

A Novel Design of an Optical Probe for Detecting Perfusion Changes in Buccal Tissue

Maryam Amini¹, Terje Gjøvaag², Jonny Hisdal³, Peyman Mirtaheri*¹

¹Faculty of Technology, Art and Design, Oslo and Akershus University College of Applied Sciences, Oslo, Norway

²Faculty of Health Sciences, Oslo and Akershus University College of Applied Sciences, Oslo, Norway

³Section of Vascular Investigations, Oslo Vascular Centre, Oslo University Hospital Aker, Oslo, Norway

Abstract- Measuring the blood perfusion and microcirculation at a specific depth in human tissue is an important method to monitor oxygenation in critically ill patients. The optical probes that are currently available in the market are not capable of monitoring the blood perfusion from a given tissue layer and at a specific depth. We have designed a novel optical probe which is able to focus the light at a specific depth of $\sim 670 \mu\text{m}$ in buccal tissue. The new probe consists of two light guides for sending and detecting the reflected light from the sampling area (tissue), and a lens to focus the light into the desired depth of the tissue and collect it back from the same area. The new optical probe has been compared to a commercial laser Doppler probe by collecting data from the same sampling area. The result from our probe showed 15% higher accuracy in detecting changes in blood perfusion compared with an existing commercial probe.

Keywords : Laser Doppler Flowmetry; Optical focus; Probe design; Vasodilatation; Carbon dioxide

I. INTRODUCTION

Inadequate tissue perfusion and oxygenation are likely to contribute to the development of organ failure and increased mortality in critically ill patients and consequently assessment of adequacy of oxygen supply to tissues and organs is essential [1]. Thus, there is a need for a real-time and accurate method to monitor the blood perfusion in the tissue of interest [2-3]. Since the heart and brain are prior organs to receive the oxygen supply, the perfusion of the digestive

* corresponding author's e-mail address: peyman.mirtaheri@hioa.no

system with oxygenated blood will be minimized due to the “luxury metabolism” in low oxygenated conditions [4]. Since the buccal tissue and the digestive system are nourished by the same blood vessels and innervated by the same nerves, the buccal tissue perfusion is considered as a global marker of tissue perfusion [5].

Laser Doppler flowmetry (LDF) is one of the most commonly used noninvasive techniques which enable sensitive and continuous real-time monitoring of the tissue perfusion [6-7]. In LDF technique the reflective light does not collect data from a specified target area and the LDF instrument measures the average blood perfusion in different layers of the tissue (see figure 1). This may result to a reduction of selectivity and sensitivity of the measurements.

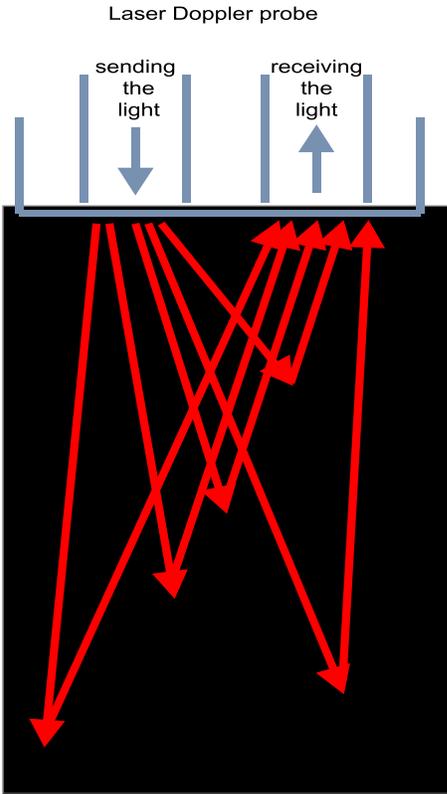


Figure 1 .Illustrates the principle of a reflective optical probe used in Laser Doppler flowmetry technique. The measurements are an average of all tissue layers [8].

There have been attempts to eliminate the error from superficial tissue layers in reflectance optical probes by using a “two-distance” fiber optic probe (see figure 2) [9, 10]. Although the arrangement of the fibers, light source or detector may be different in each design, the common idea was using a light source and two detectors for different depths (see figure 2) which may reduce the effect from the superficial tissue.

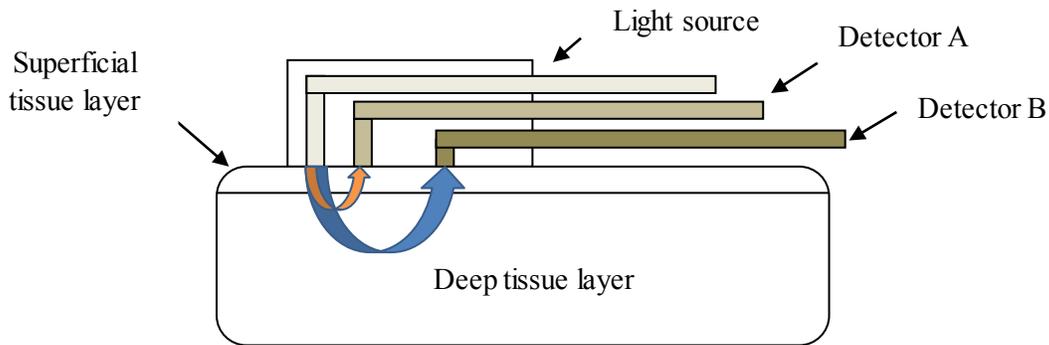


Figure 2. Reflectance probe designed for minimizing the effect of the superficial tissue layer.

Although this design solution may function in a combination of skin and muscle tissue, the distance between the light source and detector A may be difficult to achieve in other tissue layers such as buccal tissue with an epithelium layer of about 500-600 μm [5]. Moreover, the instrumentation for such a design has to either have two detectors or a multiplexer that shares the detector between two optical probes A and B which indeed increases the cost of the instrument.

In this report, we present a novel design of a noninvasive optical probe for perfusion measurements at a defined depth (i.e. in deeper tissue layers beneath epithelium), using the Laser Doppler flowmetry technique. The newly designed probe contains two optical fibers, a lens and a titanium frame. We have compared the novel probe with a commonly used optical probe by measuring the perfusion in buccal tissue during stimulation by a vasodilation agent (CO_2).

II. MATERIALS AND METHODS

A. Probe Design

In order to focus the light at the certain depth of a tissue, we arranged the fibers and a lens as illustrated in figure 3. The epithelium of buccal tissue is around 500-600 μm thick and most of the capillaries are found in a depth of 600 to 700 μm in buccal tissue [5]. In the current design we therefore chose to focus the light at a depth of 670 μm where the capillaries are located and this will enable us of a more sensitive and precise detection of changes in tissue perfusion.

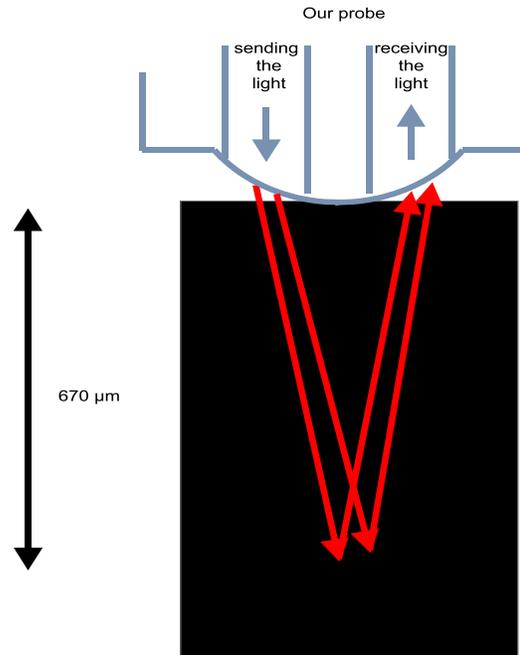


Figure 3. The expected focal point from the lens in buccal tissue and the effective measurements would be about 670 μm which is within 600-700 μm of capillary rich buccal tissue [8].

In order to achieve a focal point that is 670 μm deep in tissue, the radius of the lens, shown in figure 4, can be calculated by using the following equation:

$$\frac{f}{r} = \frac{n}{\sqrt{n^2 - 1}} - 1$$

Where f is the focal point in mm , r is the lens radius in mm and n is the index of refraction of the lens, respectively.

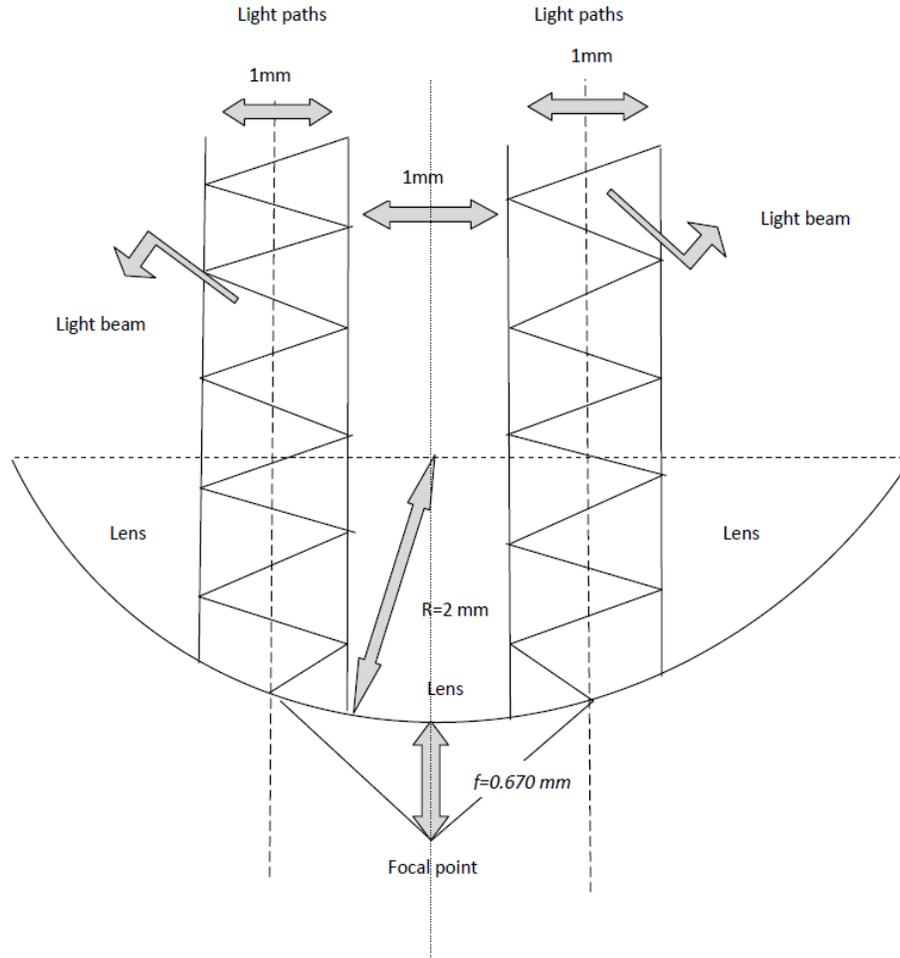


Figure 4. The arrangement of the optical fibers and the lens in order to achieve a focal point inside of $670\mu m$.

B. Probe Fabrication

A prototype probe assembly was made of medical grade titanium. Titanium was chosen due to its high biocompatibility properties and also its mechanical robustness. A computer numerical control (CNC) machine (Nexus 100-II MY, Mazak, Japan) was used to manufacture the probe parts. The probe consists of two symmetrical pieces which contain two pathways of a depth of 0.5 mm on each piece of the probe.

Guiding both the incident and the reflected light within optical fibers towards and back from the buccal tissue would demand a 90° bending of the optical fibers which would lead to optical noise and signal attenuation.

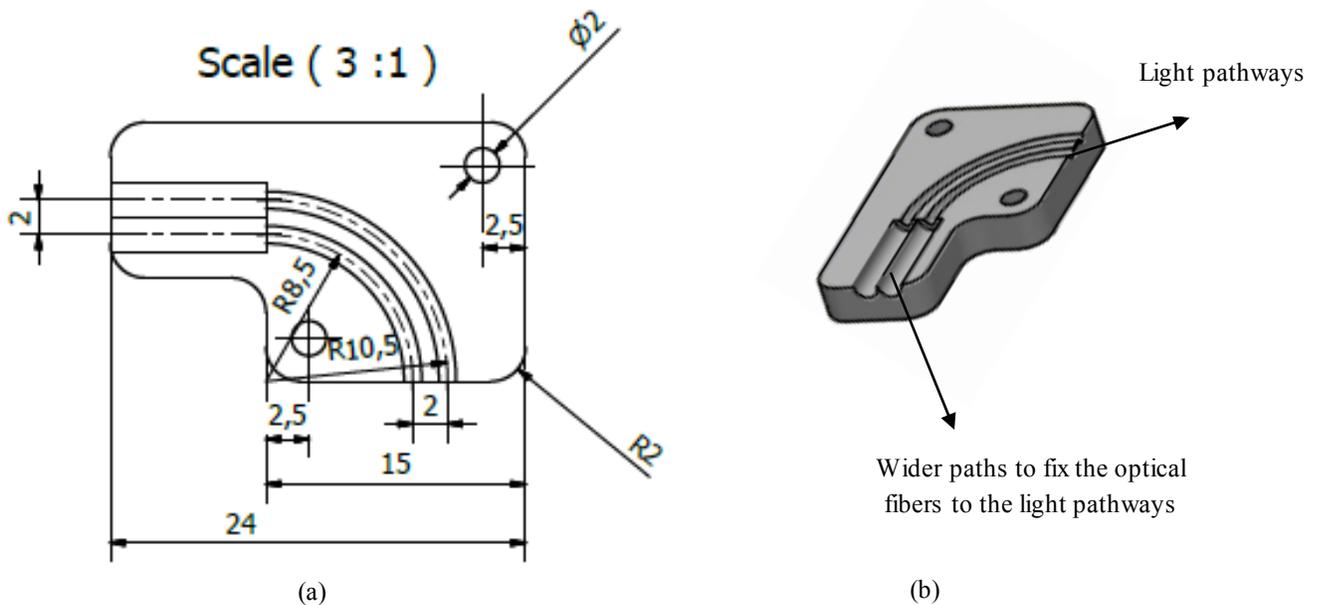


Figure 5. Showing: (a) Top view, (b) 3D view drawings of the optical probe and its respective dimensions in millimeters.

The dimensions of the cylindrical optical guides were calculated based on the dimensions of the optical fiber attaching to the probe (core: 1 mm, core with cover: 2 mm). The distance between two fiber optics was chosen such a way to prevent the cross-talk effect at the output. The two pieces of the probe were aligned with two small screws and the pathways filled with optical glue while the fibers were placed to the end of the probe. The optical glue (Norland Blocking Adhesive 107, Thorlabs, USA) has a refraction index of 1.5 which is the same index as the core of the optical fiber. The glue was then cured by UV light at 365 nm (UV curing system, Thorlabs, USA).

C. Molding the Lens and Assembly of the Probe

As indicated in figure 4, a Plano convex lens with a radius of 2 mm would focus the light from the two optical fibers in a depth of about 670 μm when the distance between the fibers is 1 mm. In order to avoid signal loss, the lens should have the same refraction index as its connective fiber optics.

We therefore manufactured the lens with the same optical glue as used in optical guides in the probe. An aluminum mold with the exact dimensions of the lens was fabricated. The aluminum mold was then filled with silicone (Elastosil RT 601 A/B, Wacker, Germany), creating a negative mold. The silicon mold was then cured in an oven with 200 °C for three hours and vulcanized at 70 °C for about 12 hours. The silicon mold was filled with the optical glue as in the optical guides in the probe (Norland Blocking Adhesive 107, Thorlabs, USA) and placed on the probe. Curing the glue was done through the silicone mold by UV light of 365 nm.

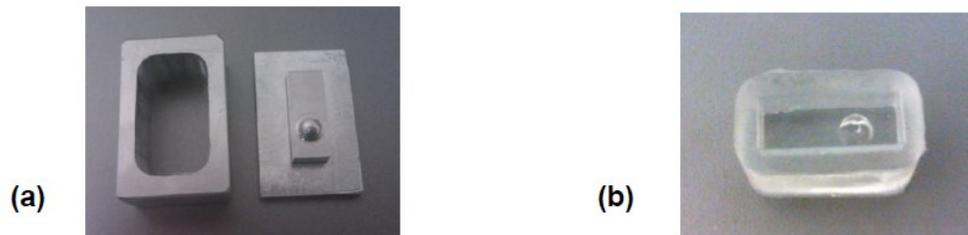


Figure 6. Showing: (a) Aluminum mold, (b) Silicone negative mold.

The lens dimension was further measured by a shadowgram (PJ-250C, Mitutoyo, USA) and the lens was manufactured again if the dimension of the lens was not within a tolerance of 5% of the lens radius. Figure 7 illustrates the assembled probe with its respected lens and two optical fibers connected.

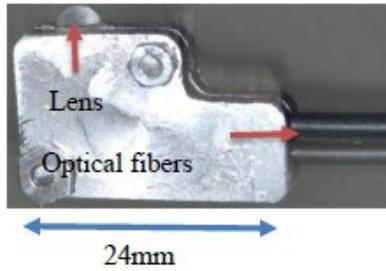


Figure 7. The assembled optical probe with its respective lens and optical fibers.

D. Application of the Probe on Tissue Bed

The high humidity inside the oral cavity as well as the movements due to breathing will make it difficult to keep the optical probe in a stable position on buccal tissue and any kind of pressure would affect the perfusion and introduce error in the measurements. To solve this problem, we took advantage of silicone gum shield used in contact sport and placed our probe in the opening area between upper and lower part of the gum shield with the lens facing the buccal tissue (figure 8). By using the teeth as a holder, we may decrease the motion artifacts, and any undesired pressure on the buccal tissue. A shrinking tube was used for stronger fixation of the optical fibers to the probe titanium mold which would also help to avoid bending of the optical fibers.



Figure 8. Fixation of the optical probe by means of a gum shield.

III. TESTING THE PROBE

A. Focal Depth Measurement

To test the focal depth of the probe, a measurement bench was made for this purpose (figure 9). The probe was fixed and aligned on an aluminum plate on one side of the bench. The optical fibers in the probe were each connected to a light source (Avalight-LED, Avantes, USA) with an LED of 780 nm (Thorlabs, USA). The reason for choosing such a wavelength was that the laser Doppler instrument which would be used later in our experiment transmits a Laser light with the wavelength of 785 nm.

The intensity of both LED's were measured with a photo detector (PDA10A, Thorlabs, USA) and adjusted by their input current in order to achieve an equal intensity level. We applied a testing probe with a diameter of 1 mm. This optical probe was aligned with the center line of the lens of our new probe and fixed on a movable wagon. Using this setup, we could measure the optical intensity of light within a distance of two centimeters (figure 9).

Further, the testing probe was connected to a spectrometer (AvaSpec-2048x14, Avantes, USA). By moving the testing probe in front of our optical probe on a straight line, we were able to detect spectra with peak at 780 nm. When changing the distance between our optical probe and the testing probe, it was possible to detect different intensities for the respective peaks of the spectra. The testing of the probe was repeated five times and the average of the measurements is presented in figure 10a which shows the highest intensity around 600 μm .

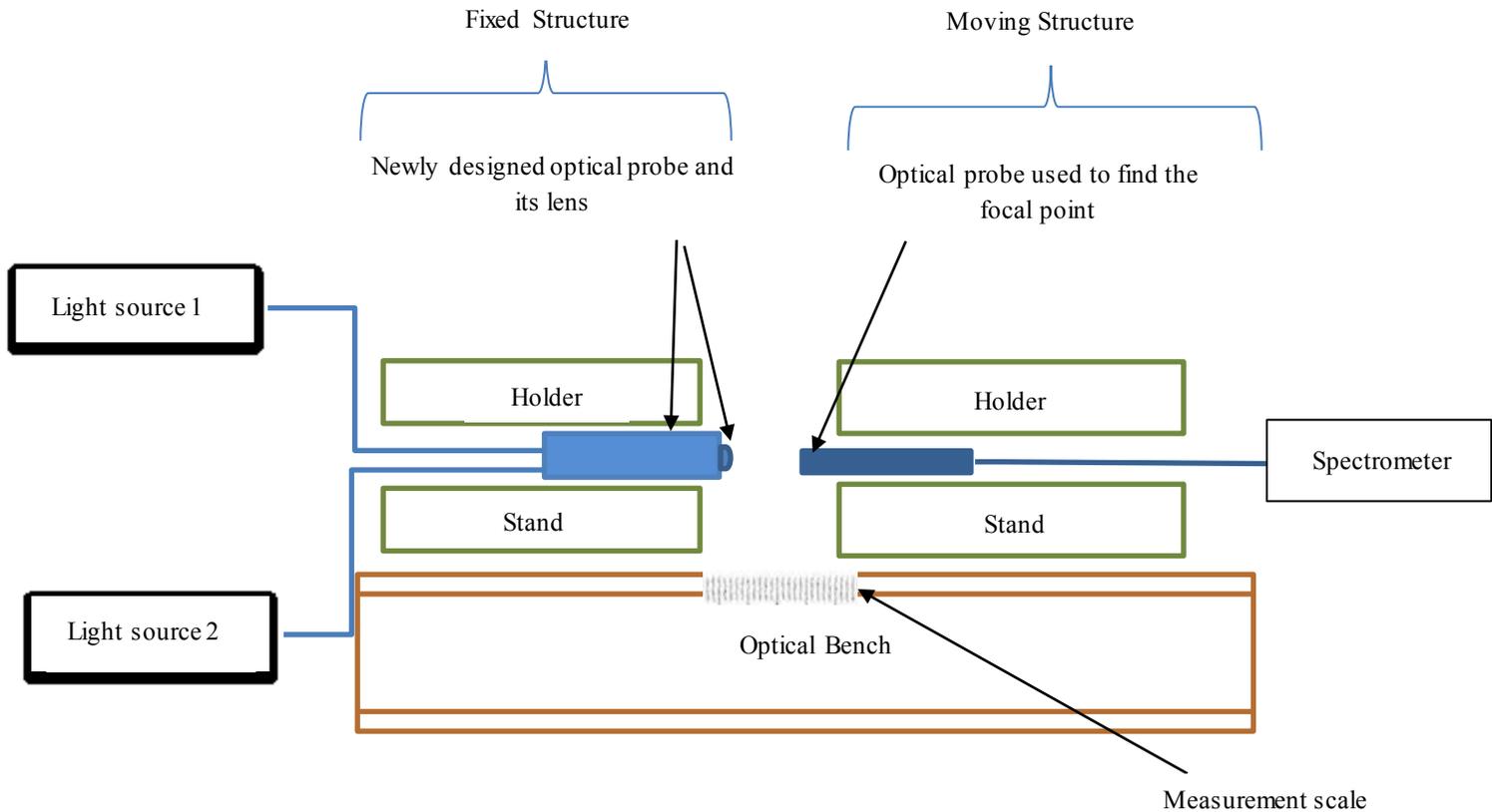
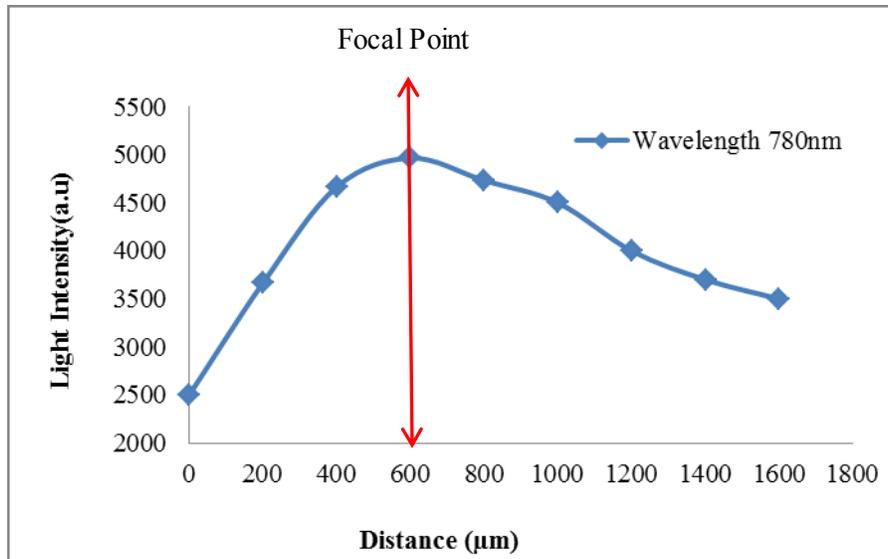
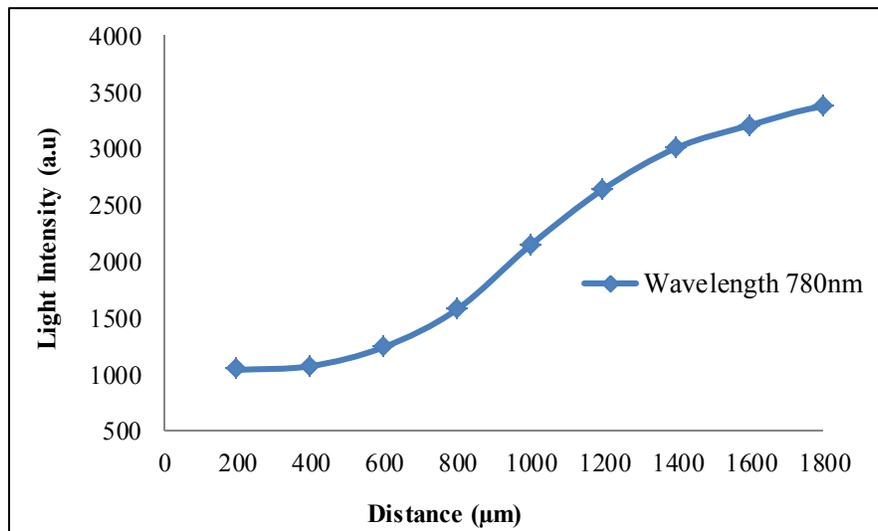


Figure 9. Schematic of the focal depth measurement setup.

In order to show the functionality of the lens, we performed another experiment without the lens. We performed three repetitions and the results as average of light intensity is presented in figure 10b. As shown in the figure, the intensity of the light is not the same at any distance which demonstrates the need for a lens to converge and focus the light to the desired depth.



(a)



(b)

Figure 10. Showing the results of the light intensity measurements when the detector optical probe was moved away from our optical probe: (a) new optical probe with lens, (b) without lens.

B. Flow Measurements in Buccal Tissue

In order to test the new probe in a biological environment, we carried out an experiment on a healthy subject, testing our new optical probe and another reference probe to measure the

perfusion, using the same laser Doppler instrument (VMS-LDF, Moor Instruments, UK) for both probes. The LDF instrument transmits a low power laser light with 785 nm wavelength through an optical probe to the tissue. The scattered light by the movement of blood cells is reflected back and collected by the optical probe. In this experiment, we applied a low profile laser Doppler probe (VP8c titanium disc, Moor Instruments, UK). The VP8c titanium disc probe has a 2.1 mm outside diameter flexible nylon sleeve with a titanium disc probe head. The probe head has 8 mm outside diameter and is 2.5 mm thick. The head has 4 holes drilled around the edges for sutures to provide stability during measurements. Light is delivered via a central window and at a right angle to the cable. The Doppler probe is described as suitable for use in the human oral cavity, with an estimated sampling depth of around 1 mm. We used a similar connector from the Moor instruments in order to connect the new designed optical probe to the Moor laser Doppler unit. The measurement setup is shown in figure 11.

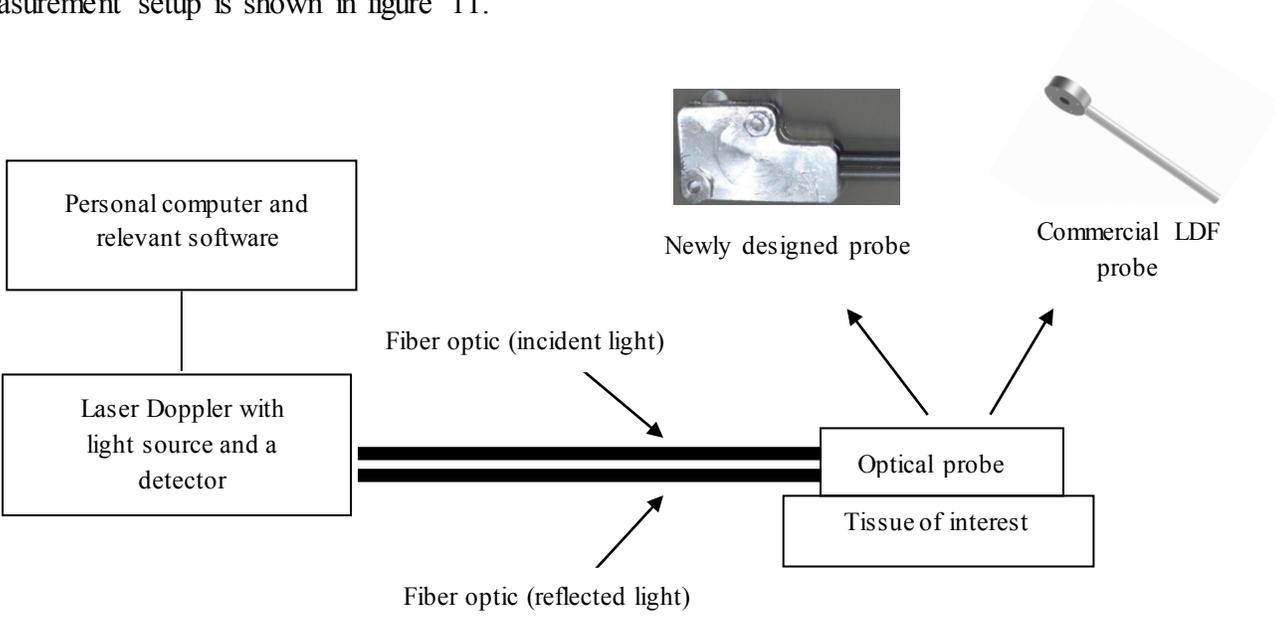


Figure 11. Schematic of the measurement setup for testing our optical probe with a laser Doppler flow meter produced by Moor Instruments, UK.

Perfusion in buccal tissue was continuously measured and recorded for a period of one minute. The subject was asked to chew an Orbit gum before and after the experiment to neutralize the pH in saliva. To minimize motion artifacts, the subject was instructed to avoid talking and moving the body. Also in order to be able to monitor only the local effects of CO₂ on the buccal tissue, the subject was asked to breath only through the nose. Carbon dioxide is known to have a powerful vasodilatative effect on the smooth muscle of blood vessels [12-13]. It also activates the sympathetic nervous system causing epinephrine and nor-epinephrine to be released [14]. The effect of CO₂ on cutaneous microcirculation has also been measured by laser Doppler flowmetry and it is concluded that CO₂ concentration is one of the factors that significantly affect blood flow [15-16]. Therefore, after one minute of recording the base line flow, 100% CO₂ (carbon dioxide) gas with a constant flow of 14.8 ml min⁻¹ was introduced in to the mouth cavity through tiny polyurethane tube for one minute. The box chart of the recorded and compared results are shown in figure 12 where perfusion changes monitored by both probes before and during application of CO₂ each for the period of 60 seconds are illustrated.

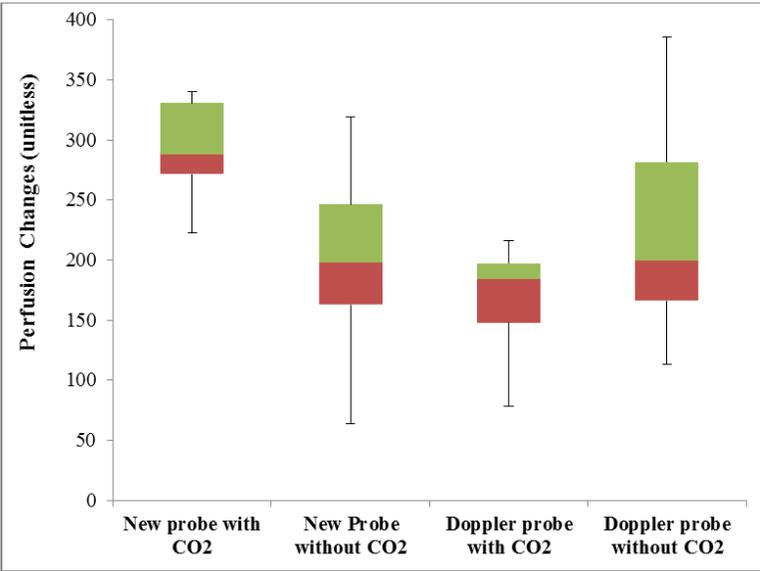


Figure 12.Box and whiskers plot of the distribution of perfusion changes.

monitored by both the new and Doppler probes, before and during the introduction of CO₂, each for the period of 60 seconds.(I am not sure about its being unit less, jonny has picked on it !!)

In order to compare the performance of the two probes, a comparison of Receiver Operating Characteristic (ROC) curves was performed. Typically, ROC curve has been used as a graphic mean for distinguishing different stimuli responses as to weak stimuli or no stimuli [11]. In a (ROC) curve the true positive rate (Sensitivity) is plotted as a function of the false positive rate (100-Specificity) for different cut-off points. Each point on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the ROC curve is a measure of how well a parameter can distinguish between two diagnostic groups e.g. diseased/normal [11]. The result obtained by using Medcalc (version 11.6) is presented in figure 13.

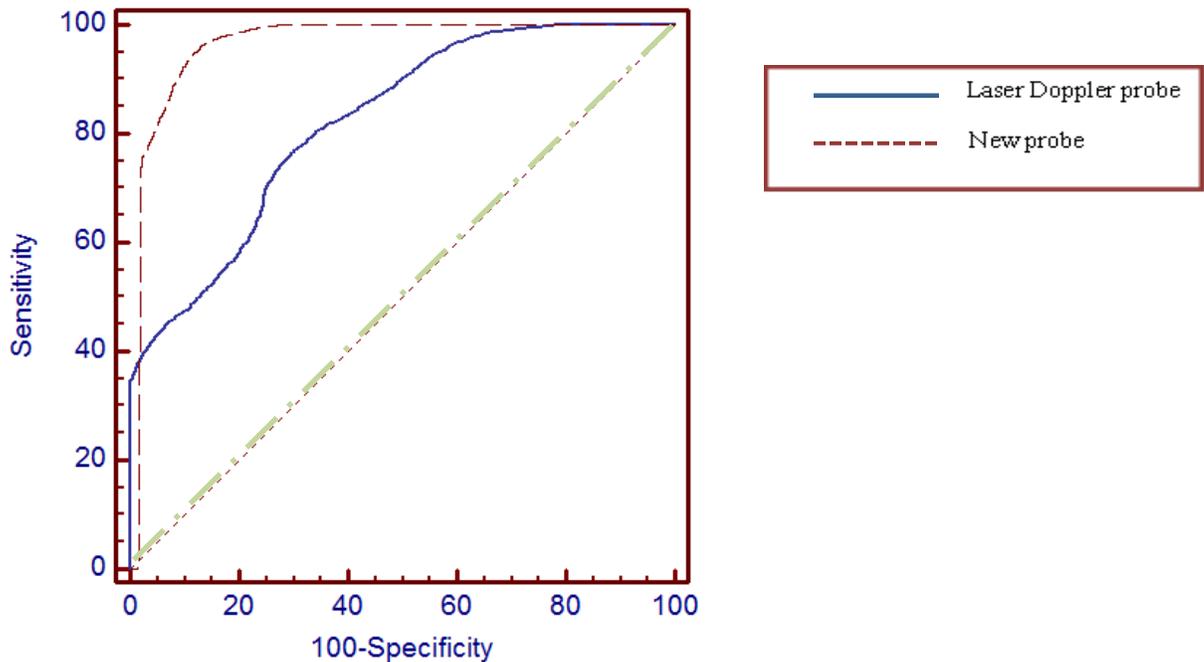


Figure 13. Showing ROC curves for laser Doppler probe and the new probe.

IV. DISCUSSION

Choosing the buccal tissue as the strategic position for monitoring perfusion, provides us with the opportunity of assessing a tissue in which the skin layer does not exist; therefore inducing the changes in perfusion with a vasodilator such as CO₂ gas would be easier.

The absence of capillaries in the epithelium of buccal tissue decreases the sensitivity of perfusion measurements with the commercial laser Doppler probes. We have now succeeded to design an optical probe with a focus point below the epithelium of the buccal tissue which enables us of sensitive and precise perfusion measurements of the capillaries in such tissues.

The results indicate that the percentage of perfusion changes detected by our probe when the tissue is stimulated by 100% CO₂ is 70% of the expected perfusion changes whereas it is only 45% by Doppler probe. Consequently, when CO₂ is not applied to the tissue, percentage of perfusion changes is 30% with our probe and 55% with the Doppler probe. Assuming that an ideal probe should enable us of measuring 100% of the perfusion changes, we can conclude that our probe has ~ 30% error in measuring the true ???perfusion changes while this error increases to 55% with the Doppler probe. Also as shown in the box and whiskers plot (figure 12), the monitored perfusion changes by the new probe when CO₂ is applied is higher than the changes detected by laser Doppler probe . overall, results confirm 25% higher sensitivity of the new probe in detection of perfusion changes in comparison to the available laser Doppler probe. Additionally, as illustrated in figure 13, the comparison of the ROC curves shows 15% increase of accuracy of the new probe compared to the commercial laser Doppler probe. A test with perfect discrimination (no overlap in the two distributions) has a ROC curve that passes through

the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test [11]. The area under the curve (AUC) which can be used as an overall estimation of the accuracy of each test [11] was 0.821 for the laser Doppler probe and 0.964 for our probe.

It should be considered that during the main test on a human subject with laser Doppler, movement of the tissue or the fiber can always lead to errors in the readings. Although the available commercial flowmeters have been designed to decrease these errors as much as possible, errors caused by movement still seem to be inevitable. This is particularly expressed when the perfusion is at normal levels shown in figure 12. Nevertheless, the new probe design proves that the perfusion changes with local stimuli may still be in the detection range even with influence of these errors.

We would like to point out that as far as we know, there are not any other publications using a similar technique to measure the perfusion changes in buccal tissue in order to eliminate the effect from the superficial tissue layer.

V.CONCLUSION

A novel optical probe with the capability of measuring perfusion changes in a given depth is successfully developed. Using light pathways filled with optical glue allows the 90° of light bending with minimum scattering and cross talk. The light converging lens made of the optical glue, made it possible for measurements to be done at a specific and desired depth in buccal tissue. The findings imply that the novel probe seems to be more accurate than the available commercial laser Doppler probes. Capability of our probe to noninvasively monitor the perfusion

changes at a specific depth can be promising for applications in emergency units and operating rooms to aid with diagnosis of perfusion abnormalities.

ACKNOWLEDGMENT

The authors are indebted to Per Ø. Staff, Dean of the Faculty of Engineering and Birger Emblem, Executive Director of Engineering Faculty, Oslo university College, Norway, for their kind support of this work. Without their support, we were not able to initiate this project. Our special thanks to mechanical engineer Rune Orderløyken at Faculty of Engineering, Oslo University College for helping us to make the prototypes and molds used in this work.

REFERENCES

1. J. D. Lang, E., D.J., Luiz Poli defigueiredo, Steve Hays, Mali Mathru, and George C. Kramerr *A Novel Approach to Monitor Tissue Perfusion: Bladder Mucosal Pco₂, Paz, and pHi During Ischemia and Reperfusion*. *Journal of Critical Care*, 1992. **14**: p. 93-98.
2. M. C. Skala, P., G.M., *An Investigation of Fiber-Optic Probe Designs for Optical Spectroscopic Diagnosis of Epithelial Pre-Cancers* *Lasers Surg Med*, 2004. **34**: p. 25-38.
3. T. J. Pfefer, S., K.T., Ediger, M.N., Nishioka, N.S, *Multiple-fiber probe design for fluorescence spectroscopy in tissue*. *Applied Optics*, 2002. **41**: p. 4712-4721.
4. Weil, *Comparisons between sublingual and Gastric Tonometry during Hemorrhagic Shock*. *Chest*, 2000. **118**(4): p. 1127-1132.
5. C. A. Squier, W., P.W., *Structure and function of the oral mucosa and implications for drug delivery*, in eds. M.J. Rathbone. *Oral Mucosal Drug Delivery*, 1996: p. 1-26.
6. Stern, M.D., *In vivo evaluation of microcirculation by coherent light scattering*. *Nature*, 1975. **254**: p. 56-58.
7. M. N. Patino, M., F., Loyola-Rodriguez, J.P., Tenorio-Govea, E., Brito-Orta, M.D., *A novel procedure for evaluating gingival perfusion status using laser-Doppler flowmetry* *J Clin Periodontol*, 2005. **32**.
8. D.Harris, J.R.R., *Drug delivery via the mucous membranes of the oral cavity*. *J.Pharm.Sci*, 1992. **81**: p. 1-10.

9. . R. Soller, Y., Y., Landry, M.R., Soyemi, O.O., Shear, M.A., Anunciacion, D.S, *Simultaneous correction of the influence of skin color and fat on tissue spectroscopy by use of a two-distance fiber-optic probe and orthogonalization technique*. Optics Letters, 2005. **30**: p. 2269-2271.
10. M. Nogawa, K., T., Takatani, S, *A Novel Hybrid Reflectance Pulse Oximeter Sensor with Improved Linearity and General Applicability to Various Portions of the Body*. Proceedings of the 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society, 1998. **20**.
11. DeLong ER, DeLong DM, Clarke-Pearson DL, *Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach*. Biometrics, 1988. **44**: 837-845.
12. Duling, B.R., *Changes in microvascular diameter and oxygen tension induced by carbon dioxide*. Circulation Res., 1973. **32**: p. 370-376.
13. R. T. Jackson, C., A.A. and Pollock, R.A. , *The effect of carbon dioxide inhalation on cerebral blood flow: A two-hour duration study in dogs with microspheres*. Stroke, 1974. **5**: p. 344-349.
14. M. Elam, Y., T., Thoren, P. and Svensson, T.H. , *Hypercapnia and hypoxia: chemoreceptor-mediated control of locus coeruleus neurons and splanchnic, sympathetic nerves*. Brain Res., 1981. **222**: p. 378-381.
15. W. Schnizer, E., R., Schoeps, P. and Seichert, N. , *The effects of external CO2 application on human skin microcirculation investigated by laser-Doppler flowmetry*. Int. J. Microcirc. Clin. Exp., 1985. **4**: p. 343-350.
16. H. Svensson, S., P., Holmberg, J. and Jacobsson, S. , *Laser Doppler flowmetry and transcutaneously measured carbon dioxide tension for observing changes of skin blood flow in fingers*. Stand. J. Plast. Reconstr. Surg., 1983. **17**: p. 183-186.