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Product Brief:

VevoCQ™ Advanced Contrast Quantification Software Analysis Tools
for the Vevo® 2100 System

Introduction

Microbubble contrast agents have been used as a method of assessing *in vivo* microvascular perfusion where velocities require enhanced sensitivities compared to those available in standard Doppler techniques. This highly sensitive contrast imaging technique was optimized with the development of the Vevo 2100 linear array based high-frequency ultrasound imaging system and subsequent release of Nonlinear Contrast Imaging Mode, in which an amplitude moldulation pulse sequencing scheme was implemented to retain the nonlinear microbubble signal at the fundamental frequency.

The VevoCQ software builds on this highly sensitive imaging technique, allowing for advanced quantification of the Nonlinear Contrast Mode images. There are two available MicroMarker™ Contrast Agent products, Non-Targeted and Target-Ready MicroMarker; the former is used to enhance the visualization of the perfused microvasculature within a tissue of interest, while the latter is used to enhance the visualization of an endothelial cell surface marker, such as vascular endothelial growth factor receptor 2 (VEGFR2). The VevoCQ software provides analysis tools to aid in the quantification and visualization of key parameters for both of these MicroMarker Contrast Agents.

When working with Non-Targeted MicroMarker Contrast Agents one is typically investigating perfusion kinetics of a tissue, organ or tumor of interest. The contrast agent can be delivered by bolus injection and a time vs. intensity curve is generated. Alternatively, the contrast agent can be infused and a destructive pulse is applied and a replenishment curve is generated. Both scenarios are included within the software. Perfusion modeling is performed with both these experimental designs in which amplitude related parameters are expressed relative to echo power, time related parameters are expressed in seconds and both are combined to produce quantities relating to blood flow and dynamics.

In addition, VevoCQ provides quantification of Target-Ready MicroMarker Contrast Agents to detect the binding of microbubbles to a cell surface marker within a tissue of interest. This is achieved via late phase targeted contrast enhancement imaging. Differential targeted enhancement is calculated by the VevoCQ software as an estimate of the bound contrast agent. When this value is generated for both a specific target as well as a negative control, an estimate of the relative expression for the target of interest can be assessed.

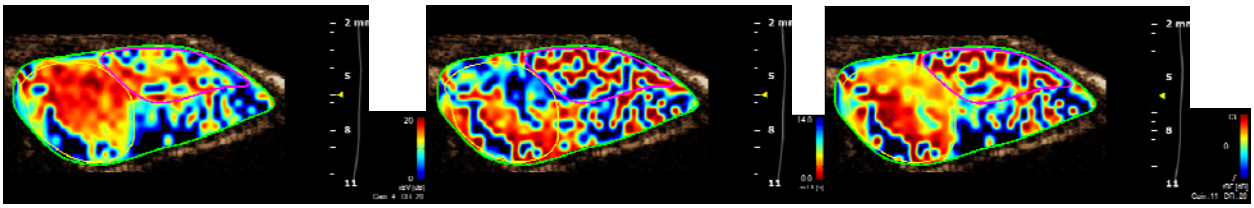

Figure 1 – Parametric images from destruction-replenishment perfusion model, (a) relative blood volume, (b) mean transit time, (c) relative blood flow

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Background Theory

This section is a brief discussion on how the VevoCQ software works and the mechanisms behind image generation.

Images acquired in Nonlinear Contrast Mode on the Vevo 2100 high-frequency ultrasound imaging system are compatible with the VevoCQ software. The software analysis tool is a post-processing tool which can be applied to the data at any point after acquisition. The software is available for both the Vevo system and for the desktop workstation.

Perfusion Modeling

As discussed earlier, there are 2 options for perfusion modeling. Both bolus and destruction-replenishment models are included and subsequent perfusion parameters are available for quantification of contrast enhancement kinetics.

Bolus Perfusion Model

In this model the Non-Targeted MicroMarker Contrast Agent is delivered as a bolus injection intravenously, the perfusion model is defined as:

$$f(t) = O + A\frac{1}{st\sqrt{2\pi}}e^{-\frac{(\ln(t)-m)^2}{2s^2}}, t > 0 \qquad (1)$$

Where O, A, m and s are fitting parameters; O is the offset, A is an amplitude parameter and m and s are the mean and standard deviation of the normally distributed natural logarithm of t, respectively.

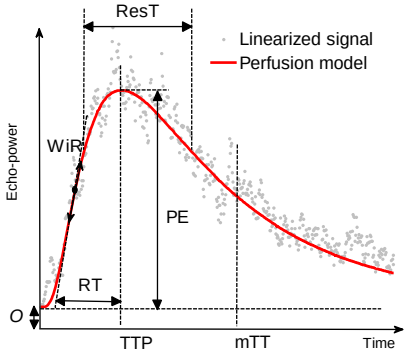

Figure 2 – Bolus perfusion model curve fit algorithm showing various calculated parameters

Figure 2 displays the bolus perfusion curve fit algorithm as outlined in equation 1, where Rise Time (RT), Time to Peak (TTP), Wash-in Rate (WiR) and Peak Enhancement (PE) are all

output parameters of the bolus perfusion model. The perfusion parameters are a combination of amplitude related parameters which are relative to echo-power, time related parameters and a combination of the two which are related to blood flow kinetics.

Destruction-Replenishment Perfusion Model

In this model the Non-Targeted MicroMarker Contrast Agent is delivered via constant infusion intravenously and then a destructive pulse is applied and the replenishment kinetics are observed. The perfusion model is defined as:

$$f(t) = O + \frac{A}{2}\left[1 + erf\left(\frac{\ln(t) - m}{s\sqrt{2}}\right)\right], t > 0 \qquad (2)$$

Where O, A, m and s are fitting parameters, which have already been defined for equation 1.

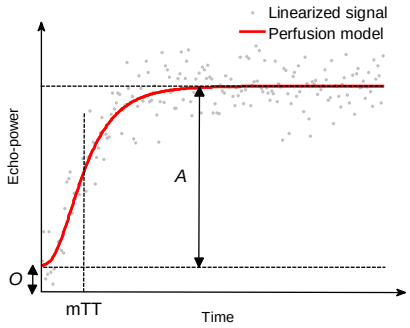

Figure 3 – Destruction-Replenishment perfusion model curve fit algorithm showing various calculated parameters

Figure 3 displays the destruction-replenishment perfusion curve fit algorithm as outlined in equation 2, Mean Transit Time (mTT) and the A (plateau) value are all output parameters. In this model the output parameters include only relative blood volume (the plateau or A value from curve fit algorithm). Mean transit time is an absolute time measurement representing filling kinetics and relative blood flow which is a ratio of these two parameters.

Targeted Contrast Imaging

Target-Ready MicroMarker Contrast Agents can be conjugated to an antibody or ligand of interest to detect the expression of a target of interest, such as endothelial cell surface receptors. The VevoCQ software analysis tool allows for the quantification of late phase differential targeted enhancement. In addition, color-coded parametric images are created

for qualitative assessment of spatial distribution of the bound contrast agent. The differential targeted enhancement (ΔTE) is defined as:

$$\Delta TE = TE_{bd} - TE_{ad} \qquad (3)$$

Where TE_{bd} and TE_{ad} refer the targeted enhancement before and after destruction respectively.

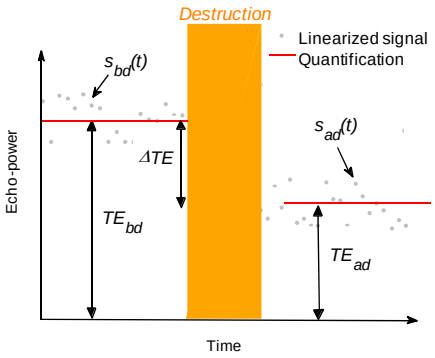

Figure 4 – Differential targeted enhancement model showing various parameters

Figure 4 displays the differential targeted enhancement model outlined in equation 3, here the s_{bd} and s_{ad} are simply the linearized signal before and after destruction which are used in subsequent calculations of variation within the software.

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Features

1. User-defined regions of interest selection (up to 4)

The ability to define multiple areas of interest allows selection and detection of anatomical regions with differing perfusion kinetics to be chosen for investigation (i.e. tumor and surrounding normal tissue or multiple areas within a tumor).

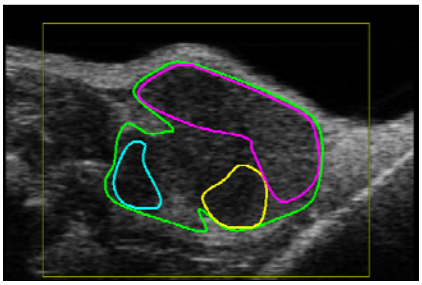

Figure 5 – Location of the four user defined regions of interest on the subcutaneous tumor

2. Curve fitting capabilities

Three options (based on what protocol for microbubble administration is used) are available such that data is plotted and generated for both perfusion and targeted molecular imaging studies.

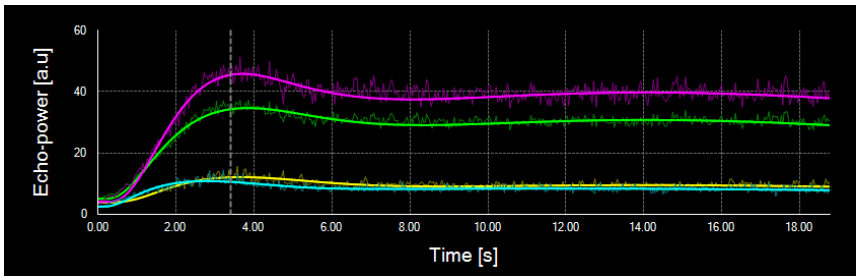

Figure 6 – Bolus perfusion model curve fit algorithm as applied to each region of interest on the subcutaneous tumor model

3. Data quantification

A multitude of quantification tools are available such that data and parameters can be generated that relate to perfusion kinetics as well as molecular imaging capabilities (this is derived from the curve fittings).

Table 1 – Selection of VevoCQ bolus perfusion model parametric outputs

	Whole Tumor (green)	Bottom Right (yellow)	Upper Area (pink)	Bottom Left (turquoise)
Peak Enhancement (a.u.)	29.61	8.21	41.73	8.32
Time to Peak (sec.)	3.84	3.66	3.70	2.78
Wash-in Rate (a.u.)	15.50	4.51	22.56	6.09

Data is output in a TSV format (Excel).

Table 2 – Output parameters for bolus perfusion model

PE	Peak Enhancement	[a.u]
AUC	Area Under the Curve, limited to peak opacification instant	[a.u]
RT	Rise Time	[s]
TTP	Time To Peak	[s]
WiR	Wash-in Rate (maximum slope)	[a.u]
PI	Perfusion Index (AUC / RT)	[a.u]

Table 3 – Output parameters for destruction-replenishment reperfusion model

rBV	relative Blood Volume (A)	[a.u]
mTT	mean Transit Time	[s]
rBF	relative Blood Flow (rBV / mTT)	[a.u]

4. Parametric imaging

Allows a visualize display of the intensity of perfusion kinetics and molecular signal.

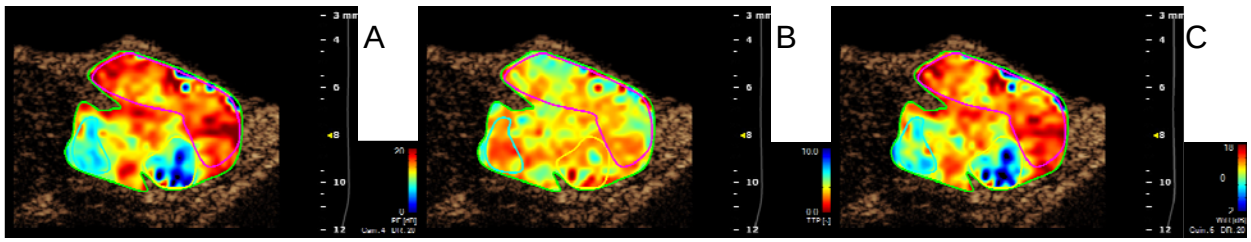

Figure 7 – Parametric images from bolus perfusion model, (a) peak enhancement, (b) time to peak, (c) wash-in rate

Images and videos can be exported in a variety of formats such as TIFF, Bitmap, and WMV.

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Summary

The VevoCQ software is an advanced software analysis tool to be used in conjunction with the highly sensitive Nonlinear Contrast Imaging Mode available on the Vevo 2100 system. This software allows for the study of contrast uptake kinetics as well as late phase targeted enhancement. It provides advanced curve fitting algorithms for quantitative assessment of perfusion parameters as well as color-coded parametric images useful for qualitative assessment of the spatial distribution of the same parameters. The software is a post-processing tool which can be used on numerous tissues, organs and tumor models, including subcutaneous tumors, abdominal organs and hind limb muscles.

Recommended Papers

Quantifying Antivascular Effects of Monoclonal Antibodies to Vascular Endothelial Growth Factor: Insights from Imaging. O'Connor, JPB, RAD Carano, AR Clamp, J Ross, et al. Clin Cancer Res 15(21):6674-82, 2009.

A New 15-50 MHz Array-Based Micro-Ultrasound Scanner for Preclinical Imaging. Foster, FS, J Mehi, M Lukacs, D Hirson, et al. Ultrasound Med Biol 35(10):1700-8, 2009.

Inhibition of Hedgehog Signaling Enhances Delivery of Chemotherapy in a Mouse Model of Pancreatic Cancer. Olive, KP, MA Jacobetz, CJ Davidson, A Gopinathan, et al. Science 324(5933):1457-61, 2009.

Sunitinib and PF-562,271 (FAK/Pyk2 inhibitor) Effectively Block Growth and Recovery of Human Hepatocellular Carcinoma in a Rat Xenograft Model. Bagi, CM, J Christensen, DP Cohen, WG Roberts, et al. Cancer Biol Ther 8(9):856-65, 2009.

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VevoCQ Product Specifications

Product number: VS-12176

Description:

- Post-processing tool for perfusion and targeted signal analysis.
- Perfusion parameters (including amplitude and time) derived from a curve fitting algorithm for bolus kinetics and replenishment kinetics following destruction reperfusion.
- Spatial rendering is available in the form of parametric maps.
- Data can be exported in .tiff, .bmp, .wmv files and .tsv formats.
- **Requires** Nonlinear Contrast Imaging Functionality on the Vevo 2100 system (VS-11953).
- Currently works with data generated with the MS200 and MS250 MicroScan™ probes.