Diabetic Complications Consortium

Application Title: Validate tissue oxygenation biomarker in diabetic foot ulcers to assess healing using a low-cost hand-held optical imager

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1. Project Accomplishments:

- Developed a near-infrared optical scanner that is capable of both static and dynamic imaging, such that it can acquire spatio-temporal maps of tissues without contact.
- Preliminary validation studies on phantoms gave >85% correlation in measurements compared to standard spectrophotometer. Ongoing studies to quantify oxygenation changes using NIROS and validate it via phantom studies.
- Preliminary in-vivo studies on controls validated NIROS' ability to observe temporal changes in oxygen saturation with ~90% correlation to a commercial device.
- The project was part of a MS student's final project in Spring 2019. The ongoing studies are part of a doctoral student's research focus.
- Publications/Presentations: Work was presented as posters at 3 venues, and submitted an extended abstract for another conference. Peer-reviewed journal manuscript (related to validation studies) is under preparation for Spring 2020 submission.

2. Specific Aims:

AIM 1: Calibration and validation of TO-based biomarker from tissue phantom studies using NIROS.

(i) Phantom validation studies (without scatterers) using Near-Infrared Optical Scanner (NIROS) ([1,2]: Phantom studies on time-varying hemoglobin concentration changes are currently performed using our custom-developed hand-held NIROS against a commercial spectrophotometer (see Fig. 1). Methemoglobin (MHb) solutions were

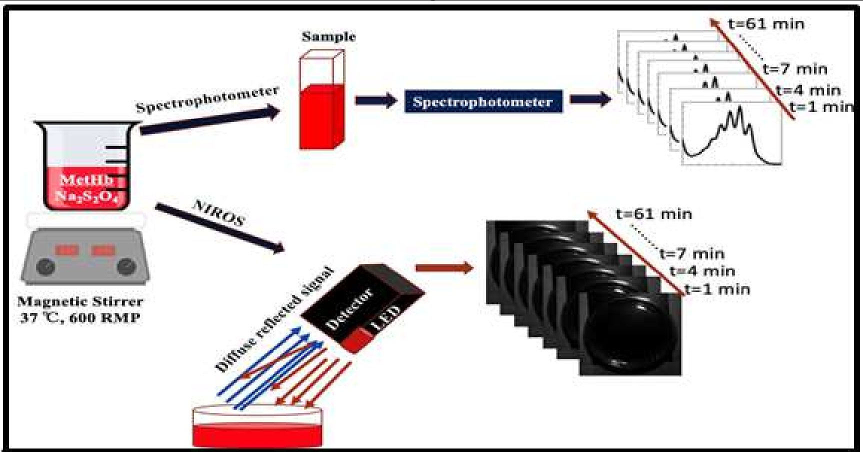

Fig. 1. Experimental set up for simultaneously implement spectrophotometer and NIROS

reduced to HbR using sodium hydrosulfite, which converted to HbO with time. From systematic phantom studies, the changes in oxygen saturation (ΔStO_2) with time were recorded using both the devices and >85% correlation was observed across the devices (see Figs. 2, 3). These studies were carried out in non-scattering medium. The study with inclusion of scatterers into the phantom medium is described in the next sub-aim.

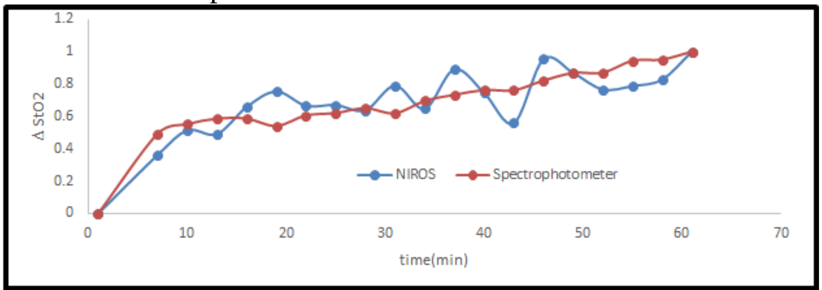

Fig 2. Change of oxygen saturation(ΔStO_2) of phantom with time using spectrophotometer and NIROS

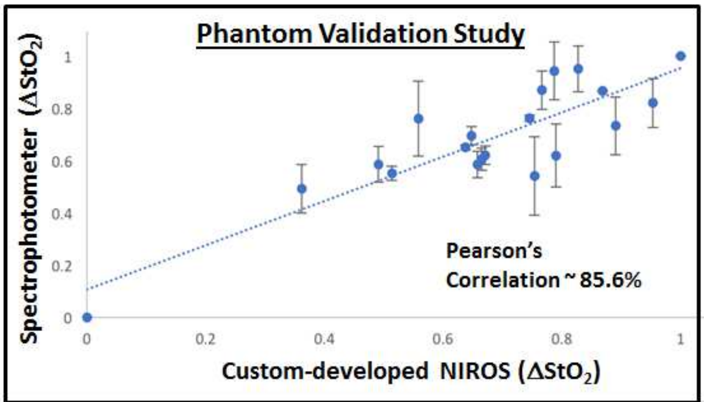

Fig. 3. Pearson's correlation of changes in oxygen saturation (ΔStO_2) from bovine hemoglobin based phantom studies using our custom NIR optical scanner (NIROS) and commercial spectrophotometer.

(ii) Phantom validation studies (with scattering agents) using NIROS (ongoing):

A scattering agent was introduced into the phantom medium (using diluted milk). Imaging was performed using NIROS, a thermometer, pH meter, and Dissolved Oxygen probe (see Fig. 4). Simultaneously during imaging, temperature, acidity and dissolved oxygen (ppm) was recorded throughout the reaction. It was found that there were no significant changes in pH throughout the reaction- which can affect the amount of oxygen in the solution. Using the temperature and dissolved oxygen measurements, oxygen saturation (StO_2) was calculated and compared over the 2 hour time span at 5 minute intervals (starting from minute 1). It was found that there was a significant loss (from 8.8 to 0.3 ppm) in saturated oxygen immediately following reduction (min 1) and that this value increased drastically until the 20 minute time stamp. The change became less significant until the 1 hour mark where no changes in StO_2 were observed. Oxygen saturated changes (ΔStO_2) measured using NIROS images were large between minute 0 and minute 1 and in the second hour shows no significant changes. Applying singular value decomposition to the NIROS data removed the effects of surface noise and the oxygen saturation changes were more distinct and consistent. Currently studies are ongoing to understand the noise floor of the NIROS device before correlating NIROS's data to DO meter's oxygen saturation results. The ongoing work in this study is to quantify the extent of change that occurs in the first hour of the reaction using NIROS, in order to develop the calibration factor and complete the validation studies.

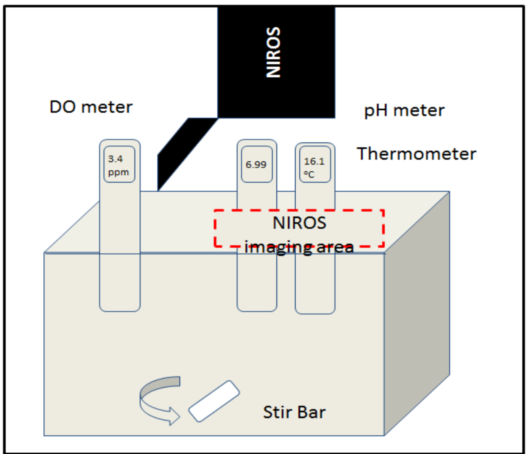

Fig. 4. Experimental set-up of phantom studies (including scatterers, via 45% whole milk in water). Imaging performed by NIROS, along with Dissolved Oxygen (DO) meter, pH meter, and thermometer.

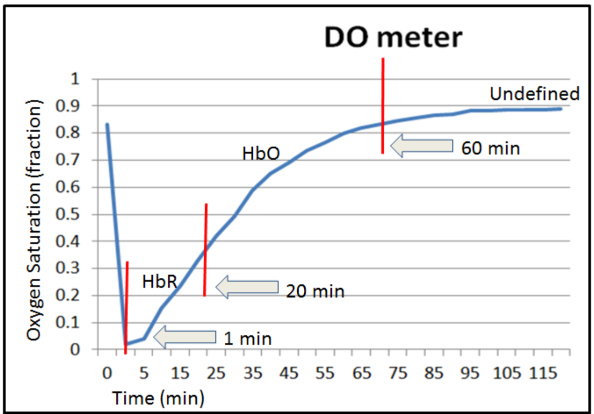

Fig. 5. Changes in oxygen saturation observed from the calibrated, standard Dissolved Oxygen (DO) meter. The red lines show when the expected hemoglobin parameters (deoxy-, HbR, and oxy-hemoglobin, HbO) tend to be performed based on the chemical reaction. Experimental studies were carried out on phantom studies (including scatterers, via 45% whole milk in water).

AIM 2: Develop a quantifiable TO biomarker for assessing healing of DFUs across weeks of treatment.

Prior to studies on DFU cases, validation studies in-vivo on control subjects was carried out using NIROS and a commercial device to validate the ability of NIROS to obtain similar tissue oxygenation changes (as that of the commercial device).

<u>(i) In-vivo validation of NIROS against a commercial device device [3,4, ongoing]</u>:

In this study, NIROS' ability to acquire 2D spatial and temporal maps of tissue oxygenation at a high sampling rate (10Hz) has been developed. The ability to obtain spatial-temporal maps was via standard occlusion studies were performed and the results validated using a commercial multispectral imaging device (capable of obtaining 2D spatial TO maps).

Fig. 6 : Near Infrared Optical Scanner (NIROS) operated using a laptop and stabilized using an articulating arm.

<u>Instrumentation of NIROS for dynamic imaging:</u> NIROS was modified to allow dynamic imaging at a high sampling rate (10Hz). NIROS utilizes multi-wavelength LEDs in the 650-900nm range to illuminate the tissue region of interest at 8mW of

optical power per wavelength per LED (see Fig. 6). In this study, 690nm and 830nm was utilized to acquire 2D dynamic diffuse reflectance signals (at each wavelength) up to 10Hz frequency. A custom MATLAB-based GUI was used to record these diffuse reflectance signals detected by NIR sensitive CMOS camera, which are in turn processed to obtain 2D tissue oxygenation maps in terms of effective oxy- (ΔHbO), deoxy- (ΔHbR), total hemoglobin (ΔHbT), and oxygen saturation (ΔStO_2) across time.

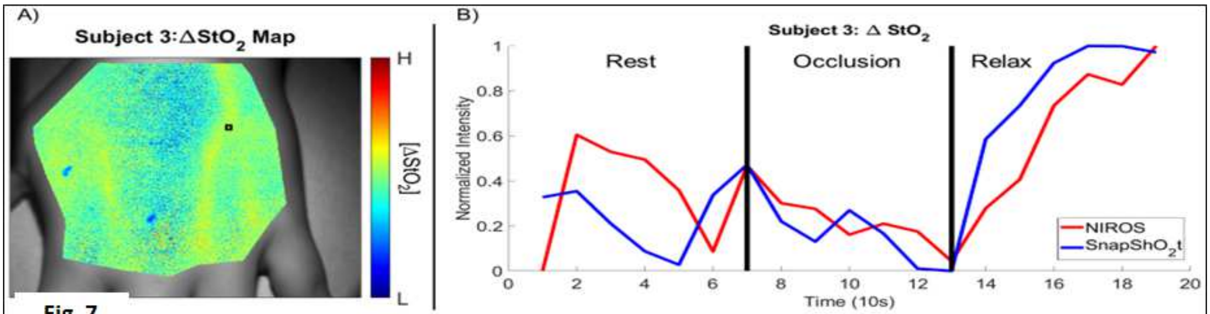

Fig. 7 A) StO_2 map for subject 3 with ROI demarcated by a black box. B) Normalized StO_2 trendline from NIROS (red) and the commercial device (blue).

<u>In-vivo studies:</u> Healthy control subjects are recruited with IRB approval from FIU, with datasets from 3 subjects provided. All subjects were imaged on the hand (dorsal) using an upper arm occlusion paradigm. The paradigm utilized is 3 minutes long and consists of a 50 sec resting period, 10 sec occluding period, 60 sec of full occlusion, and 60 sec relaxation period. The occlusion pressure set to 30 mm Hg over the subject's systolic pressure. Preliminary validation studies showed ~90% correlation in ΔStO_2 changes in response to occlusion when compared to a commercial device employing similar NIRS technology (SnapShO$_2$t, Kent) (see Fig. 7) [3,4].

Ongoing efforts are to complete the validation studies on statistically significant sample size and begin the studies on DFUs starting Spring 2020.

3. Publications:

1. M. Sailaijiang, "*Validation of a Near Infrared Optical Scanner to Measure Changes in Oxygenation: Phantom Studies,*" MS Project, Florida International University, April 2019.
2. M. Sailaijiang, K. Kaile, A. Godavarty "*Validation of a near-infrared optical scanner to measure changes in oxygenation: Phantom studies,*" BME Graduate Research Day, FIU, Feb 2019 (Poster).
3. K. Leiva, E. Robledo, D. Ortega, A. Godavarty, "*Dynamic Tissue Oxygenation Measurements from a Hand-Held Near-Infrared Optical Scanner (NIROS): In-vivo Validation Studies,*" OSA Biophotonics Congress, April 2020 (2-page abstract submitted).
4. J. Barter, K. Leiva, E. Robledo, A. Godavarty, **"***Automation of Image Analysis for Real-Time Oxygenation Imaging of Wounds,*" BMES Annual Meeting Oct 16-19, 2019 (Poster Presentation)