VISUALSONICS
FUJIFILM

Imaging Guide

2D **Target-Ready** Contrast Agent
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 Target-Ready Contrast Agent*, and all tools listed within
- Guide to Animal Preparation and Anesthesia_v1.0
- Medical air (oxygen content less than 31%)
- Timer

Imaging Protocol

A Prepare Contrast Agent

1. Prepare the contrast agent according to the instructions provided in *VisualSonics Protocol – Preparation Protocol for Target-Ready 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 and Use 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;

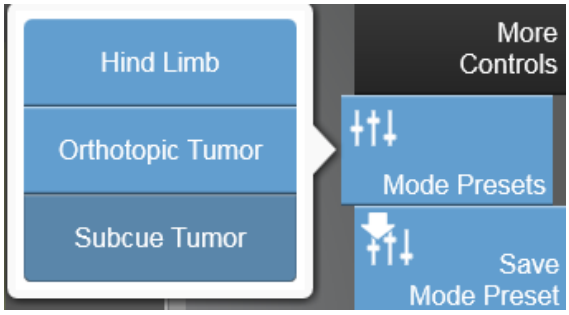

Fig.2 – Preset selection

- 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.

  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.

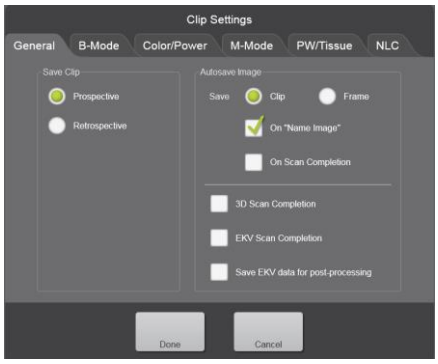

Fig.3 – Setting "Save Clip"

Image Acquisition

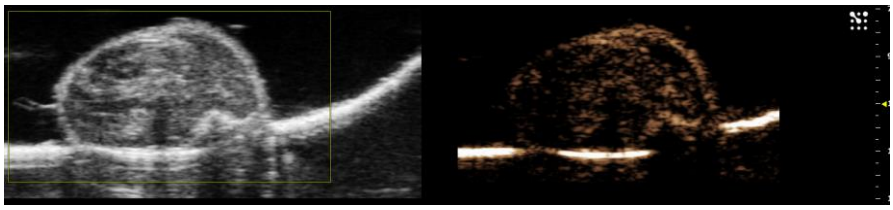

Fig. 4 – Tumor perfusion by bolus injection

Control Isotype injection

- Set Timer to 4 minutes;
- Tap the "**Burst Settings**" control;
- Set the "**Burst Duration**" to the value of choice or leave it at the default value, and "**Burst Position**" to 50%;

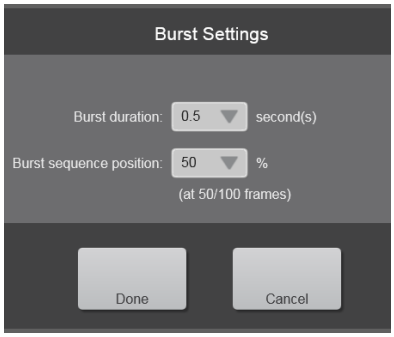

Fig.5 – Burst Settings panel

- Prepare the "Control Isotype" 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. "Control bolus injection".
- Press "Start" on the Timer and ensure that imaging is paused for the 4 minutes wait.

After 4 minutes

- Start imaging and 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**" and enter a label for the acquired cineloop, i.e. "Control sequence".

Antibody injection

The injection with the antibody of choice can be performed after 10-15 mins and the Isotype Control injection to allow clearance of the first injection from the blood stream.

After 10-15 mins repeat the steps above in the exact order to ensure consistency and data accuracy.

- Set Timer to 4 minutes;
- Prepare the "Antibody" 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. "Antibody bolus injection".
- Press "Start" on the Timer and ensure that imaging is paused for the 4 minutes wait.

After 4 minutes

- Start imaging and 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**" and enter a label for the acquired cineloop, i.e. "Antibody sequence".

Note: The injection can be repeated after 10-15 mins, however the number of injections is 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

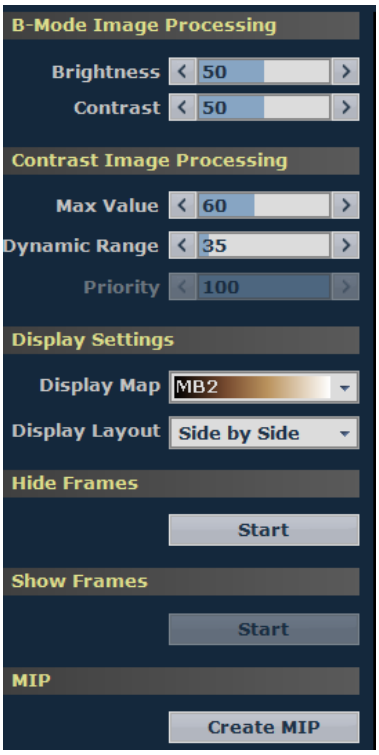

Fig.6 – Post-Processing panel in VevoLab

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. Both ways the steps are:

- Open the "Control Sequence" image and trace the **region of interest**;
  - On the Vevo 3100, start by tapping "Set" on the first point and then simply trace around the area of interest;
  - On the Vevo 3100, tap the Graph icon in the measurements display

to display the graph for the contrast data in each of the images;

- 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.

- After the measurement has been completed, right-click to edit the measurement label to "Control Seq.";

- 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.

- Select to Copy/Paste the Contrast Region measurement from the "Control Sequence" image and open and Paste the measurement on the "Antibody Sequence" image. After the measurement has been completed, right-click to edit the measurement label to "Antibody Seq.";

- Right-click the measurements label to display the **Region Graph**;

- Select "Report" and in the report check the Control and Antibody;

- The graphs from both images will be displayed and you can select the regions before and after the burst pulse to calculate the change in intensity based on the amount of contrast bound to the expressed biomarker.

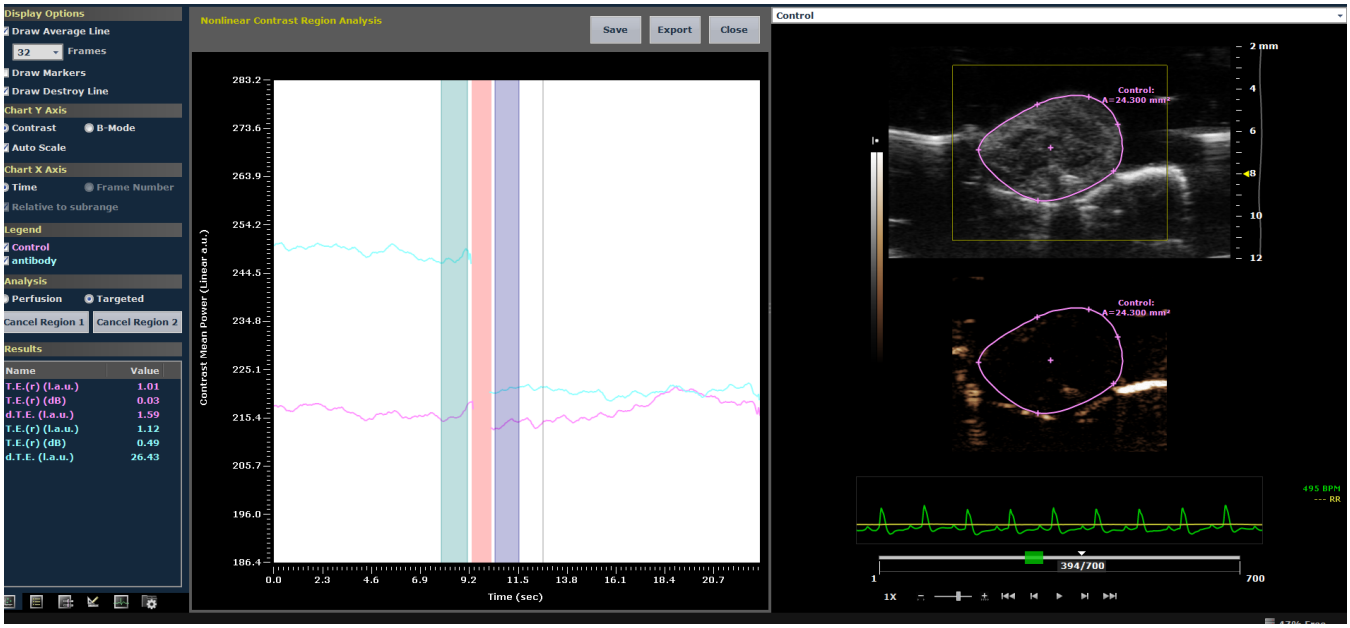

Fig.7 – Analysis window in VevoLab with Display, Analysis and Results panels on the left, Graphs panel in the middle and Image panel on the right side

The application calculates the Target Enhancement, **T.E.**, in dB units and in arbitrary units (a.u.) based on change in intensity before and after the burst. In the image above the graphs display the difference in intensity change in between the control isotype, there is insignificant binding in the region of interest, and the "antibody" where there is quite a drop in intensity after the burst.

FUJIFILM 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