

VisualSonics Application Protocol

Subharmonic Imaging using MicroMarker™ Non-Targeted Contrast Agents in the Kidney

Part Number: 11851

1 Objective

The objective of the **Subharmonic Imaging using MicroMarker Non-Targeted Contrast Agents** protocol is to describe the activities performed during a study, including:

- Software configuration for Subharmonic Imaging
- Bolus injection of MicroMarker contrast agents
- Acquisition of the bolus wash-in cine loop
- Analysis of the Subharmonic Contrast Mode data

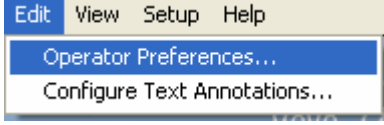

This protocol is intended for mouse imaging applications.

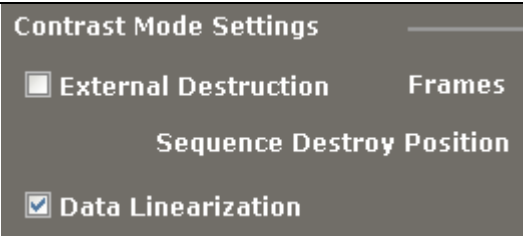
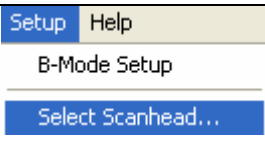
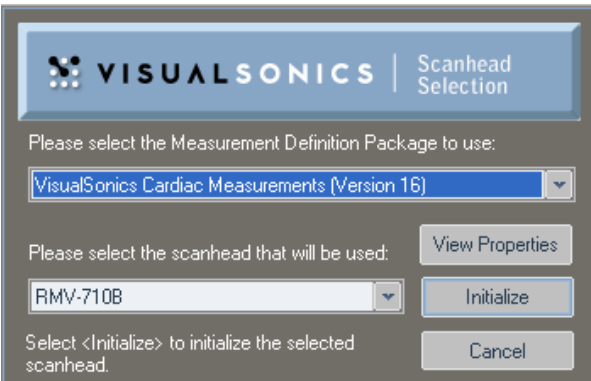
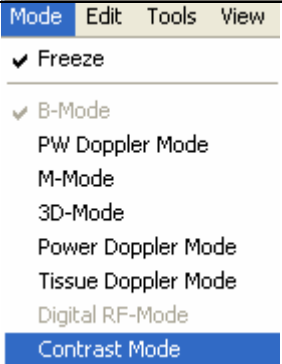
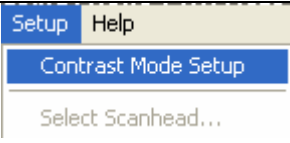

2 Tools Used During the Study

- Vevo 770® High-resolution imaging system with software version 3.0.0 or higher installed
- Vevo Contrast Mode enabled with Subharmonic option
- RMV 710B
- The PN11683 - VisualSonics Preparation Protocol - Bolus Injection of Vevo MicroMarker, and all tools listed within
- Medical air (oxygen content less than 31%)

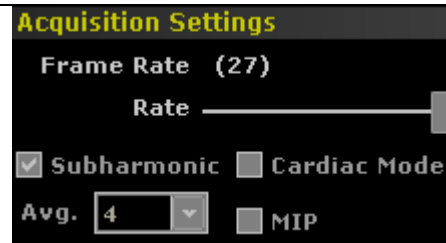
3 Preparation

3.1 Prepare the Vevo 770 system

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| <p>1. From the Edit menu, select Operator Preferences.</p> |  |
| <p>2. In the Operator Preferences dialog, ensure that the Contrast Mode cine loop size is set to 800 frames.</p> <p>This setting defines the size of the Contrast</p> |  |

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| <p>Mode cine loop when using the Pre-Trig function.</p> <p>3. Check Data Linearization "ON".</p> |  |
| <p>4. Connect RMV 710B to the Vevo 770. From the Setup menu, select Select Scanhead, or press the Select Scanhead key.</p> |  |
| <p>5. Select the 710B from the Scanhead Selection dialog, and click Initialize.</p> |  |
| <p>6. From the Mode menu, select Contrast Mode or press the Contrast Mode key on the keyboard. The system begins scanning automatically.</p> |  |
| <p>7. From the Setup menu, select Contrast Mode Setup, or press the Mode Setup key.</p> |  |
| <p>8. In the Transmit Settings, select 100% Power.</p> |  |

9. In the Acquisition Settings section, ensure that Cardiac Mode is not checked, and adjust the Frame Rate slider all the way to the right, resulting in a frame rate around 27.



3.2 Prepare contrast agent

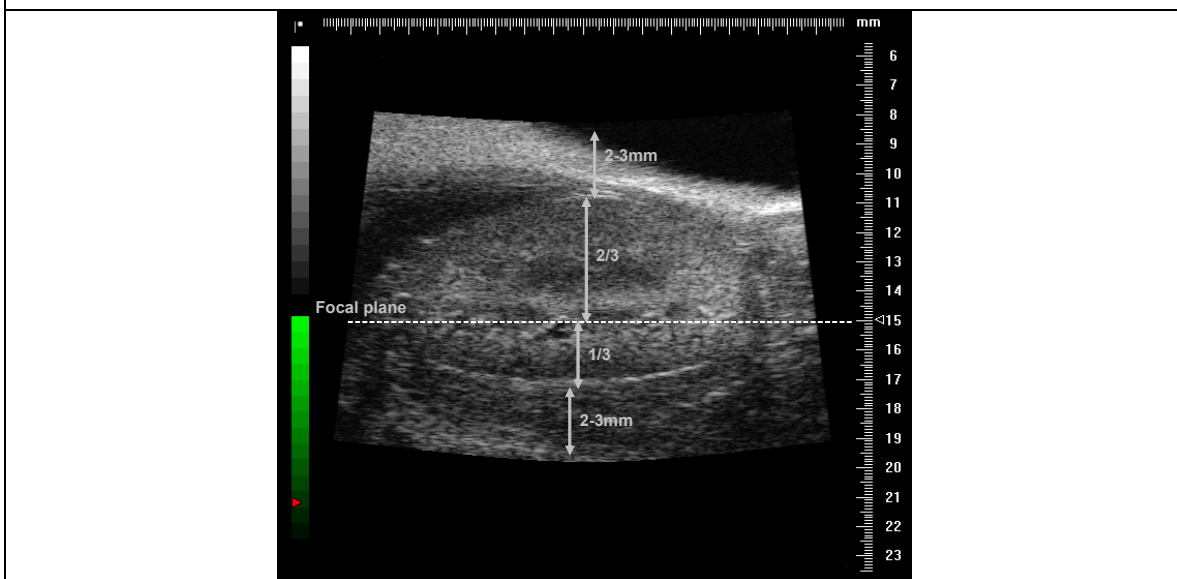
1. Prepare the contrast agent according to the instructions provided in PN11683 - VisualSonics Preparation Protocol - Bolus Injection of Vevo MicroMarker.

3.3 Prepare subject animal

1. Prepare the subject animal for contrast agent injection by gaining vascular access via a tail vein, jugular vein, or retro-orbital sinus injection.

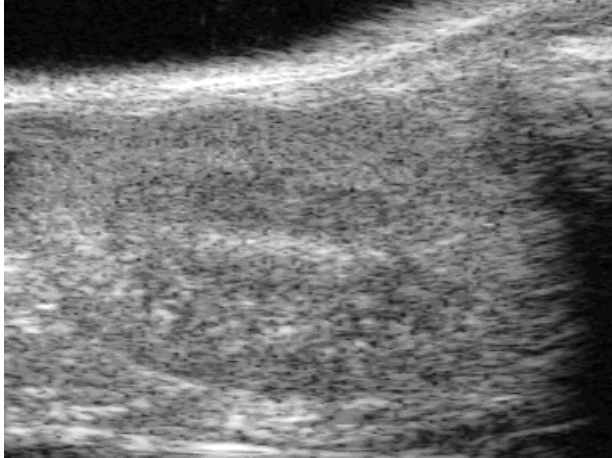

3.4 Positioning of subject animal for Subharmonic imaging

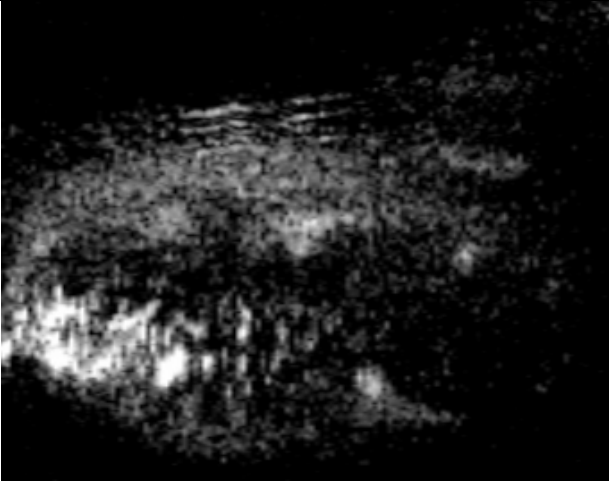
1. Remove the hair around the region to be imaged, in this case remove the hair on the back of the animal overlaying the kidney. Before starting the contrast agent imaging, adjust the imaging plane so the focal point is situated at approximately 1/3 from the bottom of the kidney (see image below). For optimal results and minimal tissue attenuation, the kidney should be less than 1.5 mm beneath the skin line, this is accomplished by imaging the kidney from the dorsal side, or back, of the animal.
2. Optimize the Field of View such that only the kidney and surrounding tissue is visible on screen.



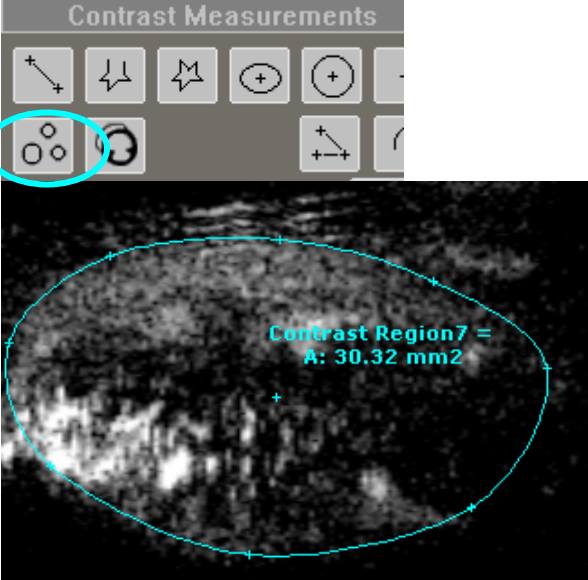
Note: Once the subharmonic imaging is initiated it is important to prevent any movement of the animal.

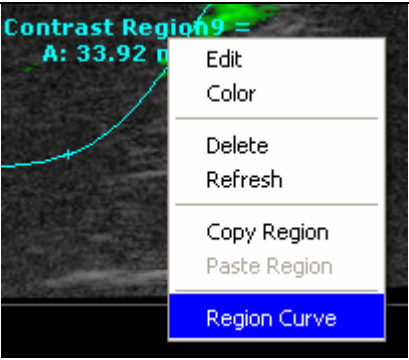
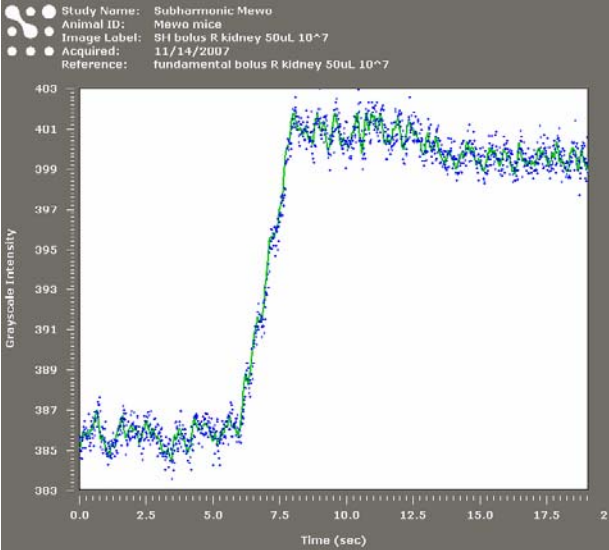
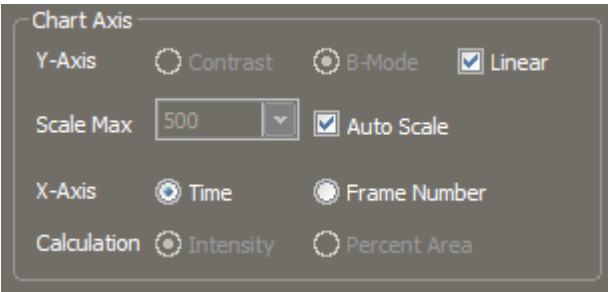
4 Subharmonic data acquisition

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| <p>1. After all preparation (3.1-3.4) steps have been completed, press Scan/Freeze to begin scanning in Contrast Mode.</p> | |
| <p>2. Acquire a short cineloop of the kidney in fundamental Contrast Mode as a guide.</p> |  |
| <p>3. Check the Subharmonic check box.</p> <p>Note: The software optimizes the parameters for subharmonic acquisition: filtering out signal from tissue (the image area is going to look black), and defaulting the TGC sliders to 22dB.</p> |  |
| <p>4. Prepare a 50ul bolus of the MicroMarker Contrast Agent diluted 1:10; briefly this is completed by diluting 20ul of stock contrast agent with 180ul of saline (for a final volume of 200ul). Draw this dilution into the syringe, and adjust the volume to 50ul.</p> <p>Please see PN11683 - VisualSonics Preparation Protocol - Bolus Injection of Vevo MicroMarker for a more detailed</p> | |

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| <p>description of how to complete this dilution.</p> <p>Note: It is critical to complete this dilution just prior to using it, and to discard any remaining contrast agent removed from the vial which is not used within 3-5 minutes of removing it from the vial.</p> | |
| <p>5. Press Pre-Trig, wait 1-2 seconds collecting baseline images, and slowly inject the 50ul bolus of contrast agent over 1-2 seconds. When the Pre-Trig acquisition is complete, label and save the cineloop.</p> |  |

5 Subharmonic data analysis

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| <ol style="list-style-type: none"> From the Tools menu, select Measurements, or press the Measurement key. Select the Contrast Measurement and trace the region of interest. |  |
| <ol style="list-style-type: none"> Right click on the measurement and select Region Curve to display the measurement graph. <p>The graph displays the Grayscale Intensity increase over time.</p> | |

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| <p>4. Ensure B-Mode is selected for Y-Axis and Linear box is checked.</p> <p>5. The intensity change in dB can be calculated from the region curve:</p> $\text{Intensity Change} = 20 \times \log \frac{\text{Max. Intensity}}{\text{Min. Intensity}}$ <p>Note: Max. and Min. Intensity values are read from the graph.</p> |  |

Need help?

Call us toll-free at 1-866-416-4636 (North America) or 416-484-5000 (other regions), or contact us via email at support@visualsonics.com