Application Note

Quantification of Tumor Vascularity using High Frequency Ultrasound

VISUALSONICS

Introduction:

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. There are various types of tumor models which are of interest, including transgenic, orthotopic, and subcutaneous, all of which are well suited for imaging using high frequency ultrasound.

Ultrasound imaging is a non-invasive, real-time imaging technique which allows for longitudinal studies on the same animal. Imaging sessions provide anatomical and functional data; sessions are preformed rapidly, allowing for short anesthesia times and quick data acquisition.

The Vevo® 2100 High Resolution Ultrasound Imaging system, using linear array technology for the transducer design, provides axial resolution down to 30µm; this type of resolution would allow for the detection of tumorigenesis well before the lesion is palpable. There are various Doppler imaging techniques available on the Vevo 2100 which allow for detection and quantification of vascularity in vessels larger than 30µm. These Doppler techniques allow for the quantification of flow in the vessels, as well as calculation of percent vascularity, as a measure of relative blood volume, in 2D as well as 3D imaging. MicroMarker™ Contrast Agents can be used to assess vascularity in vessels down to the capillary level, allowing for assessment of relative perfusion in a tumor area or tumor volume.

Ultrasound imaging is non-invasive therefore the same tumor can be studied over the course of an experiment, leading to much stronger data and requiring fewer animals to get significant results. The MicroMarker contrast agents are administered through the tail vein, or any other venous access point, and can be followed throughout the course of a longitudinal study.

The goal of this poster is to show the utility of the Vevo 2100 in tumor vascularity imaging, images will be shown from various tumor models. The various Doppler imaging modes will be explained, as will the use of MicroMarker Contrast agents.

Materials and Methods:

The Vevo 2100 High Resolution Ultrasound Imaging System (VisualSonics Inc, Toronto, Canada) was used to acquire all images. The MS-550D (center operating frequency of 40MHz, axial resolution 40um) probe was used to acquire all images.

Animals were anaesthetized using isofluorane (1.5-2.0%); the animal was secured to a heated animal handling platform which allows for monitoring of the ECG, respiration, and temperature of the animal. The hair over the tumor area was removed using a depilatory cream when necessary, and ultrasound gel was used to provide a coupling interface between the ultrasound probe and animal.

The Vevo 2100 software allows for acquisition of Color Doppler and Power Doppler images; Doppler imaging is used in ultrasound to detect the presence of blood flow and to evaluate direction and speed of flow in vessels larger than 30µm. Doppler imaging is used to detect the phase shift in the reflected ultrasound pulse; when the transmitted ultrasound pulse encounters moving blood and is reflected the ultrasound pulse is altered, this change is the phase shift or Doppler Effect. The most commonly used Doppler imaging modes in cancer research are Color Doppler and Power Doppler.

MicroMarker Contrast Agents (VisualSonics Inc, Toronto, Canada) were used for all contrast studies used to assess relative perfusion within the tumors down to the capillary level. The microbubbles have a phospholipid shell and a perfluorobutane/nitrogen gas core, and have an average diameter of 2-3µm.

Images were acquired from athymic nude mice implanted with 5x105 MeWo human melanoma cells (ATCC; Manassas, VA) subdermally in the hindlimb 3 to 4 weeks before imaging. And from PSP94 transgenic prostate mice[1], which develop fast growing prostate tumors between 4 and 8 months of age.

Doppler Imaging Modes:

Color Doppler

Color Doppler applies a color pixel where blood flow is detected; the specific color indicates direction of flow, blue is blood flowing away from the transducer while red is blood flowing towards the transducer. Information on the mean velocity of blood flow is also provided on the image. Information is provided for blood vessels which are larger than the resolution of the ultrasound probe, in most cases that is larger than 30µm in size.

Color Doppler is typically used in cancer research to provide an overview of the vascular network within a tumor, and to give an indication of the direction of flow and the mean velocity. Color Doppler can also be used as a tool to identify the presence of a tumor based on changed in blood flow patterns.

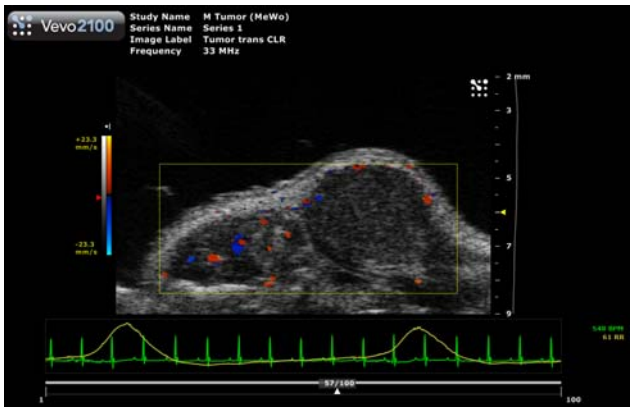

Figure 1 *– Color Doppler imaging of a MeWo tumor on the hind limb of a mouse. Blue indicates blood moving away from the ultrasound probe which is located at the top of the image, while red indicates blood moving towards the ultrasound probe. The region of interest is defined by the yellow color overlay box; while the mean velocity is located on the left hand side of the image, next to the color bars.*

Power Doppler

Power Doppler is similar to Color Doppler in that a region of interest box is drawn over the area where the overlay is to be shown. However the data in Power Doppler represents intensity of flow rather than direction of flow, so as the intensity of flow increases the color overlay changes from orange to yellow. An area can be drawn around the tumor, or around a particular area of interest, and the percent vascularization (PV) is quantified. The PV represents the percentage of pixels within the defined area which have a Power Doppler signal associated with them, indicating the presence of blood flow. Information is provided for blood vessels larger than 30µm in size.

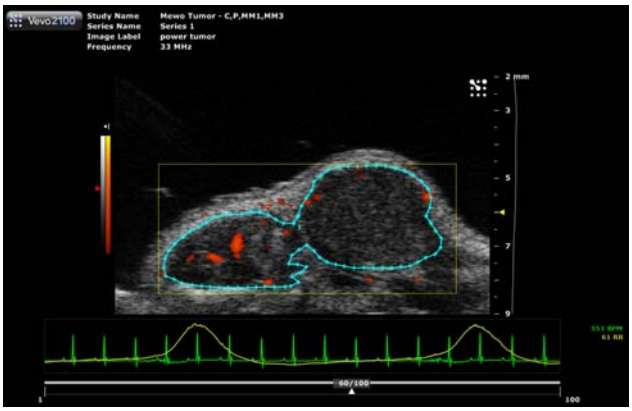

Figure 2 *– Power Doppler imaging of a MeWo tumor on the hind limb of a mouse. An area of interest was drawn around the tumor (turquoise) and the percent vascularization (PV) of the area was calculated to be 5.53% (value shown on another screen).*

Power Doppler imaging can also be completed in 3D, where a motor is used to translate the ultrasound probe over the complete area of the tumor, this technique allows for quantification of the PV for the entire tumor. The image can also be rendered, such that the vascular network within the 3D volume of the tumor can be visualized.

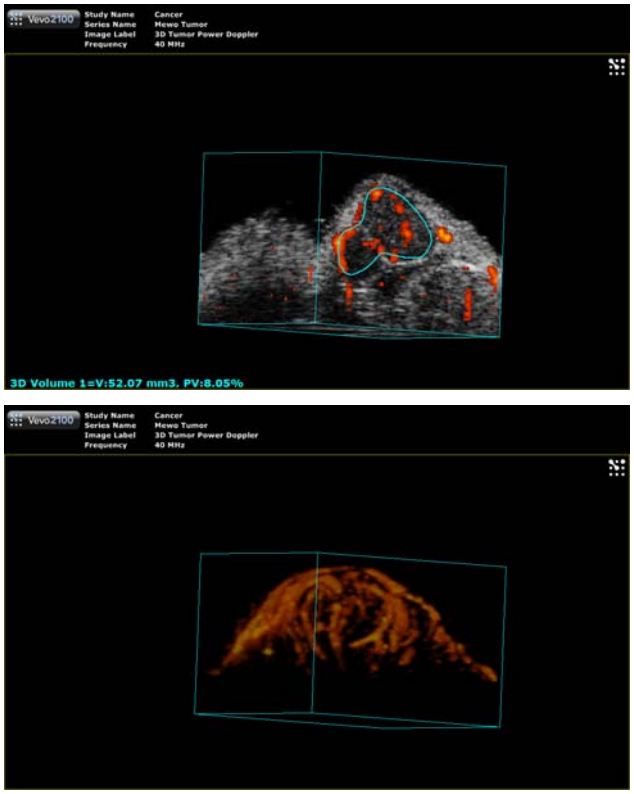

Figure 3 *– 3D Power Doppler imaging of a MeWo tumor on hind limb of mouse. The tumor volume and PV can be quantified (A), while the image can be rendered (B) to show the vascular network of a tumor. Here, as is common with many subcutaneous tumors, the vascular network is capsular, with less blood flow seen in the core of the tumor.*

MicroMarker Contrast Agents:

MicroMarker Contrast Agents are used to enhance the visualization of blood flow down to the capillary level and allow for the quantification of relative perfusion; quantification can be preformed to yield relative blood volume and relative blood velocity. They are injected IV, typically through the tail vein, and circulate through functional blood vessels.

The microbubbles are made up of a phospholipid shell containing a polyethylene glycol outer shell, along with a perfluorobutane/nitrogen gas core. The microbubbles are designed in such a way that the average diameter is 2-3µm in size, allowing for enhanced visualization of blood flow in vessels down to the capillary level.

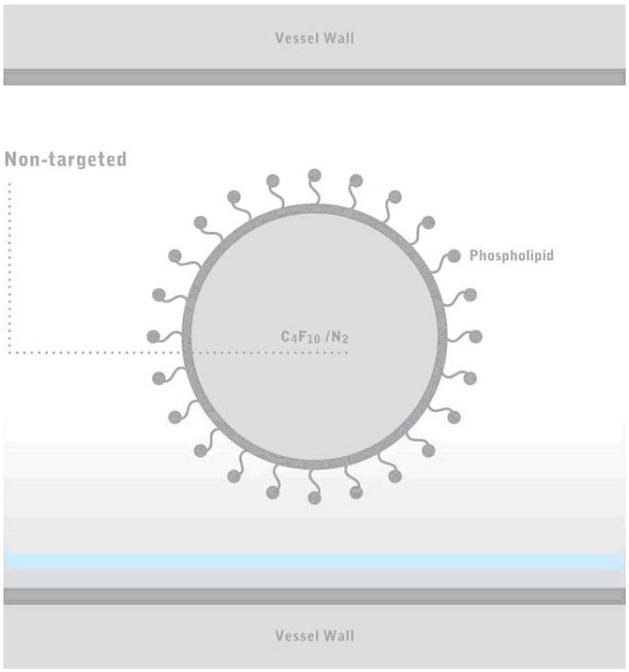

Figure 4 *– MicroMarker Contrast Agent structure. Here the microbubble is shown within a blood vessel.*

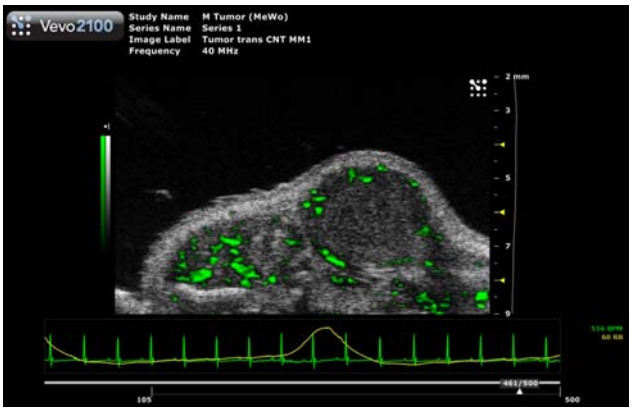

Figure 5 *– MicroMarker Contrast Agent imaging in a MeWo tumor on the hind limb of a mouse. The green contrast overlay is shown wherever the MicroMarker Contrast Agents are present enhancing the visualization of blood flow.*

The slope with which the contrast agents enter a specific region, and the plateau signal intensity they reach allow for quantification of the relative blood velocity (slope) and relative blood volume (plateau level) in a 2D area. If a region of interest is drawn around the tumor a wash-in curve is generated, the software allows for curve fit functionality, where the equation for the curve fit is[2]:

$$Y = A\ (1 - e^{-Bt})$$

Where A is the plateau value, representing the relative blood volume; and the B is the slope, representing the relative blood velocity; while Y is the contrast signal.

Figure 6 *– MicroMarker Contrast Agent bolus wash-in graph for the entire tumor volume. The curve fit functionality provides two values, A is the plateau value which is a measure of relative blood volume, and B is the slope of the curve which is a measure of relative blood velocity.*

The MicroMarker Contrast Agent imaging can also be done in 3D, where a motor is used to translate the ultrasound probe over the complete area of the tumor. This type of imaging provides a measure of relative blood volume for the entire tumor, detecting blood flow down to the capillary level. The relative blood volume is calculated as the percent agent (PA), which represents the percentage of pixels within the defined volume which have a contrast agent signal associated with them, indicating the presence of blood flow.

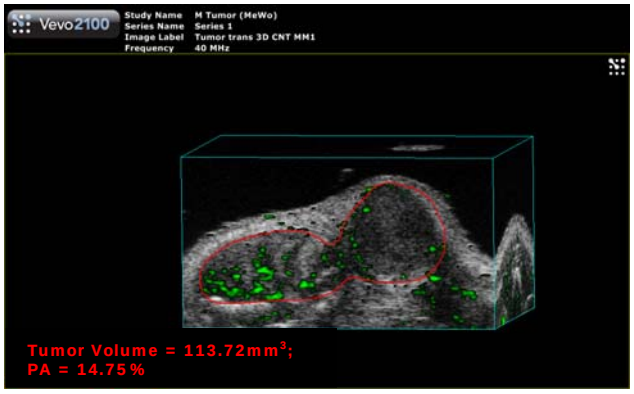

Figure 7 *– 3D MicroMarker Contrast Agent imaging in a MeWo tumor on the hind limb of a mouse. The percent agent (PA) is calculated and is a measure of relative blood volume.*

Additional Example:

The above explanations of how Doppler imaging modes and MicroMarker Contrast Agent imaging can be used to study the vascularity of tumors were done showing examples of a subcutaneous tumor on the hind limb of a mouse.

The Vevo 2100 is well suited for studying orthotopic and transgenic tumor models as shown below using a transgenic prostate tumor model.

The vascularity of the entire tumor volume was quantified using 3D Power Doppler imaging, the tumor was found to be 7.05mm3 having a percent vascularity of 2.98% (Figure 8). MicroMarker Contrast Agents were used to enhance the visualization of blood flow in the tumor; two regions of interest were drawn on the image to generate the wash-in curves, one on the tumor and the other on the surrounding prostate (Figure 9). The wash-in curve for the tumor has a well defined inflow; the slope represents the relative blood velocity, while the plateau represents the relative blood volume. The wash-in curve for the prostate tissue on the other hand is not well defined, showing very little increase in contrast signal, this indicates very little blood flow within this area of interest.

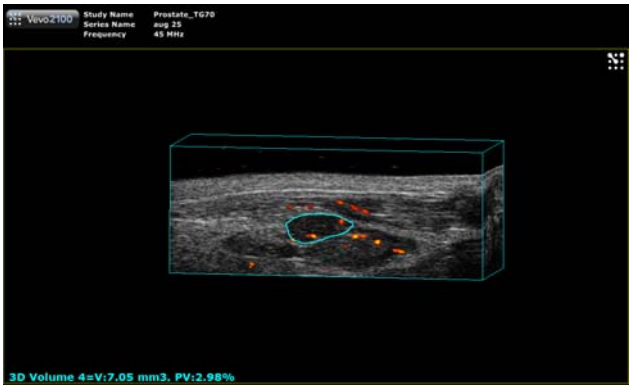

Figure 8 *– 3D Power Doppler imaging in a transgenic prostate tumor. The tumor volume and percent vascularity are calculated.*

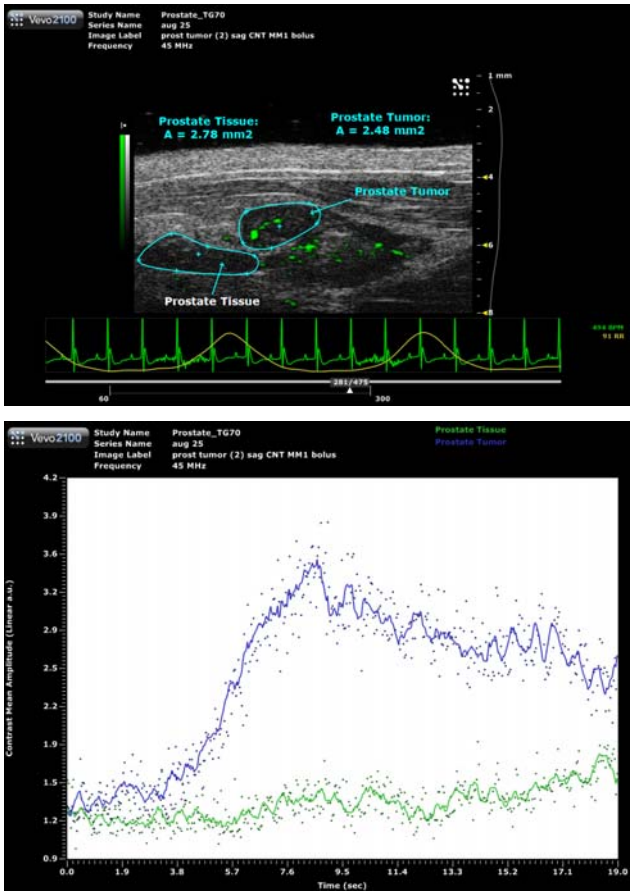

Figure 9 *– MicroMarker Contrast Agent imaging in a transgenic prostate tumor. Two regions of interest are drawn, one around the tumor and the other around the surrounding prostate tissue (A). The wash-in curves were generated (B) showing a well defined wash-in curve for the tumor (blue), while the wash-in curve for the prostate tissue (green) is not well defined.*

Conclusions:

The images presented here clearly show the utility of the Vevo 2100 High Resolution Ultrasound Imaging System as a tool for in vivo imaging and quantification of tumor vascularity. With the resolution of the Vevo 2100 pre-palpable tumors can be imaged and followed longitudinally throughout the course of a study. The non-invasive nature of ultrasound imaging allows the same tumor to be studied over the course of an experiment, leading to much stronger data and requiring fewer animals to get significant results.

Color and Power Doppler provide the ability to study the changing vascularity of a tumor over time without the use of contrast agents, however by using MicroMarker Contrast Agents the imaging session provides information on the vascularity of the tumor down to the capillary level allowing for the quantification of relative perfusion. This additional information is desirable as the smaller vessels such as the capillaries are the site of oxygen and nutrient exchange, and are therefore crucial to the growth of the tumor.

Additionally, all imaging is done in vivo and the vasculature is studied in real time allowing for quantification of actively perfused blood vessels. This becomes increasingly important when studying tumors as the vascular network of a tumor is disorganized. Vessels may be forming, and therefore counted using traditional techniques such as histology; however they are not actively perfused and are therefore not contributing to the growth or survival of the tumor.

The techniques discussed above allow for acquisition of real time data on actively perfused vessels, and therefore allow for the study of acute or chronic changes in perfusion in response to a potential therapeutic or experimental procedure.

References:

[1] Wirtzfeld, LA, G Wu, M Bygraves, Y Yamasuki, et al. A New Three-Dimensional Ultrasound Microimaging Technology for Preclinical Studies Using a Transgenic Prostate Cancer Mouse Model. Cancer Res 65(14):6337-6345, 2005.

[2] Wei, K, AR Jayaweera, S Firoozan, A Linka, et al. Quantification of Myocardial Blood Flow with Ultrasound-Induced Destruction of Microbubbles Administered as a Contrast Venous Infusion. Circulation 97(5):473-83, 1998.

Recommended Papers:

Detecting Vascular Changes in Tumor Xenografts Using Micro-Ultrasound and Micro-CT Following Treatment wit VEGFR-2 Blocking Antibodies.
Cheung, AMY, AS Brown, V Cucevic, M Roy, et al. Ultrasound in Med & Biol 33(8):1259-68, 2007.

Volumetric High-Frequency Doppler Ultrasound Enables the Assessment of Early Antiangiogenic Therapy Effects on Tumor Xenografts in Nude Mice.
Jugold, M, M Palmowski, J Huppert, EC Woenne, et al. Eur Radiol 18(4):753-8, 2008.

Nanosecond Pulsed Electric Fields Cause Melanomas to Self-Destruct.
Nuccitelli, R, U Pliquett, X Chen, W Ford, et al. Biochem Biophys Res Comm 343(2):351-60, 2006.

Recommended VisualSonics Protocols:

VisualSonics Vevo 2100 Imaging System, Operators Manual

Doppler Imaging

Vevo 2100 App Protocol – Imaging Using Color and Power Doppler (PN:12072)

Principles of Doppler: Pulsed-Wave Doppler, Color Doppler, Power Doppler

MicroMarker Contrast Agent Imaging

Vevo 2100 App Protocol 3D Imaging Using the Untargeted MicroMarker (PN:12073)

VisualSonics Application Protocol – Image Enhancement by Bolus Injection using Untargeted MicroMarker

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