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VisualSonics Application Protocol

3D Tumor Imaging using the MicroMarker Non-Targeted Contrast Agents

Part Number: 11902

1 Objective

The objective of the **3D Tumor Imaging using the MicroMarker Non-Targeted Contrast Agents** protocol is to outline the steps that are involved in quantifying the microbubble signal in a 3D volume.

- Administer contrast agent by bolus or continuous infusion
- Acquiring a pre-bolus Contrast Mode 3D cine loop
- Acquiring a post-bolus Contrast Mode 3D cine loop
- Processing of the Contrast Mode 3D data
- Analyzing of the Contrast Mode 3D data

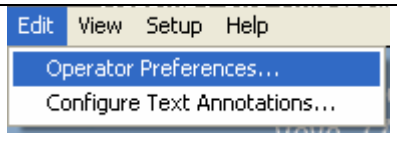
This protocol is intended for mouse imaging applications.

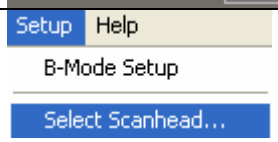
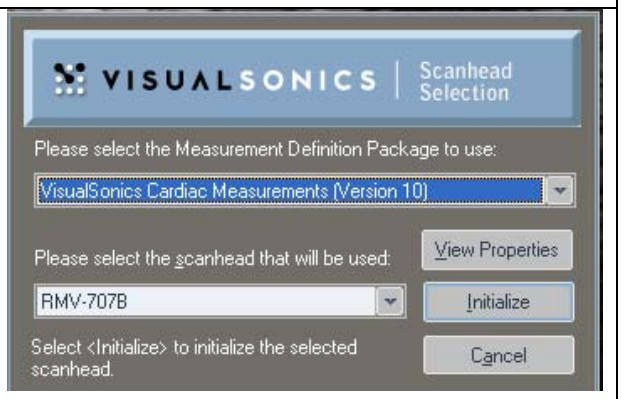
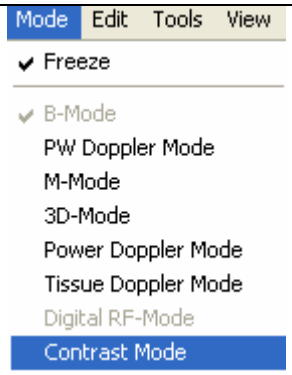
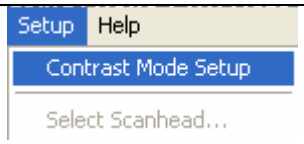
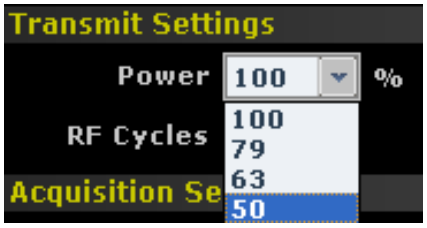
2 Tools Used During the Study

- Vevo 770® High-resolution imaging system with software version 2.3.0 or higher installed
- Vevo Contrast Mode software
- The appropriate VisualSonics Preparation Protocol for the MicroMarker Non-Targeted contrast experiment being performed, and all tools listed within
- Medical air (oxygen content less than 31%)

3 Preparation

3.1 Prepare the Vevo 770 system

1. From the Edit menu, select **Operator Preferences**.	

2. In the Operator Preferences dialog box, specify *800* for the Default Cine Loop Size for Contrast Mode.	
3. Attach the 3D motor to the Vevo 770 Rail system, connect the appropriate RMV to the Vevo770 system, and attach the RMV to the 3D motor.	
4. From the Setup menu, choose **Select Scanhead**, or press the Select Scanhead key. Select the connected RMV from the Scanhead Selection dialog box, and click **Initialize**. Although RMV-707B is shown in this example any RMV can be used. Please utilize the appropriate RMV for the tissue being imaged.	
5. From the Mode menu, select **Contrast Mode**, or press the contrast mode button on the keyboard. The system begins scanning automatically.	
6. From the Setup menu, select **Contrast Mode Setup**, or press the Mode Setup key on the keyboard.	
7. In the Transmit Settings, select **50%** Power.	
8. From the Setup menu, select **Contrast Mode Setup** or press the Mode Setup	

key on the keyboard to turn off the Contrast Mode setup panel.	

3.2 Prepare contrast agent

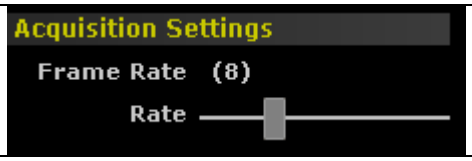
1. Prepare the contrast agent according to the instructions provided in the VisualSonics Preparation Protocol for bolus or continuous infusion.

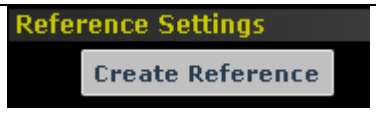
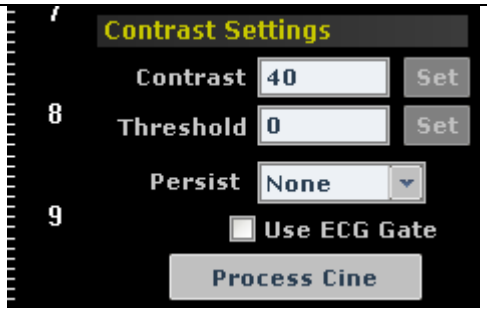
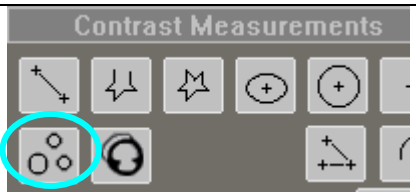
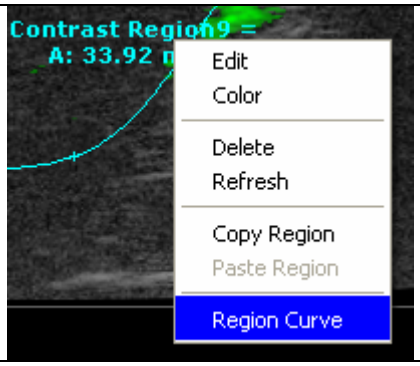
3.3 Prepare subject animal

1. Prepare the subject animal for contrast agent injection and gain vascular access via a tail or jugular vein.

4 Preliminary Steps Necessary for a New Model

Note: These steps are only necessary when establishing this protocol with a new model. The objective of this section is to ensure that the level of circulating microbubbles is constant after bolus injection of the MicroMarker contrast agent for at least the time required to acquire a 3D volume of your area of interest (approximately 20-30 seconds as a minimum). Once this has been confirmed for your model, these steps are not needed every time an experiment is conducted.
If the results show that a bolus delivery is not sufficient to maintain a constant level of circulating microbubbles for a short time, then an infusion pump will be required to infuse and maintain a constant level of circulating microbubbles.
Once this has been completed for your model, start with the steps outlined in section 5 for future studies.

1. After all preparation (3.1-3.3) steps have been completed, press **Scan/Freeze** to begin scanning in Contrast Mode.	
2. Adjust the frame rate to 8-10 frames per second. This will increase the time acquired in the 800 frame cine loop.	
3. Using the height adjustment knob on the rail system place the image of the tumor in the **focal zone**.	
4. Find the imaging plane where the tumor displays the largest area. Save a 2D cine loop of this area that is at least 300 frames in length and label as *2D baseline*.	
5. Acquire a Contrast Mode 2D cine loop	

while injecting the bolus. Press **PreTrig** and approximately 2 seconds later, inject the bolus of MicroMarker Non-Targeted Contrast Agent. Once the Pretrig acquisition is complete, save and label this cineloop as *2D bolus*.	
6. Load the *2D baseline* cine loop and create the reference by clicking on **Create Reference**.	
7. Load the *2D bolus* cine loop and process this cine loop. Set **Contrast** to **40** and the **Threshold** to **0** prior to pressing the **Process Cine** button.	
8. Draw a contrast region of interest on the tumor contour.	
9. Generate a Contrast Region Curve by moving the cursor over the measurement label then right click and select **Region Curve** option from the drop down menu.	

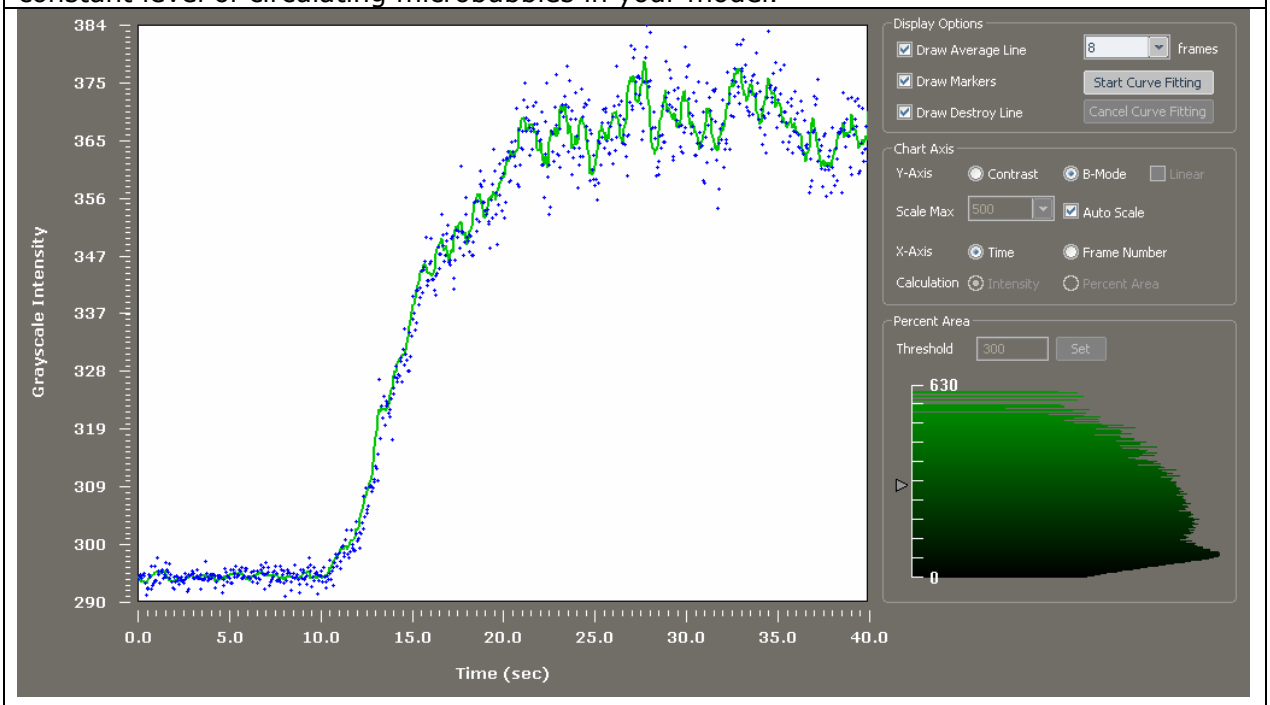
10. The region curve graph displays the level of circulating microbubbles in the region of interest as Contrast Intensity vs. Time.

Ensure **B-Mode** and **Time** are selected for Y- and X-Axis respectively.

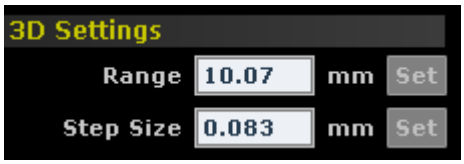
Ensure that this curve plateaus for at least 20 seconds after the bolus is delivered. If this plateau is observed in your model, proceed to the steps in section 5: Contrast Mode 3D Volume Data Acquisition

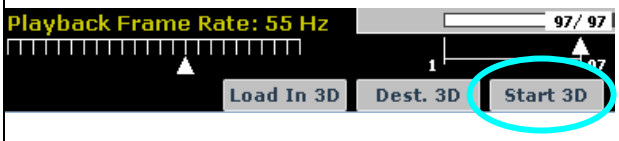
If this plateau is not apparent using a bolus injection in your model, then you will have to use an infusion pump to maintain a constant level of circulating microbubbles. Please refer to **Preparation for Infusion using the Vevo Micromarker Contrast Agent Kits, Part Number: 11684**. Flow rates must be tested to determine a rate which maintains a

constant level of circulating microbubbles in your model.



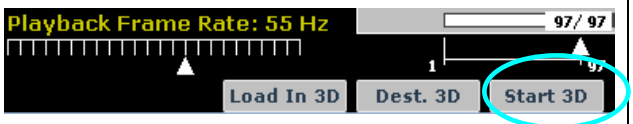
5.1 Prepare 3D acquisition

1. Initialize the 3D motor by clicking **[Init 3D]**.	
2. Using the height adjustment knob on the rail system place the image of the tumor in the **focal zone**.	
3. Determine the size of the tumor by scanning across the entire tumor using the micromanipulator knob while reading the ruler located on the micromanipulator.	
4. Set the **range** to be slightly larger than the determined tumor size and the **step size** to 0.08-0.100 mm. The 3D motor will move the RMV a total distance of the set **range**. Along the way, the RMV will take an image of the tumor at every 0.080-0.100 mm, the set **step size**. Decreasing **step size** can increase 3D image	

resolution, however will increase acquisition time. Note: Decreasing **step size** will increase 3D image resolution. However, decreasing step size to below the RMV's capable resolution will not further increase 3D image resolution.	
5. Bring the imaging plane to be exactly in the middle of the tumor.	
6. Click **[Start 3D]** to begin the 3D imaging sequence across the tumor. Ensure that this imaging sequence contains view of the entire tumor. If the imaging sequence does not cover the entire tumor, adjust the middle position of the tumor (5.1.5) and/or increase the **range** of the motor.	

Note: Once the study has been initiated it is important to prevent any movement of the animal to ensure good registration of the 3D data sets.

5.2 Contrast Mode 3D Volume Data Acquisition

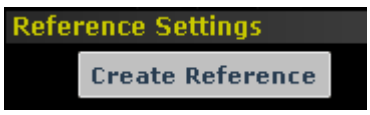
1. After all preparation (5.1) steps have been completed, press **Scan/Freeze** to begin scanning in Contrast Mode.	
2. Press **Scan/Freeze** as 3D acquisition can only be initiated from the System Paused. Press **Start 3D** and acquire a pre-bolus Contrast Mode 3D cineloop. Save and label this clip as *pre-bolus*. *Note: This cine loop will later be processed to create a 3D image.*	
3. **Bolus**: To acquire the Contrast Mode 2D cineloop while injecting the bolus: - press **PreTrig** on the keyboard and approximately 2 seconds later, inject the bolus of MicroMarker Non-Targeted Contrast Agent -Once the PreTrig acquisition is complete, save and label the cineloop as *bolus delivery*	

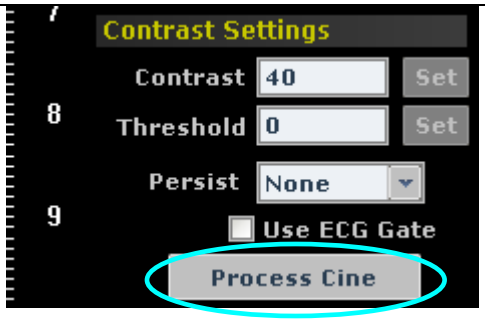
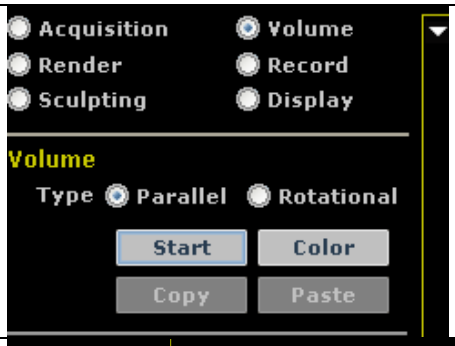
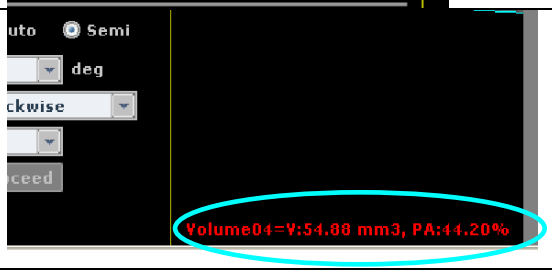
Constant Infusion: set the pump and syringe as described in the Continuous Infusion Imaging Protocol - Pre-fill the cannulation tubing with contrast agent. - Begin imaging and start the infusion pump.	
4. For injection by **bolus**: Immediately after the microcirculation stabilizes (constant flow of microbubbles in the vasculature) begin the 3D acquisition by pressing **Start 3D** to acquire a post-bolus Contrast Mode 3D cineloop. Label and save this cineloop as *post-bolus*. For **constant infusion**: Immediately after the microcirculation stabilizes (constant flow of microbubbles in the vasculature) begin the 3D acquisition by pressing **Start 3D** to acquire a post-bolus Contrast Mode 3D cineloop. Label and save this cineloop as *post-infusion*.	

6. Process 3D Contrast Mode data

The Vevo 770 processes acquired 3D Contrast Mode data using a reference 3D cine loop. It generates a "contrast overlay" that identifies the differences in the intensity between **two** 3D Contrast Mode acquisitions.

To generate a contrast overlay:

1. Load the *pre-bolus* 3D cine loop and press **Create Reference.**	
2. Load the *post-bolus* 3D cine loop *and* press **Process Cine**. Adjust the **Contrast** and **Threshold** values to minimize signals observed from bodily movements (eg. Breathing) while maintaining signals observed from the microbubbles.	

	
3. Open the processed *post-bolus* 3D cine loop and press **Load in 3D** to generate a 3D image of the tissue. Save and label this as *post-bolus 3D volume*.	
4. The standard tools within the 3D module can now be used to analyze the 3D volume. With the *post-bolus 3D volume* open, select the **Volume** tool to trace a 3D volume of the tumor. Please refer to the Vevo770 Operator Manual for instructions on using the volume trace tool.	
5. The volume trace tool will generate *volume* (V) and *percent agent* (PA) values. The volume value is the calculated volume of the tumor. The PA value calculates the percentage of pixels within the tumor which have a color associated with them	

Need help?

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