al., *Global estimates of diabetes prevalence for 2013 and projections for 2035.* Diabetes research and clinical practice, 2014. 103(2): p. 137-149.
10. Purwaningsih, T. and B. Machmud, *Influence of overweight and obesity on the diabetes in the world on adult people using spatial regression.* International Journal of Advances in Intelligent Informatics, 2016. 2(3): p. 149-156.
11. Tam, C.L., et al., *Investigating diet and physical activity in Malaysia: education and family history of diabetes relate to lower levels of physical activity.* Frontiers in psychology, 2014. 5: p. 1328.
12. Gnyawali, S.C., et al., *Retooling Laser Speckle Contrast Analysis Algorithm to Enhance Non-Invasive High Resolution Laser Speckle Functional Imaging of Cutaneous Microcirculation.* Scientific reports, 2017. 7: p. 41048.
13. van den Berg, P.J., *Integrated photoacoustic/ultrasound imaging: applications and new techniques.* 2017.
14. Harun, N.H., et al., *Fusion noise-removal technique with modified dark-contrast algorithm for robust segmentation of acute leukemia cell images.* International Journal of Advances in Intelligent Informatics, 2018. 4(3): p. 202-211.
15. Basak, K., et al., *Learning of speckle statistics for in vivo and noninvasive characterization of cutaneous wound regions using laser speckle contrast imaging.* Microvascular research, 2016. 107: p. 6-16.
16. Philimon, S.P., et al. *Multispectral imaging system for clinical assessment of superficial wound tissue oxygenation*. in *2016 IEEE EMBS Conference on Biomedical Engineering and Sciences (IECBES)*. 2016. IEEE.
17. Huong, A., S. Philimon, and X. Ngu, *Multispectral imaging of acute wound tissue oxygenation.* Journal of Innovative Optical Health Sciences, 2017. 10(03): p. 1750004.
18. Schreml, S., et al., *Oxygen in acute and chronic wound healing.* British Journal of Dermatology, 2010. 163(2): p. 257-268.
19. Dunn, A.K., *Laser speckle contrast imaging of cerebral blood flow.* Annals of biomedical engineering, 2012. 40(2): p. 367-377.
20. Bliedtner, K., et al. *Towards real time speckle controlled retinal photocoagulation*. in *Ophthalmic Technologies XXVI*. 2016. International Society for Optics and Photonics.
21. Rege, A., et al., *In vivo laser speckle imaging reveals microvascular remodeling and hemodynamic changes during wound healing angiogenesis.* Angiogenesis, 2012. 15(1): p. 87-98.
22. Li, P., et al., *Imaging cerebral blood flow through the intact rat skull with temporal laser speckle imaging.* Optics letters, 2006. 31(12): p. 1824-1826.
23. Wang, L., et al., *Anterior and Posterior Optic Nerve Head Blood Flow in Nonhuman Primate Experimental Glaucoma Model Measured by Laser Speckle Imaging Technique and Microsphere MethodOptic Nerve Blood Flow in Experimental Glaucoma.* Investigative ophthalmology & visual science, 2012. 53(13): p. 8303-8309.
24. Shiba, C., et al., *Relationship between glycosylated hemoglobin A1c and ocular circulation by laser speckle flowgraphy in patients with/without diabetes mellitus.* Graefe's archive for clinical and experimental ophthalmology= Albrecht von Graefes Archiv fur klinische und experimentelle Ophthalmologie, 2016. 254(9): p. 1801-1809.
25. Shepherd, A., *History of laser-Doppler blood flowmetry*, in *Laser-Doppler blood flowmetry*. 1990, Springer. p. 1-16.
26. Draijer, M., et al., *Review of laser speckle contrast techniques for visualizing tissue perfusion.* Lasers in medical science, 2009. 24(4): p. 639-651.
27. Wang, J., et al., *Dual-wavelength laser speckle imaging to simultaneously access blood flow, blood volume, and oxygenation using a color CCD camera.* Optics letters, 2013. 38(18): p. 3690-3692.
28. Briers, D., et al., *Laser speckle contrast imaging: theoretical and practical limitations.* Journal of biomedical optics, 2013. 18(6): p. 066018-066018.
29. 29. Goodman, J.W., *Statistical optics*. 2015: John Wiley & Sons.
30. Fercher, A. and J.D. Briers, *Flow visualization by means of single-exposure speckle photography.* Optics communications, 1981. 37(5): p. 326-330.
31. Humeau, A., et al., *Laser Doppler perfusion monitoring and imaging: novel approaches.* Medical & biological engineering & computing, 2007. 45(5): p. 421.
32. Parthasarathy, A.B., et al., *Robust flow measurement with multi-exposure speckle imaging.* Optics express, 2008. 16(3): p. 1975-1989.
33. 33. Huang, Y.C., et al., *Noninvasive blood flow imaging for real-time feedback during laser therapy of port wine stain birthmarks.* Lasers in surgery and medicine, 2008. 40(3): p. 167-173.
34. Richards, L.M., et al., *Low-cost laser speckle contrast imaging of blood flow using a webcam.* Biomedical optics express, 2013. 4(10): p. 2269-2283.
35. He, H., et al., *Lateral laser speckle contrast analysis combined with line beam scanning illumination to improve the sampling depth of blood flow imaging.* Optics letters, 2012. 37(18): p. 3774-3776.
36. Dunn, A. and W.J. Tom, *Methods of producing laser speckle contrast images*. 2014, Google Patents.
37. Kirkpatrick, S.J., D.D. Duncan, and E.M. Wells-Gray, *Detrimental effects of speckle-pixel size matching in laser speckle contrast imaging.* Optics letters, 2008. 33(24): p. 2886-2888.

38. Huong, A.K. and X.T. Ngu, *In situ monitoring of mean blood oxygen saturation using Extended Modified Lambert Beer model.* Biomedical Engineering: Applications, Basis and Communications, 2015. 27(01): p. 1550004.
39. Huong, A., S. Philimon, and X. Ngu. *Noninvasive monitoring of temporal variation in transcutaneous oxygen saturation for clinical assessment of skin microcirculatory activity*. in *International Conference for Innovation in Biomedical Engineering and Life Sciences*. 2015. Springer.
40. Brown, A.D., et al., *Effects of cardiorespiratory fitness and cerebral blood flow on cognitive outcomes in older women.* Neurobiology of aging, 2010. 31(12): p. 2047-2057.
41. Hecht, J., *Helium neon lasers flourish in face of diode-laser competition.* Laser focus world, 1992. 28(11): p. 99-108.

AUTHORS PROFILE

Sheena PunaiPhilimonis a PhD. student under the Department of Electronic Engineering, Faculty of Electrical and Electronic Engineering, UniversitiTun Hussein Onn Malaysia. Her areas of research interest are in biomedical optics and spectroscopic imaging.

Assoc. Prof. Dr. Audrey HuongKahChing is a senior lecturer at the Department of Electronic Engineering, Faculty of Electrical and Electronic Engineering, UniverstiTun Hussein Onn Malaysia.Her areas of research interest are in biomedical optics, polarization microscopy, and spectroscopic imaging.



Dr. Xavier NguTohIk is a senior lecturer at the Department of Communication Engineering,Faculty of Electrical and Electronic Engineering, UniverstiTun Hussein Onn Malaysia. His areas of research interest are in electromagnetic compatibility and biomedical imaging.