Guide to Small Animal Micro-Echocardiography Study using the Vevo 770®

Course Objectives:

After completion of this module, the participant will be able to accomplish the following:

- Recognize the main cardiac imaging “views”,
- Understand common measurements and calculations,
- Understand the ECG cycle,
- Position micro-ultrasound RMV scanhead to acquire the necessary views, and
- Review estimated values for main calculations.

This Guide to Small Animal Micro-Echocardiography Imaging will review the imaging views associated with specific cardiac function and anatomy. It will also examine the components of the ECG signal and the correlations to the mouse model.

Note on RMV Scanhead Selection for Micro-Echocardiography Imaging:

Appropriate selection of RMV scanheads for cardiovascular imaging is critical to the resolution and definition obtained. For the Vevo 770, recommended RMV scanheads are the RMV 707B for mouse cardiac imaging and the RMV 710B or RMV 716 for large rat cardiovascular imaging.

I. Parasternal Long Axis View (PLAX)
A. Long axis view in B-mode (2D imaging)
B. Long axis view in M-mode
C. Left ventricular outflow tract measurement
D. Pulmonary outflow

II. Parasternal Short Axis View (PSAX)
   A. B-mode image of the parasternal short axis view
   B. M-mode image of the parasternal short axis view
   C. Other anatomy visualized from the short axis view

III. The Apical Four Chamber View (4APC)
   A. Mitral flow velocity from the apical four chamber view
   B. Tricuspid Inflow from the apical four chamber view

IV. Right Parasternal Long Axis View
   A. Right ventricular measurements

V. Aortic Arch View
   A. Ascending aorta, 3 branches and proximal, descending aorta
   B. Doppler of ascending and descending aorta

VI. Suprasternal View
   A. Aortic Velocity Time Integral (VTI)

VII. Anatomical M-mode

VIII. Automatic Left Ventricle (LV) Trace

IX. Tissue Doppler
   A. Strain rate
   B. Mitral Annulus

X. Pressure Volume loops

XI. Simpsons Measurement

XII. Left Ventricle (LV) Mass

XIII. Electrocardiogram (ECG) Pattern

XIV. Normal Values

VisualSonics Workbook: Guide to Micro-Echocardiography Study using the Vevo 770®, Rev.1.0
I. Parasternal Long Axis (PLAX) View

A. Long Axis View in B-Mode (2D Imaging)

**Anatomy:** Visualize the left ventricle, aorta, and mitral valve leaflets.

**Scanhead position:** Using the RMV 707B, begin by obtaining a parasternal long axis view of the heart. The “notch” on the scanhead should be pointed towards the head of the animal, and rotated approximately 30-45 degrees counter-clockwise.

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**Figure 1:** A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain a parasternal long axis view. B) B-mode image of the left ventricle in the long axis view, using the Vevo 770 and a RMV 707B scanhead.
B. **Long axis view in M-mode**

**Scanhead position:** Same as the parasternal long axis.

**Target:** If the structures are clear enough and if the axis at the correct angle, an M-Mode measurement may be obtained. This view will also give you a good sense of the left ventricular function.

*Figure 2:* B-Mode image of parasternal long axis view showing placement of M-Mode line, using the RMV 707B.

*Figure 3:* M-Mode image of the left ventricle from the parasternal long axis view using the RMV 707B.
C. **Left Ventricular Outflow Tract (LVOT) Measurement**

**Anatomy:** The LVOT can be measured by placing markers from leading edge to leading edge at the level of the aortic valve leaflets (as shown below).

**Scanhead position:** Same as the parasternal long axis view.

*Figure 4: Parasternal long axis view with aorta using the RMV 707B.*
D. **Pulmonary Outflow**

**Anatomy:** Peak velocity of the pulmonary artery is measured to determine pulmonary outflow. After a Doppler waveform is obtained (see next page), measure the maximum pulmonary artery velocity at the peak of the envelope to make a trace of the entire envelope for a pulmonary artery Velocity Time Integral (VTI).

**Scanhead position:** With the scanhead angled slightly towards the animal’s left shoulder, the pulmonary artery will come into view. This is a good position to set a sample volume and obtain a pulmonary outflow pulsed wave Doppler image.

*Figure 5: Parasternal long axis view (PLAX) angled up to right outflow showing the pulmonary artery using the RMV 707B.*
The pulmonary artery Doppler wave form should look like this:

![Doppler wave form](image)

**Figure 6:** Pulse wave Doppler image of the pulmonary artery showing VTI tracing measurement and Vmax using the RMV 707B.

II. The Parasternal Short Axis View

A. B-mode Image of the Parasternal Short Axis View

**Scanhead position:** From the parasternal long axis view, rotate the scanhead 90 degrees clockwise to the short axis. In effect, you will move the scanhead notch from the position towards the head of the mouse to the left side of the mouse. A completely round view of the left ventricle should appear. To optimize this image, you may need to angle and tilt the scanhead.

![Scanhead positioning](image)

**Figure 7:** A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain a parasternal short axis view. B) B-mode image of the short axis view of left ventricle using the RMV 707B.
B. **M-Mode Image of the Parasternal Short Axis View**

**Anatomy:** The parasternal short axis is the best view to set your M-Mode cursor and obtain an M-Mode for measuring left ventricle dimensions. These measurements will provide ejection fraction, fractional shortening information and left ventricle (LV) mass calculations.

**Scanhead position:** Same as the parasternal short axis view.

*Figure 8: M-Mode image from short axis view of left ventricle using the RMV 707B.*
C. **Other Anatomy Visualized from the Short Axis View**

**Anatomy:** If you reach the short axis view at the level of the valves, the aortic valve will appear in the middle. The tricuspid valve is on the right (left side of the screen) and the pulmonary valve will be on the left (right side of the screen.) A pulsed wave Doppler may also be placed in the pulmonary artery or tricuspid valves from this view. Coronary arteries also appear in this view.

**Scanhead position:** All aspects of the left ventricle can be analyzed by scanning down to the apex, and up to the base of the heart.

![Image](image_url)

**Figure 9:** B-mode image of the high short axis viewed at the aorta and pulmonary level using the RMV 707B.
III. The Apical Four Chamber View

** Please note that apical four chamber view is the most challenging cardiovascular view to obtain. 

Anatomy: Attempt to visualize the right and left ventricles, with the atria at the bottom of the screen. This view will provide an accurate angle for pulsed wave Doppler of the mitral and tricuspid valves. Measurements of mitral inflow will be made from the Doppler of the mitral valve.

Scanhead position: An apical four chamber view is obtained from the lower left side of the animals’ thorax. Essentially, you are trying to look up from the apex, towards the base of the heart. Place the scanhead in a transverse position with the notch facing the left side of the mouse, and angle your mouse slightly with the head down. The scanhead will also be angled by 60-70 degrees. You will then move the scanhead to the lateral wall of the thorax. From this position, some fine tuning may be required to obtain the apical view.

Figure 10: A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain an apical four chamber view. B) B-Mode image of the apical four chamber view using the RMV 707B.
Figure 11: B-Mode image showing the apical four chamber view showing placement of Doppler wire frame for mitral flow using the RMV 707B.

A. Mitral flow velocity from the apical four chamber view

**Anatomical Target:** Mitral flow velocity

**Scanhead position:** Same as the apical four chamber view

Figure 12: Pulse wave Doppler image of mitral valve inflow from apical four chamber view RMV 707.
B. **Tricuspid Inflow from the apical four chamber view**

**Anatomical Target:** Tricuspid valve

**Scanhead position:** Same as the apical four chamber view

*Figure 13:* B-mode image showing placement of Doppler wire-frame for tricuspid flow from the apical four chamber view using the RMV 707B.

C. **Tricuspid Doppler**

*Figure 14:* Pulse wave Doppler image showing Tricuspid inflow from apical four chamber view using the RMV 707B.
IV. Aortic Arch View

The final cardiovascular view will be that of the aortic arch. If imaged correctly, the ascending portion, branches and descending aorta should be visible.

**Anatomical Target:** The descending aorta can be assessed from this view by pulsed wave Doppler, where aortic banding may take place.

**Scanhead position:** The aortic arch view is obtained from a modified right parasternal view. The scanhead is positioned with the notch pointing towards the chin of the animal. The scanhead may need to be rotated slightly clockwise to perfect the image.

![Image of aortic arch view](image_url)

**Figure 15:** A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain an aortic arch view. B) B-Mode image of the high right parasternal view showing the aortic arch using the RMV 707B.
Additional Measurements and Calculations:

- A pulsed wave Doppler sample volume is placed through the ascending aorta to measure peak aortic velocities. The waveform may also be traced to obtain a VTI calculation.

- Performing the VTI, along with the LVOT diameter measurement, made earlier, will provide calculations of stroke volume and cardiac output.

Figure 16: Pulse wave Doppler mode image of the aorta outflow from high right parasternal view using the RMV 707B
V. **RIGHT PARASTERNAL LONG AXIS VIEW**

**Anatomical Target:** The right parasternal long axis view is done to evaluate the right ventricle. The positioning is a long axis view just to the right of the sternum.

**Scanhead position:** You may need to adjust this accordingly to the breed of mouse that you are using. Sometimes a caudal angle will also help open up the right ventricle.

When you obtain the image you are able to measure the thickness of the wall as well as the ventricle. This view is particularly useful when studying pulmonary hypertension, or any cardiac disease that affects the right ventricle.

![Image 17](image17.jpg)

**Figure 17:** A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain a right parasternal long axis view. B) B-Mode image of the right parasternal long axis view using the RMV 707B.
VI. SUPRASTERNAL VIEW

Anatomical Target: The suprasternal view is done to obtain a velocity of the aortic valve. The reason we do this view is to obtain the correct Doppler shift, to get accurate velocities within the valve.

Scanhead position: You place your probe in the midline of the mouse at the level of the thoracic inlet. You angle caudally and you will visualize a tube which is the ascending aorta. At the distal end of this tube you will see the aortic valve. You will place your pulse wave Doppler sample volume just above the leaflets and you will obtain an aortic velocity.

The velocity range of the pulse wave Doppler is 100-120 cm/sec, in a normal model.

**Figure 18:** A) Image showing the positioning of the RMV 707B scanhead on a mouse to obtain a suprasternal view. B) B-Mode image of the suprasternal view using the RMV 707B.
Here is what the Doppler sample of the aortic valve should look like.

Figure 19: Pulse wave Doppler mode image of the aortic valve outflow from a suprasternal view using the RMV 707B
VII. ANATOMICAL M-MODE

The regular M-mode line sample comes from one direction at 0 degrees. With anatomical M-mode we are able to steer this sample line 360 degrees, allowing us to access parts of the anatomy which are currently not perpendicular to the line sample now. You may also access anatomical m-mode tracing post processing which you cannot do with regular M-mode that has to be done in a live session.

The trick with anatomical M-mode is that you must capture a 2D cineloop of the area of interest and this must be done at 100 or over frames per minute. You can change the frames per second on the left upper hand of the screen, and you can also do compression of the image longitudinally which too will increase the frame rate. The higher the frame rate the better the anatomical M-mode trace.

Once you have done this then you may then go into the tools section and pick the M-mode trace and by right clicking on the arrows you are able to move the line to where you want. You will need to move the line by using the anterior and distal arrows, which will allow you to angle the sample line. Once you have positioned the line you must hit AM-mode on the bottom right hand corner of the screen, and your trace will appear.

![Anatomical M-mode image from short axis view of left ventricle using the RMV 707B.](image)

**Figure 20:** Anatomical M-mode image from short axis view of left ventricle using the RMV 707B.

You may also get an anatomical M-mode on a live scanning session by placing your M-mode sample line in the region of interest and by right clicking you will see the two arrows appear on the line. You are then able to get the steering function in order to move the sample, and you will obtain your M-mode tracing at that position.
VIII. AUTOMATIC Left Ventricle (LV) TRACE

The automatic trace function allows you to get ventricular function measurements by tracing the actual endocardium of the left ventricle, and continually tracing it as it moves from systole to diastole. You are able to do this in 2D or M-mode. By doing this measurement, you will obtain ejection fraction, fractional shortening, cardiac output, stroke volume and volume measurements of the left ventricle in systole and diastole, without having to measure it in the M-mode tracing.

You get this measurement by first doing an EKV of the parasternal long axis view of the heart. Make sure you acquire a good EKV of the view and save a cineloop of the image. You can then hit the heart symbol in the measurements package and it will bring up the trace measurement.

You must set your first measurements at the level of the aortic valve leaflets and then go to the middle of that measurement and extend it to the apex of the heart. You may then adjust the measurement to reach the endocardium both anteriorly and posteriorly.

Once you have the trace in the right place you will hit the right click button on the measurement and the line will change from broken to solid. By right clicking again you will get a list of options. You will pick proceed forward, and the image will move through the cineloop in the forward direction with the trace following the border of the endocardium as the heart steps through systole and diastole.

Once the trace has followed a complete cycle (one systolic and one diastolic phase) your measurement will be complete, and a list of the measurements will be displayed.

![Automatic LV trace image from an EKV of a parasternal long axis view of left ventricle using the RMV 707B.](image)

**Figure 21:** Automatic LV trace image from an EKV of a parasternal long axis view of left ventricle using the RMV 707B.
IX. TISSUE DOPPLER

Tissue Doppler uses pulse wave Doppler to detect and display the health of the myocardium wall of the left ventricle or to assess the mitral annulus, which assists the mitral valve in its function.

We now can use tissue Doppler to get strain rates on tissue, which means we are able to see the difference between healthy tissue and diseased tissue. The strain values are used to determine disease progression and treatment. It allows us to do regional myocardial strain rate analysis by quantifying the distance and velocity relationship between two tissue Doppler spectrums.

Figure 22: Tissue Doppler image to assess mitral valve annulus from an apical four chamber view using the RMV 707B. The Doppler sample volume is placed on the septal wall at the mitral annulus. In this example, the E’ and A’ waves are measured and the ratio is calculated as an indication of myocardium health.
X. PRESSURE –VOLUME MEASUREMENTS

The Vevo 770 system can integrate a blood pressure signal from a 3rd party continuous pressure monitoring system. Examples of this include:

- Conductance catheter pressure monitor from Millar Instruments
- Telemetry pressure transducer from Data Science International

The integrated blood pressure can be combined with the Vevo ultrasound image data and corresponding measurements to perform:

- Pressure-volume analysis
- Vascular strain analysis

Figure 23: Example of a pressure-diameter loop generated from the blood pressure signal and the left ventricle trace of the endocardial wall. (Note: the bright line inside the ventricle is the Millar catheter)
XI. Simpsons measurement

Simpsons measurement allows the calculation of both volume and mass based on image acquisition in systole and diastole from multiple planes.

A. Workflow

1. Acquire a minimum of three short axis images at three different levels
   - Trace the endocardium in diastole and systole

   **Note:** At the papillary muscles level, trace the endocardial border, do not include the papillary muscles in the trace.

2. Acquire a parasternal long axis view
   - Measure the left ventricle length from the aortic annulus to the endocardial border at the apex level in diastole and systole

**Figure 24:** Example of a Pressure-Volume analysis from the integrated blood pressure image
B. Methods

The standard measurement package needs to be selected then into Tools/Measurements/Simpson’s, the various areas need to be traced:

1.1.a  **Simpsons 1 area 1, diastole**

![Figure 25](image1.png)

**Figure 25:** From a parasternal short axis view at the level of the papillary muscles, the endocardium is traced in diastole without including the papillary muscles.

1.1.b  **Simpsons 1 area 1, systole**

![Figure 26](image2.png)

**Figure 26:** From a parasternal short axis view at the level of the papillary muscles, the endocardium is traced in systole without including the papillary muscles.
1.2.a Simpson's 1 area 2, diastole

Figure 27: From the same parasternal short axis view but at a different level (either higher or lower with respect to the papillary muscles), the endocardium is traced in diastole.

1.2.b Simpson's 1 area 2, systole

Figure 28: From the same parasternal short axis view but at a different level (either higher or lower with respect to the papillary muscles), the endocardium is traced in systole.
1.3.a  Simpsons 1 area 3, diastole

Figure 29: From the same parasternal short axis view but at a different level (either higher or lower with respect to the papillary muscles), the endocardium is traced in diastole.

1.3.b  Simpsons 1 area 3, systole

Figure 30: From the same parasternal short axis view but at a different level (either higher or lower with respect to the papillary muscles), the endocardium is traced in systole.
2.1 Length 1, diastole

Figure 31: In a parasternal long axis view, the left ventricle length is measured from the aortic annulus to the endocardial border at the apex level in diastole.

2.2 Length 1, systole

Figure 32: In a parasternal long axis view, the left ventricle length is measured from the aortic annulus to the endocardial border at the apex level in systole.
C. Summary

- Averaged left ventricular end-diastolic/systolic volumes are then obtained from these measurements.
- Calculation of end-diastolic volume, end-systolic volume, stroke volume, cardiac output, cardiac index and ejection fraction are calculated from the summation of areas from the diameters of 20 cylinders or discs of equal height.

\[ V = (A_1 + A_2 + A_3) \frac{h}{2} + \frac{\pi}{6} h^3 \]

**Figure 33:** Schematic of areas and length measurements necessary to obtain Simpson’s measurements.
XII. **Left Ventricle Mass**

**A. Objectives**

1. Acquire a parasternal short axis view in diastole
   - Trace the left ventricle endocardium (LV area endo)
   - Trace the left ventricle epicardium (LV area epi)
2. Acquire a parasternal long axis in both diastole and systole
   - Measure the left ventricle length in both diastole and systole (LV length d/s)

**B. Methods:**

The standard measurement package needs to be selected then into Tools/Measurements/LVmass/

1.1 Left ventricle area endocardium
1.2 Left ventricle area epicardium

*Figure 34: From a parasternal short axis view in diastole, the endocardium and epicardium are traced.*
2.1 Left ventricle length diastole

![Image](image1.png)

Figure 35: In a parasternal long axis view, the left ventricle length is measured from the aortic annulus to the endocardial border at the apex level in diastole.

2.2 Left ventricle length systole

![Image](image2.png)

Figure 36: In a parasternal long axis view, the left ventricle length is measured from the aortic annulus to the endocardial border at the apex level in systole.

C. Summary

Left ventricle mass = volume enclosed by epicardial surface (total volume) - left ventricular volume enclosed by endocardial surface (cavitary volume)

Note: The specific weight of the myocardium is also considered
XIII. Electrocardiogram (ECG) pattern

The ECG tracing is obtained in real-time when imaging.

![ECG Image]

Description on the ECG pattern:

**P wave:** represents the stimulation of the sino-atrial node on the right atrium

- Atrial depolarization/activation

**PR interval:** is the time from the onset of the atrial activation to the ventricular activation.

**QRS:** is the depolarization/activation of the ventricles

- Duration of ventricular activation

**T wave:** is the repolarization/recovery of the ventricles.

**U wave:** is rarely present. It is the continuation of the recovery of the ventricles.

XIV. Normal Values for Mice and Rats

<table>
<thead>
<tr>
<th>Value</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ejection Fraction</td>
<td>55-85%</td>
<td>55-80%</td>
</tr>
<tr>
<td>Fractional Shortening</td>
<td>30-50%</td>
<td>30-50%</td>
</tr>
<tr>
<td>Left ventricle Mass</td>
<td>65-90mg</td>
<td>60-100mg</td>
</tr>
<tr>
<td>Stroke volume</td>
<td>45-55ml/min</td>
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</tr>
<tr>
<td>Cardiac Output</td>
<td>20-35ml/min</td>
<td>40-55ml/min</td>
</tr>
</tbody>
</table>

The above values vary according to animal strain, size, sex, and health. This is only a guideline.
For more information or assistance with any imaging procedures, please contact VisualSonics at:

support@visualsonics.com or T. 416.484.5000