Application Note

Imaging Vascular Oxygen Saturation in an Acute Hindlimb Ischemia and Reperfusion Model with the Vevo LAZR Photoacoustic Imaging System

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Introduction:

Ischemia - or the lack of blood supply to a tissue - and subsequent reperfusion induces physiological and biochemical changes in the affected tissue and is an important area of study since the damage that occurs as a result is clinically important in diabetes and stroke. The ability to image total hemoglobin in addition to oxygen saturation in an ischemic or reperfused tissue could prove to be a valuable research tool in the study of ischemia and potential interventions and treatments.

Currently, ischemia is measured using techniques such as Laser Doppler, near infrared spectroscopy and magnetic resonance. Laser Doppler images tissue blood flow. Near infrared spectroscopy is capable of measuring blood flow oxygenation. However, these two techniques have poor spatial resolution. Lastly, magnetic resonance is expensive.

Photoacoustics (PA) is an imaging modality which combines the sensitivity of optical imaging with the low acoustic scattering and resolution of micro-ultrasound (µUS). It takes advantage of the photoacoustic effect, whereby an acoustic wave is generated by an object which is illuminated by pulsed electromagnetic radiation. A major advantage of this technique is that optical absorption is sensitive to biological processes so that photoacoustics may be used for functional imaging. One such biological process is the oxygenation of hemoglobin, the blood protein which carries oxygen to tissues. Hemoglobin, which has bound oxygen molecules (HbO_2), has different absorption characteristics than deoxygenated hemoglobin (Hb). By imaging with different wavelengths of light, an estimate of percent oxygen saturation (sO_2) of blood can be derived and displayed as a parametric map which can then be superimposed on a regular B-Mode ultrasound image to localize the photoacoustic signal to specific anatomy.

The Vevo® LAZR platform is a combined high-resolution photoacoustic and micro-ultrasound system which uses pulsed laser light at wavelengths from 680 to 970 nm to generate acoustic waves which are detected by a linear array transducer. Hemoglobin in red blood cells absorbs light at the wavelengths mentioned above (called the near-infra red or NIR range) and thus imaging of vasculature and calculation of oxygen saturation and total hemoglobin can be performed independent of blood flow. The system's Doppler imaging capabilities can also give blood flow information. This can all be performed non-invasively in real-time*.

In this study, we investigated the use of the Vevo LAZR photoacoustic imaging system to assess oxygen saturation and total hemoglobin content in a mouse model of acute hindlimb ischemia.

* Oxygen saturation at 1Hz and single wavelength PA imaging at 20 Hz.

Materials and Methods:

The Vevo LAZR photoacoustic imaging system (VisualSonics Inc, Toronto, Canada) was used to acquire all images. The array was retrofitted with a housing that held rectangular fiber-optic bundles (25.4 x 1.25 mm) to either side, at an angle of 30° relative to the imaging plane. The rectangular bundles were bifurcated ends of a single bundle that was coupled to a tunable laser (680-970 nm). The µUS system was synchronized with the laser and photoacoustic signals were acquired with a fluence < 20 mJ/cm^2, beamformed in software, and displayed at 5-20 Hz. The LZ550D (center operating frequency of 40 MHz, axial resolution 40µm) probe was used to acquire all images.

The animal was anaesthetized using isofluorane (1.5-2.0%) and secured to a heated animal handling platform which allows for monitoring of the ECG, respiration, and temperature of the animal. The fur was removed from the hindlimb using a depilatory cream and ultrasound gel was used to provide a coupling interface between the ultrasound probe and the animal.

The Vevo LAZR software allows for the acquisition of photoacoustic images to detect the presence of haemoglobin and other absorbers, and co-register it with B-Mode (2D) images. The wavelength of the pulsed laser light used to generate the photoacoustic effect can be changed anywhere from 680 nm to 970 nm. Images were acquired using 'Oxyhemo' mode, which collects data at 750 and 850 nm and creates and displays a parametric map of estimated oxygen saturation at a rate of 1 Hz.

The ischemic model involved using a suture thread encased by a length of polyethylene tubing (PE-20) and threaded through a piece of plastic to form a loop through which a normal CD1 mouse's hindlimb was inserted. Ischemia was induced by tightening the loop around the proximal thigh and securing it with a haemostat. Two bouts of ischemia were induced, the first for 3.5 minutes and the second for 17.2 minutes, allowing for reperfusion after each bout. For each bout, sO_2 and Hbt were calculated and plotted against time.

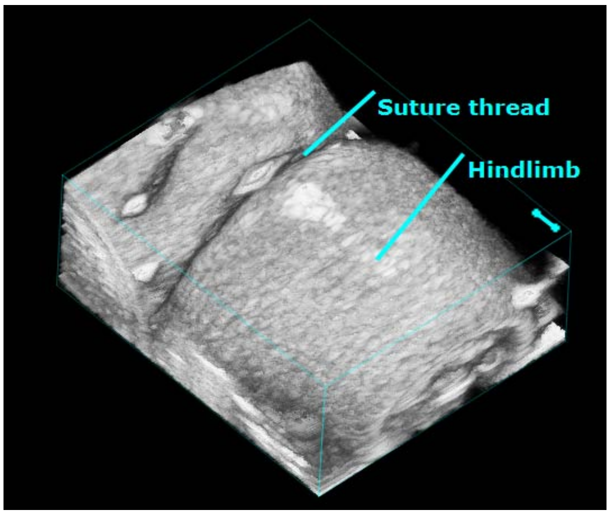

Figure 1 *–3D image of the hindlimb of a mouse showing the thigh and how the tourniquet is applied to induce ischemia.*

Photoacoustic Imaging Mode:

While pure optical imaging methods have limited depth and spatial resolution due to scattering of light, pure ultrasound is limited in its functional imaging capabilities since sound is not sensitive to chemical changes. The Vevo LAZR technology combines optical imaging and ultrasound methods to offer increased imaging depth due to the low scattering and high-resolution of ultrasound while offering functional imaging due to the different absorption spectra of oxygenated and deoxygenated hemoglobin.

The Vevo LAZR platform simultaneously collects photoacoustic and micro-ultrasound data and displays the image data side-by-side or co-registered. The intensity of the photoacoustic signal corresponds to the degree to which a substance absorbs light at the particular wavelength being used. The wavelength range of the Vevo LAZR technology lies in the NIR range, also referred to as the 'therapeutic window' since few biological molecules absorb light in this range[1]. Endogenous absorbers include hemoglobin and melanosomes[2]. For this reason, blood can be imaged effectively with photoacoustics and ischemic and normally perfused tissue may be distinguished.

In addition, the software allows the user to select a region of interest and calculate the average estimated sO_2 and Hbt there, possibly indicating the extent of ischemia. This oxyhemo measurement tool may also be used to detect changes over time when applied to images collected at different time points or on a cine loop. Oxygen saturation information can also be collected in 3D where a motor is used to translate the probe over the complete area of interest.

Hindlimb Ischemia:

2D and 3D scans of the hindlimb were performed before, during and after induction of ischemia as well as during reperfusion. A region of interest was selected proximal to (non-ischemic portion of the limb) and distal from (ischemic portion) the tourniquet during ischemia and reperfusion and the sO2 and Hbt values from these regions in each acquired frame were plotted against time.

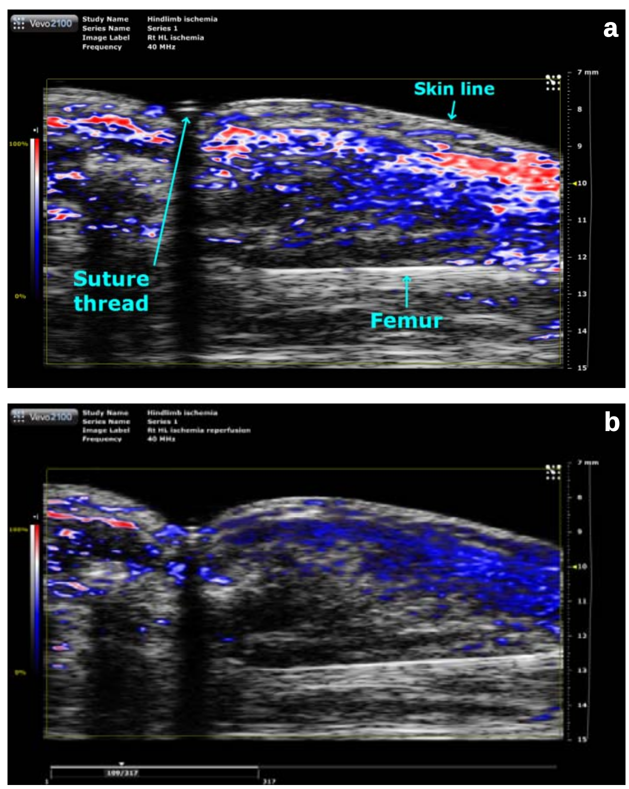

Figure 2 *– Oxyhemo image with 2D overlay of the hindlimb of a mouse under non-ischemic (a) and ischemic (b) conditions.*

The average sO_2 signal from the distal (ischemic) portion of the hindlimb in the 2D scan performed during ischemia decreased immediately and steadily to a minimum of approximately 25% in the first bout (after approximately 3.5 minutes) while the proximal (non-ischemic) portion of the limb showed relatively constant sO_2 during ischemia. Following reperfusion, the sO_2 in the distal portion rose steadily to come back close to the pre-ischemic level. The Hbt remained relatively constant in the proximal portion of the hindlimb while the distal portion showed an increase upon reperfusion.

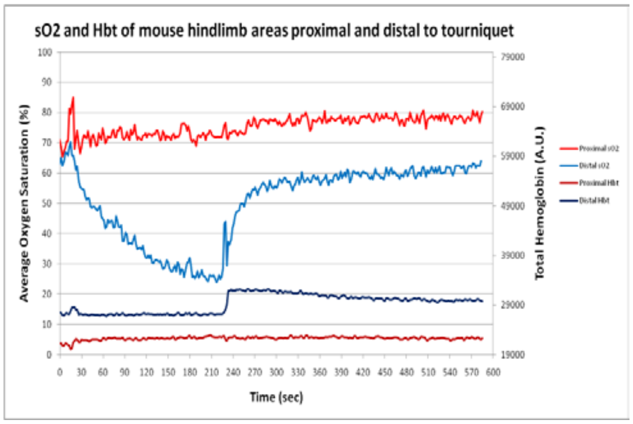

Figure 3 *– A graph that plots average relative sO_2 and Hbt as measured by selecting a ROI that encompasses the proximal (red) or distal (blue) region of the hindlimb against time. Ischemia was induced at approximately 5 seconds and alleviated at approximately 218 seconds.*

A similar trend was shown for the second extended bout of ischemia where the distal portion showed a drop in sO_2 down to 20% before rising to pre-ischemic levels after reperfusion. This increase in Hbt may be reflective of reactive hyperemia whereby increased blood flow occurs in a tissue which has undergone a brief period of ischemia.

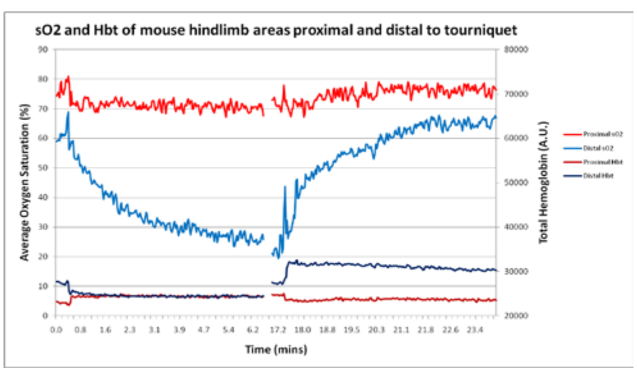

Figure 4 *– A graph that plots average relative sO_2 and Hbt as measured by selecting a ROI that encompasses the proximal (red) or distal (blue) region of the hindlimb against time. Ischemia was induced at approximately 5 seconds and alleviated at approximately 17.2 minutes.*

3D renderings of the hindlimb before and during ischemia show that the entirety of the limb was affected since the ischemic limb shows low sO_2 values (indicated by the darker blue color) throughout the limb whereas the reperfused limb shows higher sO2 signal, especially in the area of major vessels confirmed by comparison with a 3D Power Doppler rendering.

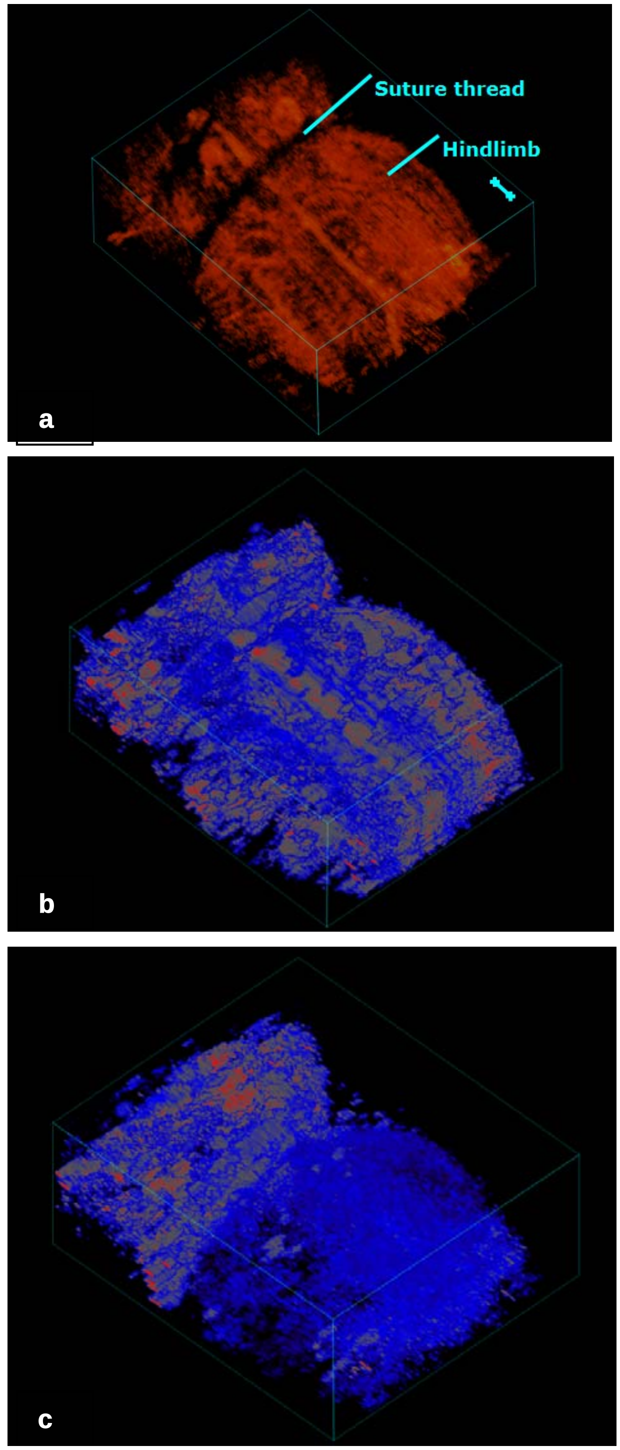

Figure 5 *– 3D images of the hindlimb of a mouse showing the perfused (Power Doppler Mode (a) and sO_2 (b) under normal conditions) and ischemic conditions (c).*

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Conclusions:

In a tourniquet-based mouse hindlimb model of acute ischemia/reperfusion, the Vevo LAZR platform was able to perform real-time measurement of estimated sO_2 and Hbt in the affected hindlimb. The platform also allowed for the comparison of non-ischemic and ischemic regions of the hindlimb in the same plane, over the same timecourse and with high anatomical and temporal resolution. By combining this data with blood flow data obtained with Pulsed-Wave, Color or Power Doppler Modes, an accurate, real-time picture of the physiology underlying hindlimb ischemia can be made available to the researcher. Future validation and development may provide absolute measures of sO_2 along with simultaneous acquisition of blood oxygenation and blood flow measurements.

References:

[1] Emelianov, S.Y *et al*. Photoacoustics for molecular imaging and therapy. *Physics Today*. **62**(8), 34-39, 2009.

[2] Li, C., Wang, L.V. Photoacoustic tomography and sensing in biomedicine. *Physics in Medicine and Biology*. **54**(19), R59-97.

Recommended VisualSonics Protocols:

VisualSonics Vevo LAZR Imaging System, Operators Manual

PA Imaging

Vevo LAZR Photoacoustic Imaging Protocols

VISUALSONICS

VisualSonics Inc.
T.1.416.484.5000
Toll Free (North America) 1.866.416.4636
Toll Free (Europe) +800.0751.2020
E. info@visualsonics.com
www.visualsonics.com