Determining the optimal site for imaging the microcirculation in neonates using Sidestream Dark – Field imaging

D. Sklia[1], P.A. Kyriacou[1] and A.J. Petros[2]

[1] School of Mathematics, Computer Science and Engineering, City University London, London, UK
[2]Great Ormond Street Children's Hospital, London, UK

Abstract— **Sidestream dark field (SDF) spectral imaging has been used to take pictures of capillaries within the skin. The most common location to place the SDF is the mucosa area in the mouth. However, in neonates the sublingual area is not easily accessible, thus an optimal location on the skin should be found. This feasibility study aims to determine the optimal site, out of five body areas on the neonate from which to obtain image of capillary microcirculation. These preliminary results indicate that the chest wall can be considered as the optimal measurement location on the skin when using the SDF modality.**

Keywords— **Sidestream dark field, imaging, neonate, capillaries**

I. Introduction

Several investigators have reported microcirculatory alterations in critically ill patients and particularly in patients with severe sepsis and septic shock. These alterations include a reduction in vessel density and an increased proportion of non-perfused or intermittently perfused capillaries. The persistence of these alterations is related to the development of organ failure and death [1].

The lack of adequate techniques to investigate the microcirculation has previously hampered human studies, as the study of microcirculation has long required the use of bulky microscopes, limiting the investigation to the nailfold area. Laser Doppler and photoplethysmography have demonstrated compromised microvascular blood flow in septic patients, but these modalities provide only a global measurement of microvascular blood flow [2].

Orthogonal Polarization Spectral (OPS) imaging is a tool that allows direct observation of living tissue and the vascular networks therein and can detect treatment-associated changes in microcirculation in accessible mucosal surfaces in humans [3]. OPS provides images of blood vessels allowing visualization of the capillary architecture in the mucosal layer in adults. Sidestream Dark-Field (SDF) imaging is a further development of OPS.

Sidestream Dark-Field spectral imaging has been used to take pictures of capillaries within the skin. In adults, the underside of the tongue is the preferable site as the blood vessels are easily seen. The mouth of the neonate is too small to get under the tongue easily so other sited have to be explored.

Changes in microvascular perfusion are more obvious in premature neonates than any other patient group [4]. Neonates can constrict their cutaneous circulation dramatically and it has been suggested that the skin of neonates acts as a 'shock organ' [5].

This pilot study investigates five body areas using SDF in order to determine the optimal site on the neonates from which to obtain good quality images of the capillary microcirculation. The nature of the study is purely observational based on the visibility of the videos followed by the reports generated by the software.

II. Materials & Methods

A. Principle of SDF imaging

Sidestream Dark Field imaging utilizes a novel method of reflectance avoidance in which the illuminated and reflected light travel through independent pathways. SDF's aim of development was to improve microcirculation image quality and eliminated the need of an external light source for illumination. In this modality, a light guide is surrounded by green (520 nm) light emitting diodes (LEDs), chosen to correspond to an isobestic point in absorption spectra of oxyhemoglobin and deoxyhemoglobin to ensure optimal optical absorption by hemoglobin (Hb) in circulation red blood cells (RBCs) independent of the Hb oxygenation state [6]. The light from the LEDs is absorbed by the hemoglobin of erythrocytes and results in the ability to observe the flowing cells. The LEDs are concentrically placed at the tip of the probe, protected by a disposable cap, send penetrating green light deep into the tissue, illuminating the microcirculation from within [7]. The LEDs at the tip of the guide are optically isolated from the inner image-conducting core, and pump light. By not being in direct optical contact with the sensing central core of the probe, no direct surface reflections are allowed to interfere with the image of the microcirculation, thus it gives clear images of the microcirculatory structures and red, as well as white, blood cell flow. A 5 or 10 times magnifying lens is used to project the image onto a video camera, providing clear images of the capillar-

ies without blurring and allowing for the better computer automatic analysis of the images. The increased depth of light penetration into the tissue using SDF allows the deeper arterioles to be clearly observed in the sublingual area where it is usually placed. The imaging output on a monitor portrays RBCs flowing as a dark moving globules against a white/grayish background [8]. Imaging optimization correcting for motion-induced hemoglobin blurring (i.e., flowing RBCs) has been resolved by synchronizing the LED light pulse illumination with the CCD camera frame rate, resulting in intravital stroboscopy using short illumination intervals (13 ms). SDF imaging has the benefit of low-power LED illumination. This enables battery and (portable) computer operation for ambulatory and on-site (emergency) applications, where high modality is of great importance [9].

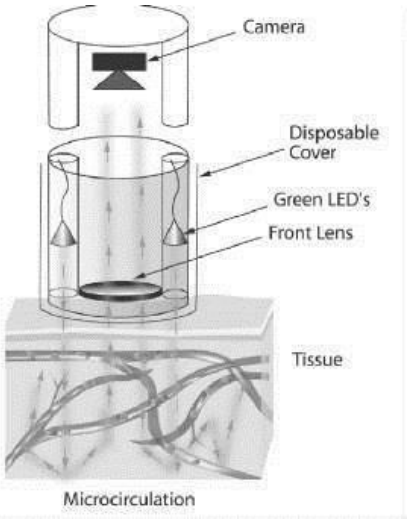

Fig. 1 Sidestream Dark Field imaging configuration [10]

B. Automated Vascular Analysis

Sidestream Dark-Field imaging is incapable to define blood vessels walls specifically; however, the change in contrast between hemoglobin within the red blood cells at the very edge of blood vessels and the surrounding tissue deduces their position. Computerized offline analysis with Automated Vascular Analysis (AVA) 3.2 software (Microvision Medical, Amsterdam, Netherlands) was used to determine blood vessel density and velocity in the videos acquired [11].

AVA 3.2 software helps analyzing microcirculatory video sequences in order to obtain quantitative and semi-quantitative (classification by eye) measurements of vessels density and of erythrocytes velocity [12].

C. Patients & Measurements

The protocol and study described were approved by the CRAC committee and the Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK (R&D ref. 13CC36), and carried out in accordance with the local Standard Operating Procedures to ensure compliance with good clinical practice.

A total of 10 neonates (4 males and 6 females, age range between 2-27 weeks) were recruited after consent by parents, in this observational study. All participants were virtually fully recovered and about to leave the unit. Some of the main exclusion criteria included neonates that are in an unstable condition, those required inotropes, being oscillated and being critically/terminally ill. Their pathological disease background (chest infection, respiratory failure, gastroschisis, hypoventilation, subdural hemorrhage from delivery, etc.) and their ethnic origin varied.

After enrollment, the SDF imaging measurements were performed to rule out the presence of clear images of capillaries.

Five body locations were chosen in order to obtain microcirculatory videos. The sites of measurement were (a) the bottom lip, (b) the upper ear, (c) the upper inner arm above the elbow, (d) the chest wall and (e) the fingertip nail bed. The duration of the videos acquired, was maximum 1 minute while the stable cuts lasted for a maximum 10 seconds.

The setup of the study included the SDF imaging device connected to an analog-to-digital converter (Blackmagic Design, Intensity Shuttle USB 3.0) displaying the images on a laptop. The videos were recorded on a secure database and stored on a secure Great Ormond Street Hospital computer for further analysis.

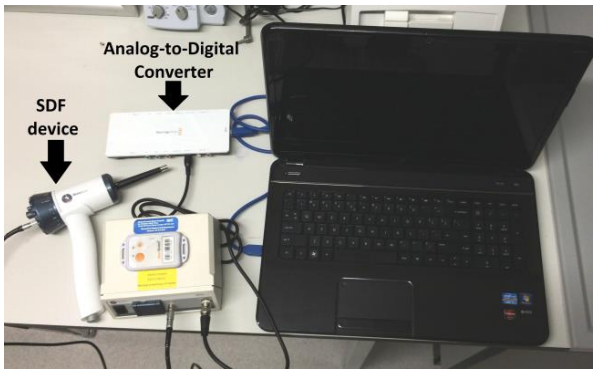

Fig. 2 The setup of the experiment includes the SDF imaging device (left, the ADC (up-left) and the laptop (right) running the AVA 3.2 software

III. RESULTS

A. SDF sampling images

The results showed that the *chest wall* was the most appropriate place on the skin to capture microcirculatory videos. This decision was mainly based on the quality of the images obtained while the verification came from the reports generated after semi-quantitative analysis using the AVA 3.2 software. Blurry videos due to several artefacts were not analyzed.

The following figures (Fig. 3, Fig. 4) are random screenshots from all measurement areas on the skin showing the capillaries and vessels of the microcirculation.

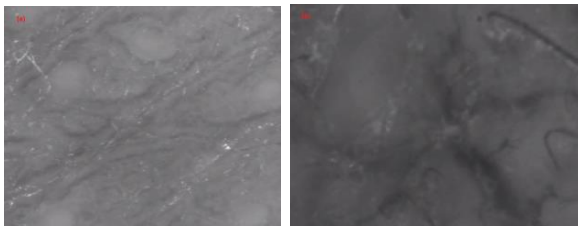

Fig. 3 (a) Chest wall and (b) Upper ear

Fig. 3(a) and 3(b) display clear images from the *chest wall* and *upper ear* respectively of two neonates. As we can see, the vessels are obvious and thus further analysis could be carried out by AVA.

The reports generated, include numerical velocity and density parameters and graphs that followed the sequence of each video.

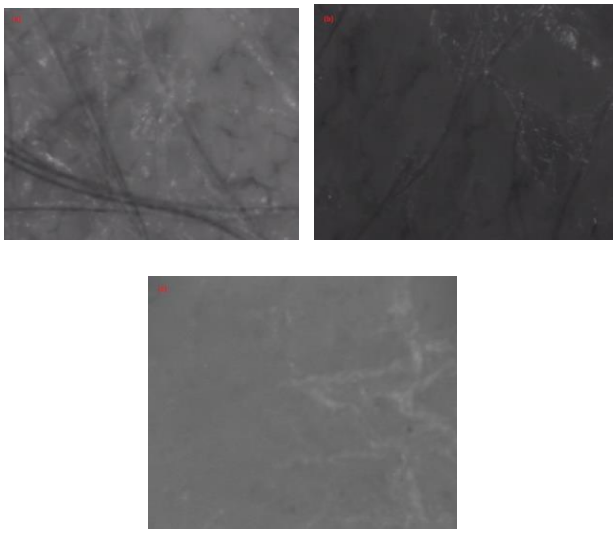

Fig. 4 (a) Upper arm, (b) Bottom lip and (c) Fingertip

The image from the *upper inner arm* (Fig. 4a) was obtained from only one patient who had thin skin at this body location.

Fig. 4(b) shows a relatively clear image of a neonate's *bottom lip*. However, we couldn't capture a stable and clear video because saliva bubbles and movement of the patient interfered with the measurement. The *finger* as presented in Fig. 4(c) showed no capillaries or red blood cells moving in any of the participants.

Table 1 summarizes the results of the measurements in respect to the images obtained. The X indicator symbolizes the body locations of each patient where no signal was captured due to several artefacts. The check mark reflects those sites that provide a sufficient image quality for further analysis. According to Table 1, the *chest wall* was the optimal measuring site, as 8 neonates out of 10 presented a relatively clear image, while the *bottom lip* and the *fingertip* considered to be the least adequate body locations for placing the SDF to measure microcirculation.

Table 1 Summary of patients versus the successful and unsuccessful measuring sites

Patients	**Body Locations**				
	Chest Wall	**Upper Ear**	**Upper Arm**	**Bottom Lip**	**Finger**
1	✓	✓	✗	✗	✗
2	✗	✓	✗	✗	✗
3	✗	✗	✗	✗	✗
4	✓	✓	✗	✗	✗
5	✓	✓	✗	✗	✗
6	✓	✓	✗	✗	✗
7	✓	✗	✗	✗	✗
8	✓	✗	✗	✗	✗
9	✓	✗	✓	✗	✗
10	✓	✗	✗	✗	✗

B. Artefacts

Melanin played a critical role as at the visible spectrum, where SDF operates (520 nm), the light absorption from melanin is high enough to interfere with the measurements on the skin [13] [14] [15]. Thus, in deep dark skin pigmented participants the imaging device couldn't display any image at the chosen body locations.

An additional parameter that affected the results was the thickness of the skin, mainly at the finger where no signal was captured on any volunteer leading us to the conclusion that the fingertip is a part of the neonatal anatomy that should be avoided for microcirculation measurements using the SDF.

Saliva on the bottom lip was creating bubbles on the tip of the probe therefore not allowing the capture of clear videos, and finally movement artefacts also restricted the capture of stable videos taken for longer times.

IV. CONCLUSION & DISCUSSION

The visibility of the videos (qualitative observations) captured by SDF allowed to correlate with the offline analysis, suggesting that the neonatal chest wall can be considered as the optimal measuring site on the skin when using the SDF technique.

The sample of the study was too small to perform any statistical analysis, however the numerical results via AVA software can be used to analyze the density and velocity of the vessels for each patient. Further studies need to take place in order to investigate this technique more rigorously in a neonatal population.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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