Application Note

Imaging of Vasculature with the Vevo LAZR Photoacoustic Imaging System

VISUALSONICS

Introduction:

Vascular research covers a wide variety of topics including vascular structure, vascular function, hemodynamics, mechanics, cellular communication, growth and differentiation encompassing diseases like atherosclerosis, diabetes, stroke and cancer. A crucial research tool in this field is the ability to image the vasculature *in vivo*. Current vascular imaging technology includes MRI, CT, ultrasound 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 biological tissue illuminated with pulsed electromagnetic radiation.

The Vevo® LAZR platform is a combined high-resolution photoacoustic and micro-ultrasound system. This system uses pulsed laser light at wavelengths from 680 to 970 nm to generate acoustic waves which are then detected by a linear array transducer. This range is known as the near-infra red or 'NIR' range

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, and therefore vasculature, can be imaged effectively with photoacoustics.

Nanoparticles (NPs), such as gold nanorods (GNRs) and single-walled carbon nanotubes (SWNTs) also absorb within the NIR range and are biologically inactive allowing for long circulation times *in vivo*[3]. These NPs can act as contrast agents to not only enhance the endogenous vascular signal, but they can be targeted to specific intravascular receptors such as alpha-5, beta-3 integrins which are overexpressed in tumor neovasculature[4]. An arginine-glycine-asparagine (RGD) conjugate binds specifically to alpha-5, beta-3 integrins.

Another major advantage of this technique is that optical absorption is sensitive to biological processes such that photoacoustics may be used for functional imaging. Hemoglobin, with its bound oxygen molecules (HbO_2), has different absorption characteristics compared to deoxygenated hemoglobin (Hb). By imaging with different wavelengths of light, an estimate of percent oxygen saturation (sO_2), as well as total blood hemoglobin (Hbt), can be derived and displayed as a parametric map. The sO_2 map can then be superimposed on a regular B-Mode ultrasound image to localize the photoacoustic signal to specific anatomy. These measurements can be performed independently of blood flow information, which can be acquired with the system's Doppler imaging capabilities. All image acquisitions, measurements and assessments can 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, total hemoglobin content and nanoparticle detection in mice.

* Oxygen saturation at 1 Hz and single wavelength photoacoustic imaging at 5-20 Hz.

Materials and Methods:

Animal Models

Photoacoustic imaging was performed on normal and tumor-bearing mice. Adult female CD1 mice were used for sO_2 measurements. Nanoparticles were visualized in athymic NUDE mice with hindlimb tumors.

Animal Handling

The animal was anaesthetized using isofluorane (1.5-2.0%) and secured to a heated animal handling platform which allowed 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.

Vevo LAZR Photoacoustic Imaging System

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 Hz. The LZ250D (center operating frequency of 21 MHz, axial resolution 75 µm) and LZ550D (center operating frequency of 40 MHz, axial resolution 40 µm) probes were used to acquire all images.

The Vevo LAZR software allows for the acquisition of photoacoustic images to detect the presence of hemoglobin and other absorbers, and co-register them with 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 in 'Single' mode using light at 680, 800 and 850 nm and 'Oxyhemo' mode, which collected data at 750 and 850 nm to create and display a parametric map of estimated oxygen saturation or total hemoglobin content at a rate of 1 Hz.

Nanoparticles

Single-walled carbon nanotubes conjugated with RGD (SWNT-RGDs; Stanford University, Palo Alto, CA) were administered to enhance signal contrast. They have a peak absorbance at 690 nm, an axial diameter of 1-2 nm (without the RGD molecule) and a length of 50-300 nm, and a concentration of 1.2 µM. The nanoparticles were coated with polyethylene glycol (PEG) to increase biocompatibility.

2D scans of a subcutaneous hindlimb tumor were performed before and after tail-vein injection of a 150 µl bolus of SWNT-RGD (described previously). In addition, a 2D scan was performed during and for approximately 3 minutes after the bolus injection.

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 with the low scattering and high-resolution of ultrasound while offering functional imaging by exploiting the differential 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 in an overlayed fashion. The intensity of the photoacoustic signal corresponds to the degree to which a substance absorbs light at the particular wavelength being used.

2D Vasculature 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 in several ways. A 2D scan at a wide variety of single wavelengths allows for the discrimination of large and small vessels within a given tissue purely by virtue of the hemoglobin signal.

Tumor vascularity is an important aspect of cancer research, with numerous potential therapeutics or experimental procedures being tested for their ability to alter the vascularity of tumors and surrounding tissue. Photoacoustic imaging can reveal tumor vascularity independent of blood flow, allowing for the identification of poorly vascularized and possibly necrotic or cystic regions of the tumor, as well as areas of neovascularization.

In addition, contrast agents such as gold nanoparticles or carbon nanotubes can be used to enhance the vascular photoacoustic signal or serve as targets to specific receptors within the vasculature (Figure 1).

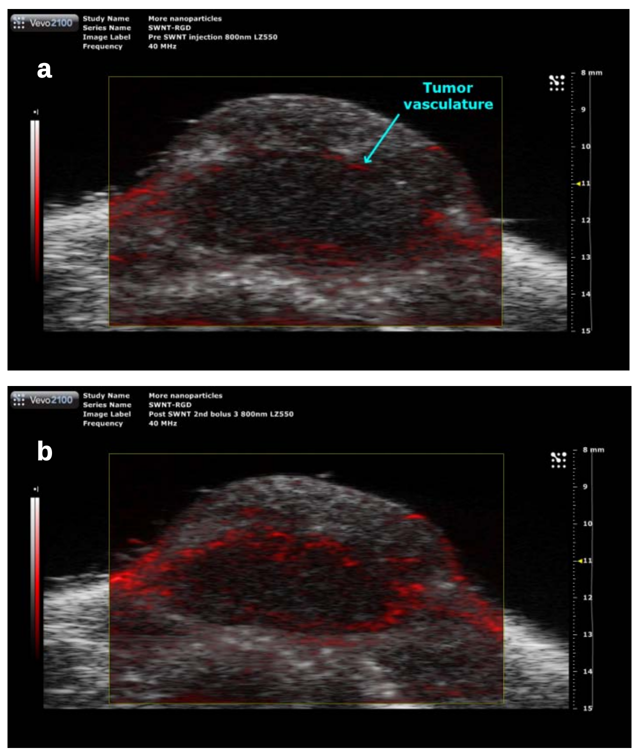

Figure 1 *– B-Mode image with 800 nm photoacoustic overlay of a subcutaneous tumor before (a) and 5 hours after (b) a 150 µl bolus of carbon nanotubes(Stanford University) targeted to tumor neovasculature.*

The average photoacoustic signal in the 2D scan performed during the bolus increased to a maximum of approximately 55% at 15 seconds, before dropping down to close to baseline after approximately 2.5 minutes after bolus injection (data not shown).

3D Vasculature Imaging:

Photoacoustic imaging can also be completed in 3D, where an LZ-Series transducer is used to rotate over an area of interest, such as a tumor or the abdominal surface. The image can also be rendered, such that the vascular network within the 3D volume can be visualized (Figures 2 & 4).

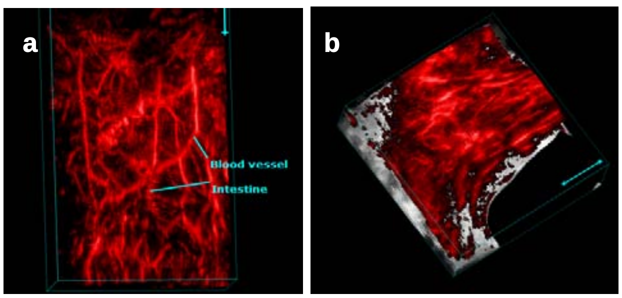

Figure 2 *–3D photoacoustic images at 680 nm of (a) superficial abdominal vasculature showing blood vessels and signal for the contents of the intestine and (b) axilla of a mouse showing signal from blood vessels. The scale bar represents 5 mm.*

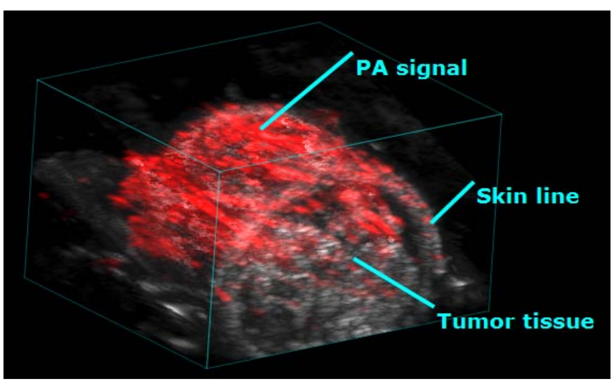

Figure 3 *– Photoacoustic imaging at 750 nm of a LLC tumor on the hindlimb of a mouse. The red signal is primarily derived from the absorption of light by hemoglobin in red blood cells.*

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, as shown in Figure 4) and Hbt, thereby 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.

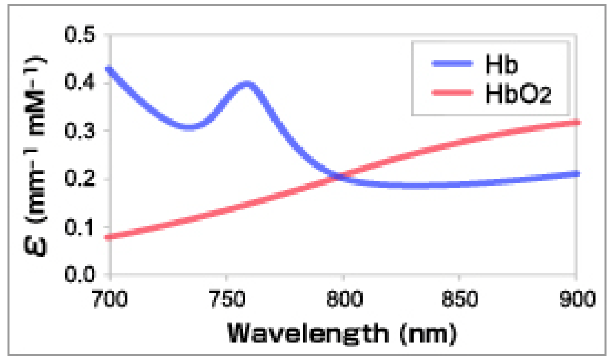

Figure 4 *- Absorption spectra for oxygenated (HbO_2) and deoxygenated (Hb) blood. The wavelength in nanometers is on the x-axis and the absorbance coefficient is on the y-axis.*

Tumor hypoxia is of great interest in cancer research, positioning the oxyhemo mode on the Vevo LAZR platform as a valuable tool. As described above, 2D and 3D scans can be performed where the sO_2 parametric map is displayed over the B-Mode image to localize the blood signal to specific parts of the tumor identified with B-Mode (Figure 7).

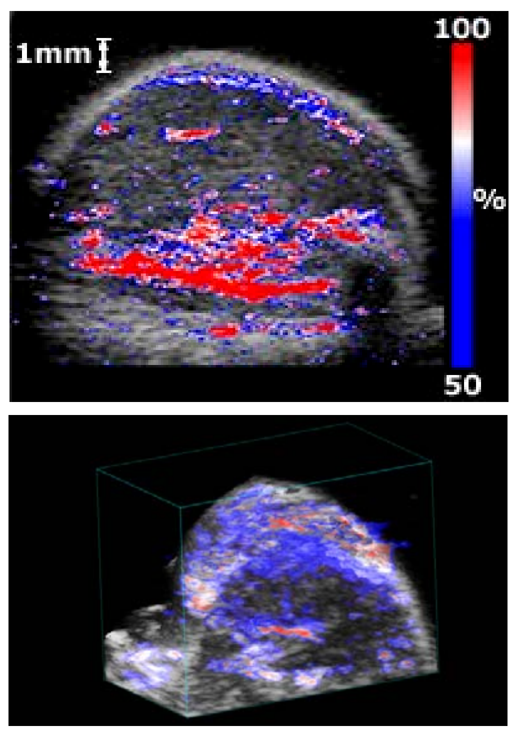

Figure 7 *– Co-registered B-Mode and oxyhemo (750 and 850 nm) photoacoustic images of a subcutaneous LLC hindlimb of a mouse in 2D (a) and 3D (b). A region of interest is drawn on the 2D image and the estimated average sO_2 value is displayed.*

VISUALSONICS

Conclusions:

The images presented here clearly show the utility of the Vevo LAZR photoacoustic imaging system as a tool for *in vivo* imaging of vascularity, quantification of oxygen saturation and total haemoglobin within 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 achieve significant results.

The use of contrast agents such as nanoparticles can further enhance the visualisation of blood vessels and allow for targeting of specific intravascular receptors in a wide variety of tissues. The techniques discussed above also allow for acquisition of real time data on blood and blood vessels in the presence or absence of blood flow. This permits studies of acute or chronic changes in vasculature in response to potential therapeutic or experimental procedures.

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.

[3] De La Zerda, A. *et al.* Carbon nanotubes as photoacoustic molecular imaging agents in living mice. *Nature nanotechnology*. **3**, 557-562, 2008.

[4] Janssen, M.L. *et al.* Tumour targeting with rediolabelled alpha(v) beta(3) integrein binding peptides in a nude mouse model. *Cancer Res.* **62**, 6146-6151, 2002.

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