Application Note

Brain Imaging with the Vevo LAZR Photoacoustic Imaging System

Introduction:

Functional neuroimaging can provide valuable information to researchers investigating brain function and disease. For example, oxygen saturation (sO_2) and total hemoglobin (Hbt) of blood in vessels in the cortex and other brain areas can be used to detect changes in neuronal activity in response to external stimulation[1]. Current brain imaging technologies includes functional magnetic resonance imaging (fMRI), positron emission tomography (PET), and recently developed photoacoustic modalities.

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.

The Vevo® LAZR photoacoustic imaging system 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, the blood protein in red blood cells which carries oxygen to tissues absorbs light at the wavelengths mentioned above (called the near-infra red or NIR range) and thus imaging of vasculature can be performed. Another major advantage of this technique is that optical absorption is sensitive to biological processes so that photoacoustics may be used for functional imaging. Hemoglobin which has bound oxygen molecules (HbO_2) has different absorption characteristics than deoxygenated hemoglobin (Hb)[2]. By imaging with different wavelengths of light, an estimate of percent sO_2 as well as Hbt 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. These measurements can be performed independent of blood flow, information which can be acquired with the system's Doppler imaging capabilities. 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 vascular structure, oxygen saturation and total hemoglobin content in the mouse brain.

* Oxygen saturation at 1 Hz and single wavelength PA imaging at 5-20 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 PA signals were acquired with a fluence < 20 mJ/cm^2, beamformed in software, and displayed at 5 Hz. The LZ-250D (center operating frequency of 21 MHz, axial resolution 75 µm) and LZ-550D (center operating frequency of 40 MHz, axial resolution 40 µm) probes were used to acquire all images.

The animal was anaesthetized using isoflurane (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 hair was removed from the imaging area using a depilatory cream and ultrasound gel was used to provide a coupling interface between the ultrasound probe and the animal. In some cases, additional imaging was performed with the skin removed from the skull and with a partial craniotomy where a section of the skull was removed to expose the surface of the cortex.

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 B-Mode 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 in 'Single' mode using light at 680, 800 and 850 nm and using 'Oxyhemo' mode, which collects data at 750 and 850 nm and creates and displays a parametric map of estimated oxygen saturation or total hemoglobin at a rate of 1 Hz.

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. Photoacoustics combines these two 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 it 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[3]. Endogenous absorbers include hemoglobin and melanosomes[4]. For this reason, blood can be imaged effectively with photoacoustics.

2D Brain Imaging:

The brain, being a highly vascularized structure, is an ideal target for photoacoustic imaging. Since blood is the dominant absorber in tissue, endogenous signal represents the vasculature, regardless of blood flow and can be imaged with the Vevo LAZR platform.

A non-invasive 2D scan at a wide variety of single wavelengths shows vasculature within and beneath the skin. The co-registration of the B-Mode and photoacoustic images allows for discrimination between signal which is in the skin and that which lies below the skull and consists of vasculature on the surface of the cortex. In some cases, cerebral vasculature as deep as 2 mm below the cortical surface can be seen.

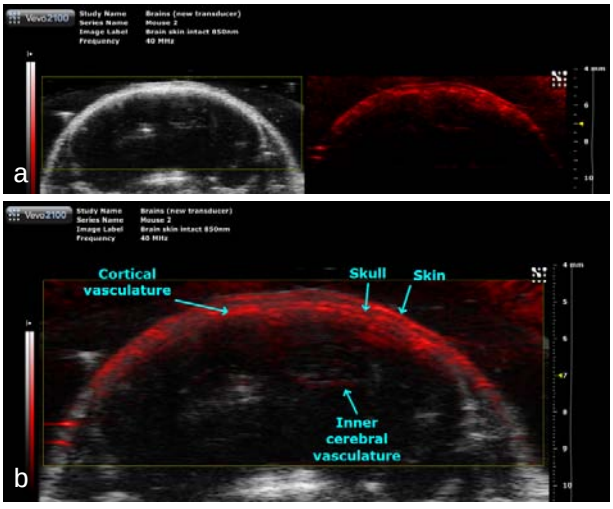

Figure 1 *– a) Side-by-side and b) Co-registered B-Mode and photoacoustic images of a 2D coronal section of a mouse cranium showing skin and cerebral vasculature. The inner cerebral vasculature is likely at the level of the dorsal thalamus and medial region of the hippocampus.*

In order to investigate deeper brain vasculature, a craniotomy was performed (whereby a section of skull was removed). Not only was the system able to detect photoacoustic signal as deep as 5 mm, but the ultrasound image acquired at the same time allowed for the localization of the signal to specific mouse neuroanatomy (estimated from the Brain Explorer software from the Allen Institute for Brain Science[5]).

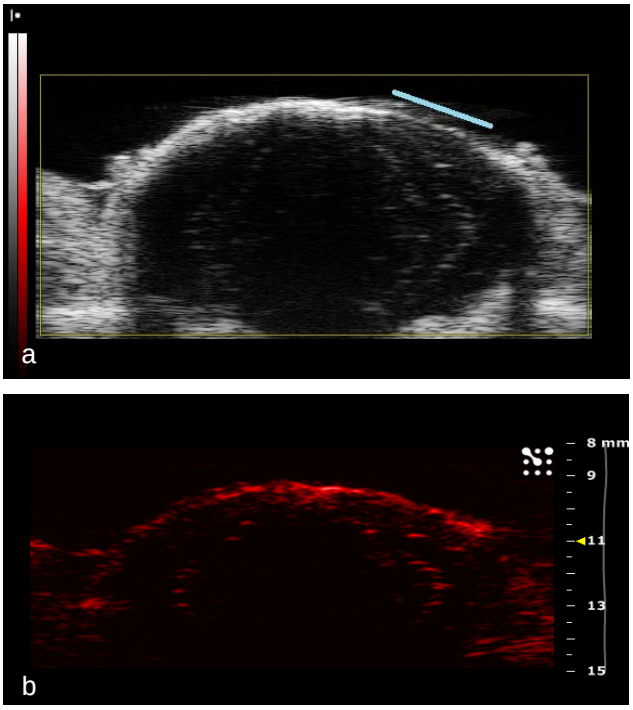

Figure 2 *– (a) 2D and (b) photoacoustic image at 680 nm of a coronal section of a mouse cranium with a section of skull removed (denoted by the blue line). Vascular signal can be seen at the medial retrohippocampal region, motor and sensory regions of the superior colliculus, and ventromedial and other cortical areas.*

3D Brain Imaging:

Photoacoustic imaging can also be completed in 3D, where a motor is used to translate the LZ-Series transducer over the entire cranium. The image can also be rendered, such that the vascular network within the 3D volume can be visualized. A 3D scan of a mouse cranium was performed non-invasively and cortical vasculature was clearly visible.

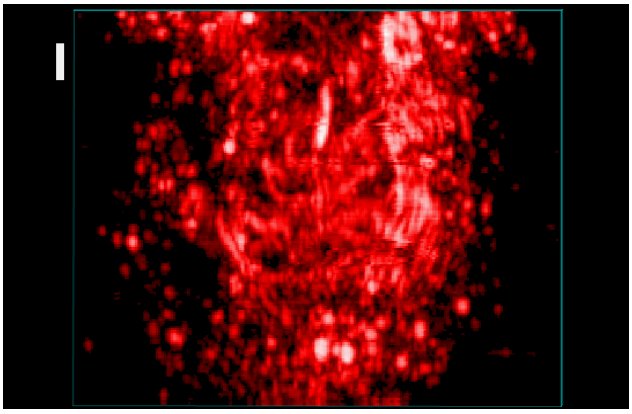

Figure 3 *– 3D photoacoustic image at 850 nm of mouse cortical vasculature. The image was acquired with the skin and skull intact. Scale bar represents 1 mm.*

A 3D scan was also done on a mouse on which a craniotomy had been performed, revealing some detail of inner cerebral vasculature. The fact that the surface of the skull was clearly visible with the co-registered ultrasound image allows for precise identification of anatomy using landmarks on the skull such as Bregma and Lambda and a mouse neuroanatomy atlas such as that of Paxinos and Franklin[6].

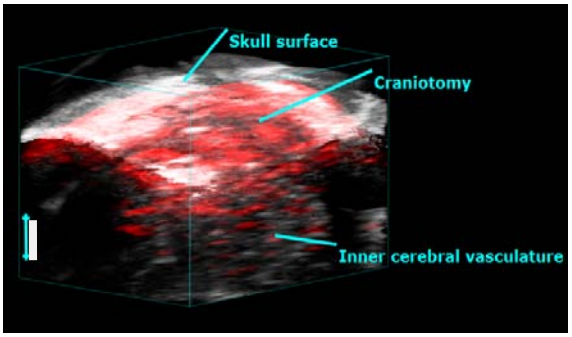

Figure 4 *– Co-registered 3D photoacoustic and ultrasound image at 680 nm of mouse cranium with a craniotomy showing skull surface and inner anatomical structure and vascular signal. Scale bar represents 1 mm.*

Oxyhemo Imaging (oxygen saturation):

The Vevo LAZR software allows the imaging and quantification of estimated sO_2 (based on the different absorption characteristics of oxygenated and deoxygenated hemoglobin) and Hbt, potentially allowing the user to distinguish between venous and arterial blood based on differences in sO_2. 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.

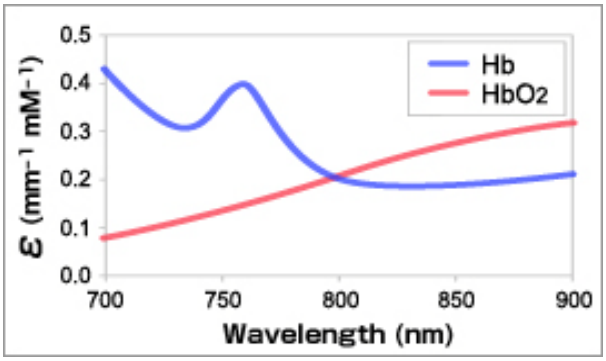

Fig 5 *- Absorption spectra for oxygenated (HbO_2) and deoxygenated (Hb) blood[2]. The wavelength in nanometers is on the x-axis and the absorbance coefficient is on the y-axis.*

A 2D scan was performed on a mouse non-invasively and with a partial craniotomy. The signal acquired with the skin and skull intact was similar to that obtained at a single wavelength. Vasculature in the skin and cortical vasculature could be distinguished by the sO_2 signals localized by comparison with the ultrasound image. The parametric map of total hemoglobin was also generated and displayed from the same image data.

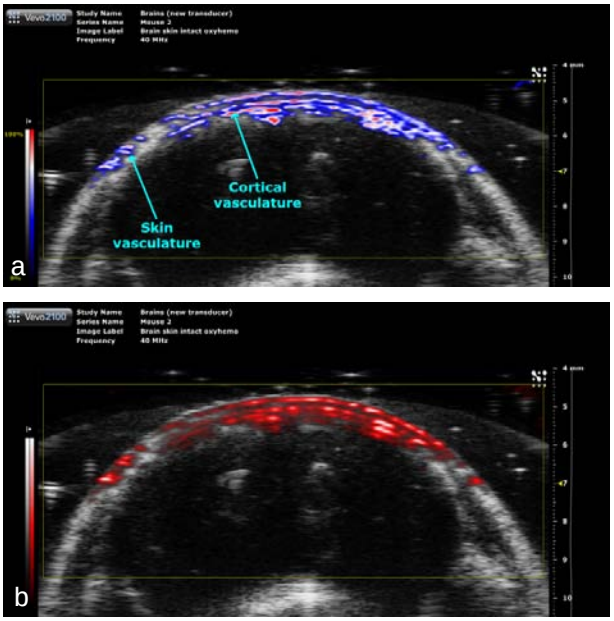

Figure 6 *– Co-registered photoacoustic and 2D ultrasound images with (a) sO_2 and (b) Hbt maps of mouse cortical vasculature. The image was acquired with the skin and skull intact.*

In order to investigate real-time changes in oxygen saturation in the brain vasculature, images were acquired on an animal with a partial craniotomy while the inhaled oxygen concentration was varied. A region of interest was selected to include

vascular signal likely originating around the intersection of the pretectal area with the lateral habenula of the thalamus and the medial dentate gyrus. While the animal breathed room air, the estimated average sO_2 within the region of interest was 76%. When the animal was switched to 100% breathed oxygen, the estimated average oxygen saturation increased to 92%.

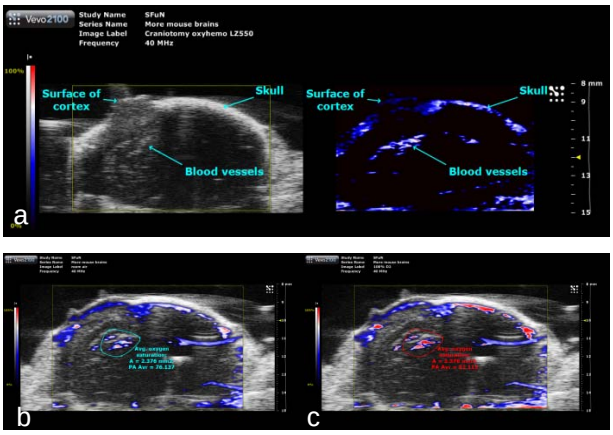

Figure 7 *– (a) B-Mode and oxyhemo photoacoustic image with a craniotomy over the left hemisphere showing an oxygen saturation map of cortical and inner cerebral vasculature. (b) Co-registered B-Mode and photoacoustic image showing oxygen saturation within the measurement region of 76% with the animal breathing room air. (c) Similar image showing an oxygen saturation of 92% within the same region with the animal breathing 100% O2.*

A 3D scan was performed in oxyhemo mode non-invasively. The non-invasive scan revealed vascular structure allowing for the identification of major cerebral arteries such as the superior sagittal sinus which appears as a distinct, highly oxygen saturated (red) vessel on the midline of the brain. In order to confirm that these signals were not originating from the skin as opposed to the cortex, the scalp was removed and a 3D scan was performed at 850 nm, a wavelength at which oxygenated blood absorbs more light than deoxygenated blood and therefore strong signal in this scan more likely represents arterial blood. A similar signal to that obtained in the non-invasive oxyhemo scan was observed at 850 nm. Comparison to a photograph of the exposed skull and underlying vessels showed that the photoacoustic signal correlated very well with the vascular structure in the cortex.

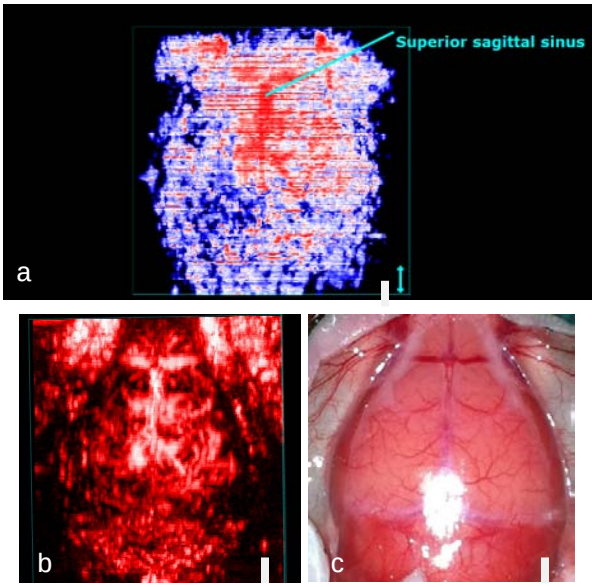

Figure 8 *– (a) 3D MIP oxyhemo image of mouse cortical vasculature with skin intact. (b) Photoacoustic MIP image at 850 nm of the cortical vasculature of the same mouse with skin removed. (c) Photograph of the same mouse showing cortical vessels visible through the intact skull (only skin removed). The scale bar represents 1 mm.*

These investigations demonstrate the use of the system for functional neuroimaging in mice non-invasively. Furthermore, materials such as Rexolite may be used to create a cranial window[7] allowing the laser pulse and the returning acoustic waves to be transmitted, increasing depth of penetration.

Conclusions:

The images presented here clearly show the utility of the Vevo LAZR photoacoustic imaging system as a tool for *in vivo* imaging of cortical and sub-cortical vasculature and quantification of oxygen saturation and total hemoglobin within the vessels. The non-invasive nature of photoacoustic imaging allows the same tissue to be studied over the course of an experiment, leading to much stronger data and requiring fewer animals to get significant results. The fact that real-time changes in oxygen saturation of blood in the cortex can be observed highlights the functional nature of photoacoustic imaging with the Vevo LAZR platform and also allows for the investigation of cerebral hypoxia and its causes such as stroke, ischemia and cerebral infarction. In addition, contrast agents such as gold nanoparticles or carbon nanotubes can be used to enhance vascular photoacoustic signal or be targeted to specific receptors within the cerebral vasculature to identify molecular targets or provide therapeutic approaches.

References:

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Recommended VisualSonics Protocols:

VisualSonics Vevo LAZR Imaging System, Operators Manual

PA Imaging

Vevo LAZR Photoacoustic Imaging Protocols

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