VISUALSONICS
FUJIFILM

Imaging Guide

2D **Non-Targeted** Contrast Agent Perfusion imaging using the **Vevo 3100** System

Vevo® 3100

System Compatibility: This guide contains instructions and suggestions for work on the Vevo 3100 and transducers from the MX series.

Objective

The objective of this protocol is to describe the activities performed during a study, including:

- injecting contrast agent by bolus or constant infusion
- acquiring a cine loop during the wash-in of the contrast agent
- creating contrast region measurements on the cine loop
- visualization, analysis and data management post-processing image optimization

This protocol is intended for mouse imaging applications.

Tools Used During the Study

- Vevo® 3100 high-resolution imaging system with
  - Nonlinear Contrast Mode enabled
  - MX201 or MX250 ultrasound transducers
- *VisualSonics Protocol - Preparation Protocol for Non-Targeted Contrast Agent*, and all tools listed within
- Guide to Animal Preparation and Anesthesia_v1.0
- Medical air (oxygen content less than 31%)

Imaging Protocol

A Prepare Contrast Agent

1. Prepare the contrast agent according to the instructions provided in *VisualSonics Protocol – Preparation Protocol for Non-Targeted Contrast Agent*.

Note: *Suggested contrast agent concentration and doses are outlined in the preparation protocol*.

B Prepare subject animal

1. Prepare the animal for contrast agent vascular access via tail vein, jugular vein or retro-orbital sinus. A cannulation technique is strongly preferred to prevent movement of the subject during imaging.

2. Prepare the subject animal for ultrasound imaging as per Institution's Animal Care Committee approved SOP.

Note: *It is important to prevent motion of the target tissue during the experiment as much as possible. Prepare materials and methods prior to starting the study and avoid touching or moving the animal in any way.*

C Prepare the Vevo 3100 Imaging System

Application Start up

Login on the system;
Connect transducer **MX250** to the system and start by initializing the transducer;
Select the appropriate application, in this case Mouse Contrast, from the Application drop-down menu;

Fig.1 – Transducer initialization and application selection

Image Optimization

- Start imaging in **B-Mode** and select the appropriate preset, i.e. Subcue Tumor, from the **Presets** fly-out list;

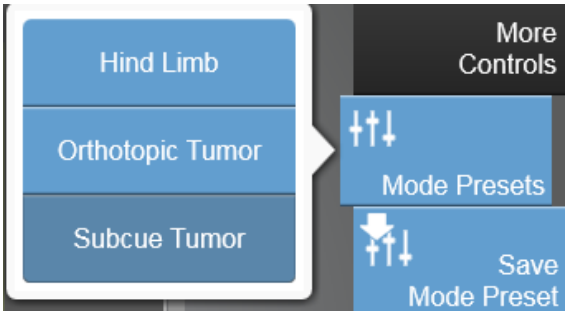

Fig.2 – Preset selection

- Place the transducer in the imaging stand and obtain the desired imaging plane;

- Adjust the acquisition parameters as required for tumor imaging and tap the **Save Preset** control to save the custom preset specific to your tumor model for future imaging sessions.

  Note: In order to maximize tissue signal suppression, use only a small amount of gel stand-off in between the transducer surface and the skin.

- Tap "**More Controls**" in the Controls bar and select the **General Tab**.

- Set the Cineloop Mode to "**Prospective**".

- Tap the **NCL** control on the Control Panel to start data acquisition in Nonlinear Contrast Mode.

- Adjust the size of the Contrast box as required for the region of interest;

- Set the **Frame Rate** to 15 or 20 fps such that the full wash-in can be observed for the bolus to be injected. With a Frame Rate of 15, adjust the Contrast Box to have at least 600 frames in the cineloop. If more frames are needed, navigate to the Cineloop Mode and check "**Extended Buffer**". This feature will extend the buffer up to 10 000 frames.

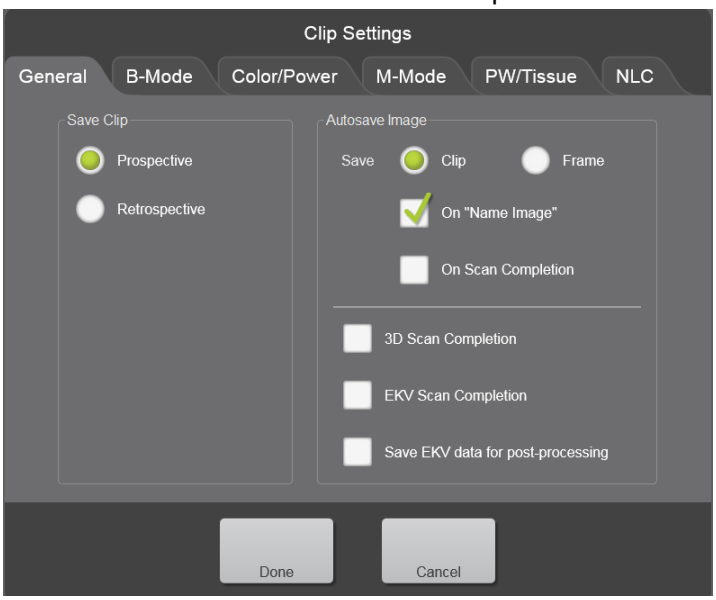

Fig.3 – Setting "Save Clip"

Image Acquisition by Bolus Injection

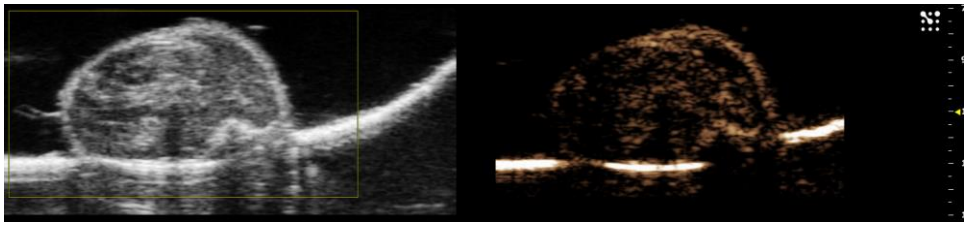

Fig. 4 – Tumor perfusion by bolus injection

- Prepare the bolus to be injected;
- Tap "**Save Cine**" and inject the bolus over 5 sec.;
- Keep the "Bolus" syringe in the cannula until the entire cineloop is acquired and saved;
- When imaging has stopped, replace the bolus syringe with the Flush syringe and inject approximately 20-30ul of saline;
- Tap "**Name Image**" and enter a label for the acquired cineloop, i.e. "First bolus injection".

Note: The injection can be repeated after 10-15 mins. The number of injections has to be limited by the maximum volume of fluid the animal can be administered during the imaging session. Refer to the approved animal protocol for specifications.

Image Acquisition by Constant Infusion

- Prepare the volume to be injected;
- Start imaging in **NCL** Mode;
- Tap the "**Burst Settings**" control;
- Set the "**Burst Duration**" and "**Burst Position**" as required;

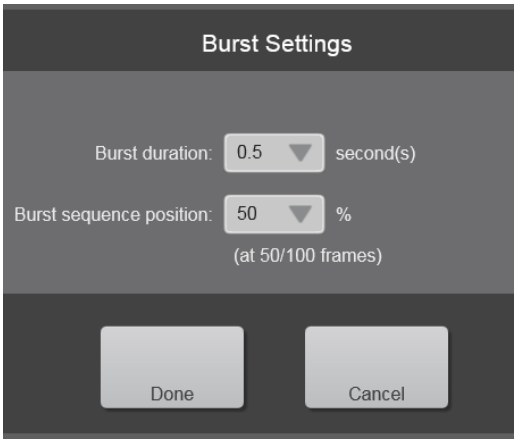

Fig.5 – Burst Settings panel

- Start the Contrast perfusion and observe the image;
- When the contrast has fully perfused the area of interest tap the "**Burst Sequence**" control;
- The system will restart acquisition, run the burst sequence and continue data acquisition for the length of the buffer.
- Tap "**Name Image**" to label the image;
- Attach the Flush syringe and inject the 20-30ul of saline after the entire volume has been injected and the cineloop acquired and saved ;
- Tap "Name Image" and enter a label for the acquired cineloop, i.e. "First replenishment injection".

Note: The injection can be repeated after 10-15 mins. The number of injections has to be limited by the maximum volume of fluid the animal can be administered during the imaging session. Refer to the approved animal protocol for specifications.

Image Post-Processing

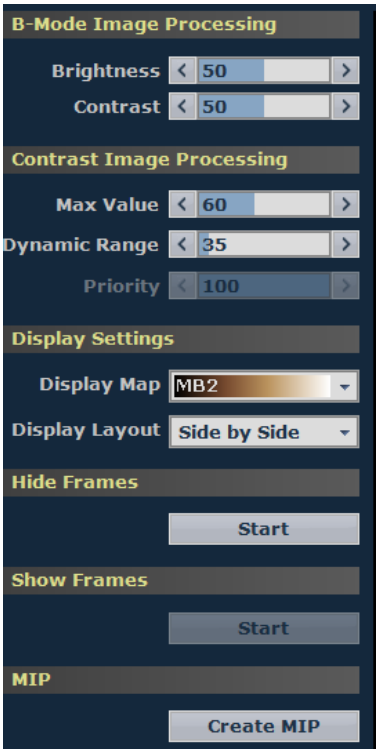

Fig.6 – Post-Processing panel in VevoLab

Image processing tools are available on the Vevo 3100 system with the image in review or from the Post-Processing Panel in VevoLab. These features can help with visual enhancements in the acquired images, especially for presentations.

- For the B-Mode image the **Contrast** and **Brightness** can be adjusted on the system by sliding the cursor in the fly-out control or in VevoLab by clicking the ">" or "<" or by simply dragging the cursor in the percentage bar.
- For the Contrast Image, in VevoLab, there are 3 parameters:
  - Max Value (dB)
  - Dynamic Range (dB)
  - Priority

All these parameters can be adjusted similar to Contrast and Brightness. Max Value and Dynamic Range should be adjusted in pairs as either one of them could apply drastic changes to the image.
Priority is only adjustable when "Both" is selected as the display layout. This layout overlays the nonlinear contrast image on top of the B-Mode image. Priority adjustment controls the degree of transparency of the Nonlinear Contrast image.

Note: All parameters adjusted in the post-processing tool are for visual image enhancement only. The intensity values and analysis are **not** affected by these adjustments.

Data Analysis

Nonlinear Contrast data analysis is available on the Vevo 3100 by tapping the Contrast Region measurement or in VevoLab from the measurements panel. The steps are:

- Trace the **region of interest** on the NLC image;
  - ➢ On the Vevo 3100, start by tapping "Set" on the first point and then simply trace around the area of interest;
  - ➢ In VevoLab left-click to start the contour and right-click to end. With every left-click you can add additional points to refine the contour.

- The application supports up to 5 contrast region measurements on every image and these measurements can also be replicated with **Copy/Paste** on multiple images.

- In VevoLab right-click the measurements label to display the **Region Graph**;

- On the Vevo 3100, tap the Graph icon in the measurements display;

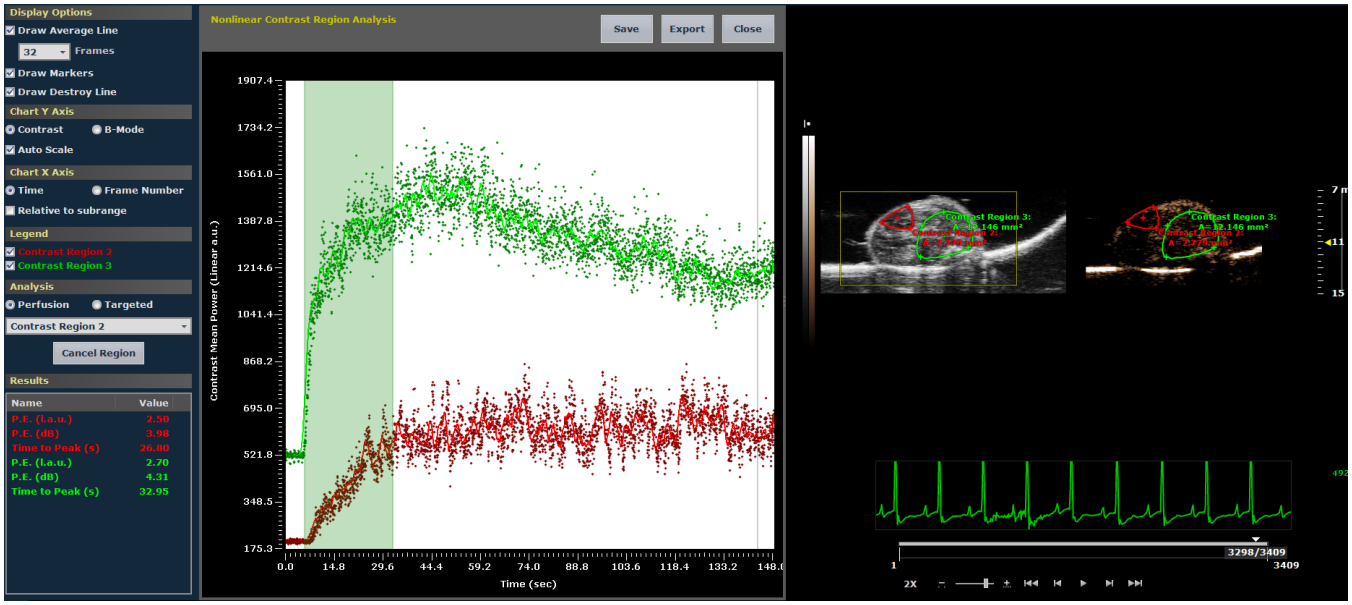

Fig.7 – Analysis window in VevoLab with Display, Analysis and Results panels on the left, Graphs panel in the middle and Image panel on the right side

Peak Enhancement, **P.E.**, in dB units and in arbitrary units (a.u.) and **Time to Peak** values in seconds.

Peak Enhancement is the ratio of the plateau value to the baseline contrast value and it translates into a measure of **relative blood volume**.

Time to Peak is the amount of time it takes the contrast intensity to reach a plateau value. This is a measure of relative **blood velocity**.

The analysis can be completed on each of the measurement contours, the results are displayed on the screen and can also be exported as a comma separated value file (can be read by Microsoft Excel) along with all the contrast plot data.

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