

## Application Brief: Imaging Oxygen Saturation with Photoacoustic Technology

### Executive Summary

Oxygen saturation ( $sO_2$ ) is a measure of the amount of hemoglobin binding sites occupied by oxygen and is expressed as a percentage value of total binding sites. Oxygen saturation measurements have important therapeutic applications in widespread clinical use. In humans, low  $sO_2$  levels in tissues could indicate a hypoxic environment, permitting tumor proliferation, inhibiting tissue perfusion or even indicating the presence of necrotizing fasciitis (Lima et al., 2009; Wang et al., 2004). Furthermore,  $sO_2$  measurements can provide information on anemia, fetal development, and ischemia/stroke.

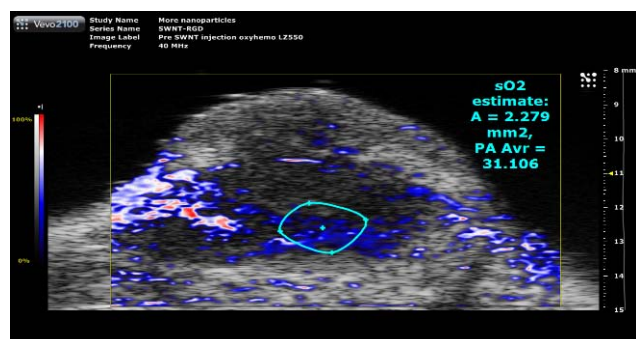
The main  $sO_2$  measurement modalities currently used for quantification within a tissue are pimonidazole staining, glucose transporter-1 (Glut-1) staining, and  $O_2$  microelectrode measurements. The invasiveness of  $O_2$  microelectrode measurements makes this modality less than ideal for determining  $sO_2$  within a tissue, especially since the needle electrodes must be inserted subdermally. Furthermore, the hypoxic tissue must be accessible to the needle to assess oxygenation status, limiting its use to tissues close to the skin surface.

Pimonidazole is a reductively activated drug marker for hypoxia, which binds covalently to thiol-containing proteins within hypoxic cells and forms intracellular adducts (Coer et al., 2007). It must be administered to the tissue of interest prospectively (~16 hours prior to excision), is extremely invasive and cannot be used on studies of archival tissues. Glut-1 is a protein upregulated in response to hypoxic conditions through the hypoxia inducible factor-1 (HIF-1) pathway, making it an ideal endogenous marker for low  $sO_2$  (Coer et al., 2007). Glut-1 expression staining holds the advantage of being detectable on retrospectively-obtained tissues and does not require drug-binding. For both pimonidazole and Glut-1 staining, areas containing positive staining are quantified immunohistochemically on excised tissue sections.

An indirect method of measuring  $sO_2$  is pulse oximetry, whereby readings are recorded by a sensor placed on a thin anatomical point (ie. earlobe or fingertip). A photosensor detects light passing through a tissue and determines the percentage of oxygenated to total hemoglobin based on the differential absorption characteristics of oxyhemoglobin and deoxyhemoglobin. Pulse oximetry has been used for diagnosing cyanosis, dyspnea and tachypnea (Schutz and Saunders., 2001). However, it is limited by accuracy-inhibiting factors, such as; decreased peripheral pulses, body temperature and blood pressure.

All the methods outlined for measuring oxygen saturation have some advantages but confer significant disadvantages. Among them, the user cannot determine the  $sO_2$  at a specific anatomical point, or visualize where the signal is coming from. With high-frequency ultrasound, the user has the ability to view the internal mammalian anatomy in a non-invasive fashion. When combining the high spatial resolution and deep tissue penetration offered by ultrasound with the optical contrast of photoacoustic laser technology,  $sO_2$  can be measured precisely, non-invasively and in real-time while the user can see exactly where the signal is coming from within a tissue.

Photoacoustics (PA) can measure  $sO_2$  in deep tissues by placing external probes on the skin surface and scanning. This technology works in a manner similar to pulse oximetry, by exploiting the differential light absorption spectra of hemoglobin. However, the cursor can sample the percentage value of oxygenation anywhere within the image of the tissue under study with high sensitivity and anatomical specificity. Not only does photoacoustics increase the areas amenable to study, but it also displays an oxygen saturation map across the tissue landscape to visually discern the  $sO_2$  status (Figure 1). The linear transducer array technology enables co-registration of both ultrasound and photoacoustic modalities in 2D or 3D, allowing for studies of anatomical structures and the functional parameters within them.



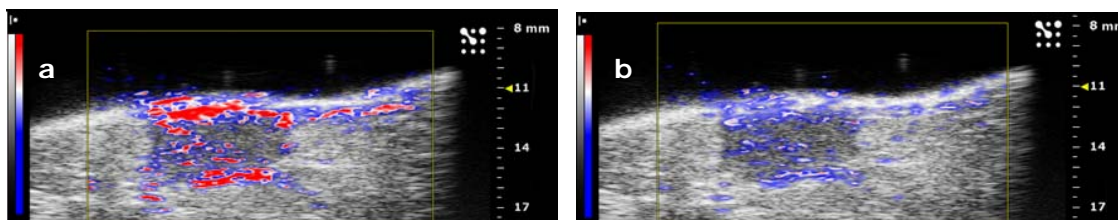
**Figure 1** – Mouse hindlimb tumor visualized at 40 MHz indicating hypoxic conditions. The sampled area has an  $sO_2$  of 31%.

### **Background on $sO_2$ Imaging Possibilities with Photoacoustics**

Oxygenation status can be achieved across multiple tissues and organs with the help of the Vevo<sup>®</sup> LAZR photoacoustics imaging system. Designed for preclinical imaging in small mammalian systems such as rats, mice and rabbits, the Vevo LAZR platform is capable of visualizing  $sO_2$  in many tissues and organs including testicle, brain, tumors, embryo and muscle.

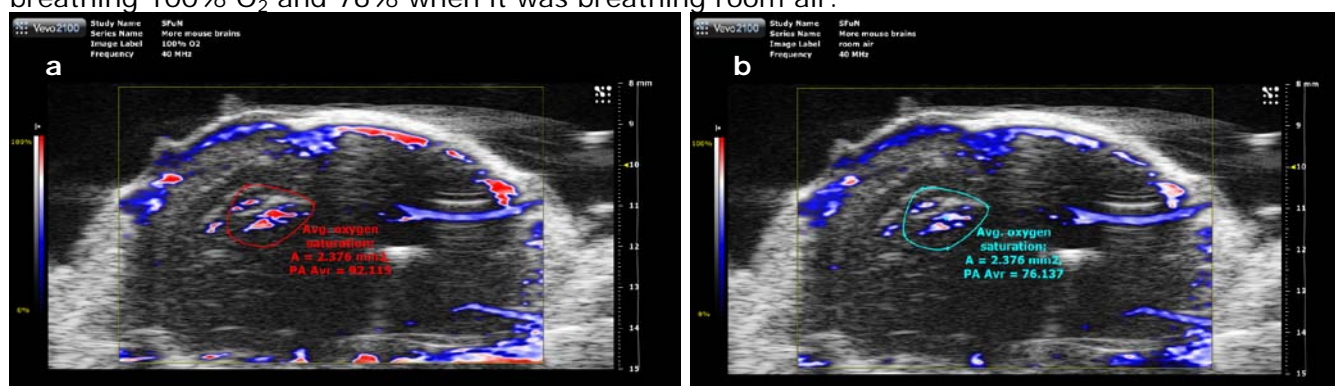
The Vevo LAZR software allows for the acquisition of photoacoustic images to detect the presence of hemoglobin and other absorbers, and co-register it with 2Dultrasound images. The wavelength of the pulsed laser light used to generate the photoacoustic effect can be changed anywhere from 680 nm to 970 nm. Images are 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 0.5 Hz. To analyze hemodynamics, the HemoMeaZure and OxyZated tools of the Vevo LAZR platform are used to measure hemoglobin content and  $sO_2$  respectively.

Rather than viewing data as a linear output of numbers via pulse oximetry, the Vevo LAZR photoacoustic imaging system offers the ability to see an *in vivo* map of oxygen saturation. Figure 2 shows an image of a mouse testicle under two oxygenation conditions – at 100% and 5%  $O_2$  inhalation .



**Figure 2** – Real-time oxygen saturation in vivo in a mouse testicle at (a) 100%  $O_2$  inhalation and (b) 5%  $O_2$  inhalation.

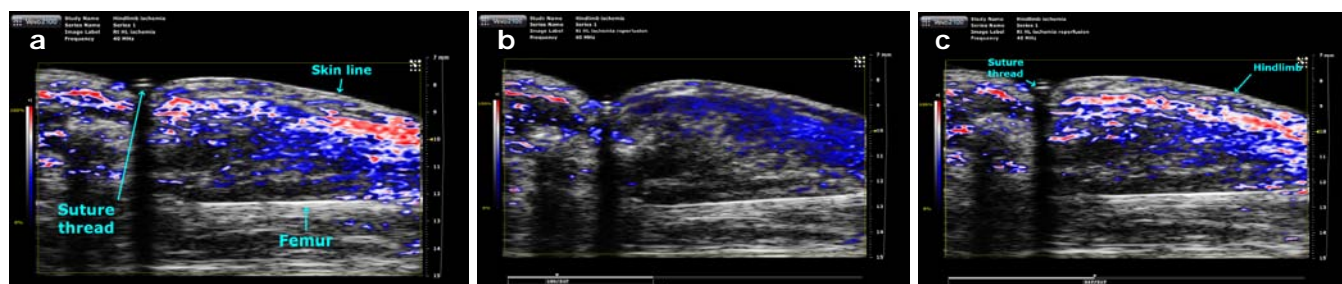
Similar conditions are easily visualized within a mouse brain breathing 100% and 21%  $O_2$  in Figure 3, a and b, respectively. These images also illustrate the ability to average the  $sO_2$  within a specific area of the tissue, which in this case reached 92% oxygenation when the animal was breathing 100%  $O_2$  and 76% when it was breathing room air.



**Figure 3** – Images of the mouse brain with a partial craniotomy. Oxyhemo measurements are taken in a brain region while the animal is breathing (a) 100%  $O_2$  (92% oxygenation) or (b) room air (76% oxygenation).

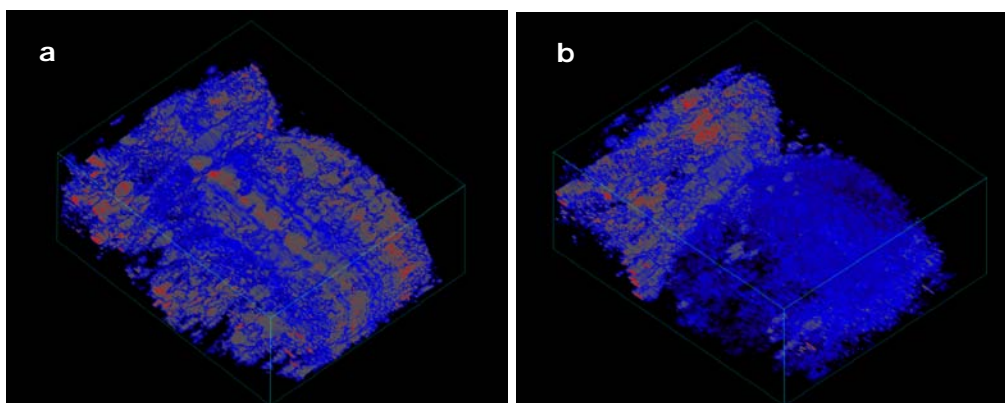
The brain can also be visualized through an intact skull with an imaging depth of  $\sim 3.5$ mm (see  $sO_2$  Application Note).

Induction of ischemia and subsequent reperfusion in the mouse hindlimb generates dramatic visible changes in  $sO_2$  with the Vevo LAZR platform, as shown in Figure 4.



**Figure 4** – Oxyhemo image with 2D2D overlay of the mouse hindlimb under (a) non-ischemic, (b) ischemic and (c) reperfused conditions.

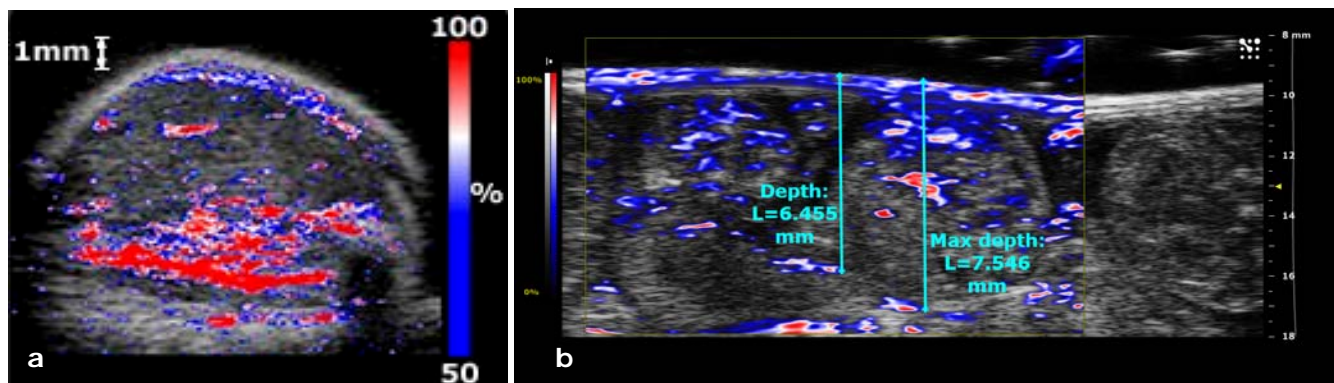




**Figure 5** – 3D images of the hindlimb of a mouse showing (a) ischemic conditions and (b)  $sO_2$  reperfusion.

Pre-ischemic, ischemic and reperfused tissues show varying degrees of oxygenation. These conditions can also be displayed in a 3D rendering by the Vevo LAZR platform, as shown in Figure 5.

In studies of tumor and embryo development, photoacoustics offers the ability to monitor hemodynamics *in vivo* and in real-time. Moreover, the imaging is completely safe and non-invasive, and will not harm the embryo. The tumor microenvironment will also be preserved, allowing for longitudinal studies to be performed. Photoacoustic imaging with  $sO_2$  mapping in the tumor and embryo is shown in Figure 6.



**Figure 6** – (a) Photoacoustic oxygenation map superimposed on a 2D image of a subcutaneous tumor and (b) Oxyhemo image of an ~E18 intact embryo with LZ250 showing depth of various signals to a max depth of 7.5 mm

Maternal blood oxygenation levels can also be measured within the placenta when studying hemodynamics in the embryo. Tumors can be easily detected early on within tissues based on their highly disorganized formations of neovasculature, which absorb the illuminated pulsed laser light at much higher levels. The 2D registration renders rapid information about its location based on its increased hemoglobin count, which acts as an endogenous signal for photoacoustics.



### **VisualSonics' Value Proposition**

sO<sub>2</sub> measurements have in the past been limited to invasive, immunohistochemical, drug-induced, surface sensor or molecular assay detection methods. These modalities, however, are incomplete. Those which allow visualization of the tissue oxygenation status, such as pimonidazole and Glut-1 staining, are invasive and require excision, which inhibits longitudinal studies. Those which offer real-time monitoring, such as pulse oximetry, are non-visual and limited only to superficial or very shallow subdermal anatomy. Pulse oximetry cannot detect tumors if their position is not already known, and will provide sO<sub>2</sub> values but will not source their location on parametric map of the tissue under study. They are also subject to variables such as blood pressure, circulation and peripheral cyanosis (Schutz and Saunders, 2001).

The Vevo LAZR photoacoustic imaging system combines anatomical and functional information of the tissue being imaged to address the shortcomings of current sO<sub>2</sub> measurement methods. It seeks to address the needs of researchers aiming to obtain quick, accurate and visually-mapped imaging information on oxygen saturation across a variety of tissues to assess a variety of conditions. This document has shown photoacoustic imaging with the Vevo LAZR platform in testicular glands, embryos, tumors, hindlimbs and brain. Within these tissues, conditions of ischemia, anemia, hypoxia, and reperfusion (among others) can be discerned with photoacoustic imaging. The imaging is precise, non-invasive, anatomically localized and conducted in real-time.

Below is a summary of the unique value proposition VisualSonics delivers to researchers with the Vevo LAZR photoacoustic imaging system for oxygen saturation quantification:

1. Inherent co-registration of the oxygen saturation signal and the 2D anatomical image.
2. High specificity via the use of the endogenous heme signal for detecting location and oxygen saturation level of blood within anatomical structures.
3. Real-time visualization permits quick rendering and longitudinal monitoring studies for embryo development, maternal blood oxygenation levels and tumor development.
4. Endogenous photoacoustic signal tools HemoMeaZure and OxyZated measure hemoglobin content and oxygen saturation, respectively, without the use of contrast agents. HemoMeaZure aids in assessment of anemia, while the OxyZated tool evaluates tumor and tissue hypoxia.
5. Live 2D and 3D imaging for tissue morphology, vasculature mapping and oxygen saturation measurements.
6. LAZRTight enclosure ensures optimal safety for the operator, and consists of physiological monitoring, integrated anesthesia, transducer mounting system, 3D image controller, and microinjection system ensures efficient and reproducible imaging.



**VISUALSONICS**

### **Bibliography**

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sO<sub>2</sub> Application Note.