VISUALSONICS

Imaging Guide

Guide to Small Animal
Echocardiography
using the Vevo® 2100 Imaging System

Vevo**2100**

Guide to Small Animal Echocardiography using the Vevo® 2100 Imaging System

Course Objectives:

This guide is designed to aid the user in:
- Recognizing the main cardiac imaging "views"
- Understand common measurements and calculations
- Position the probe appropriately to acquire the necessary views
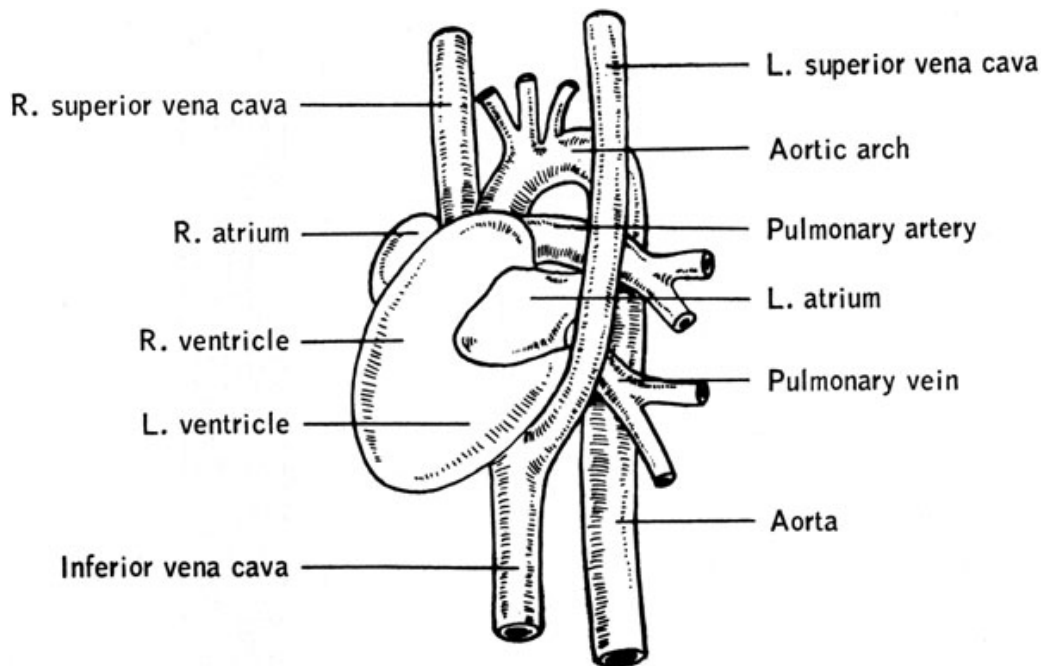- Review estimated values for main calculations

Figure 1. Mouse cardiac anatomy.

http://www.informatics.jax.org/greenbook/figures/figure13-2.shtml

Overview of the Imaging Modes to be used in this Guide

B-Mode:

B-Mode or brightness mode, imaging is used to acquire two dimensional images of an area of interest. The grayscale image allows for identification of anatomical structures and the software allows for various measurements in this mode of imaging.
A motor can be attached to the ultrasound probe to translate the probe over a defined range with a defined step-size, the software then recompiles the various 2D slices into a 3D volume. The 3D software allows for various measurements and imaging planes in this type of imaging.

M-Mode:

M-Mode or motion mode, imaging is used to acquire an image in one dimension over time. Simply, a sample volume is placed over an area of interest and the movement of that area is displayed in the spectrum over time. For example the movement of the carotid artery walls over time can be examined using this type of imaging. The diameter of the vessel can be measured, as also the wall thickness, using various software measurement tools.

Pulsed-Wave Doppler Mode:

Pulsed-Wave (PW) Doppler Mode imaging is used to analyze blood flow within a vessel of interest. The sample volume is placed within the vessel and the system generates a PW Doppler spectrum which provides the information necessary to make velocity, acceleration and time measurements with the software.

It is crucial for PW Doppler Mode and all other Doppler imaging modes that an appropriate angle be achieved between the transmitted ultrasound beam and the blood flowing through the vessel. The imaging plane should be adjusted such that the angle between these two is less than 60°; this will provide an accurate velocity measurement.

Please see the Quick-Start Guide: *Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes* for further information on the various Doppler imaging modes.

Simultaneous B-Mode and PW Doppler Mode is a very useful mode for evaluating small vessels in real-time. Absolute velocity measurements are limited to slower flows in simultaneous mode due to PRF issues. Optimizing the display parameters in Preferences to make the spectral display small will greatly help one see small vessels and the Doppler data.

Color Doppler Mode:

Color Doppler Mode allows for quick visualization of blood flow and provides information on the direction of flow, the color is assigned accordingly with blood flowing towards the transducer colored on a scale from red to white and blood flowing away from the transducer colored on a scale from blue to white. BART is an acronym commonly used to remember the colors associated with the direction of flow; Blue Away, Red Towards.

Please see our Quick-Start Guide: *Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes* for further information on the various Doppler imaging modes.

Contrast Mode:

Contrast Mode is a mode very similar to B-Mode in that it is used to look at 2D anatomical structures however it includes specific analysis tools to allow a reference subtraction of two images to apply a copper (or green) contrast overlay to the injected contrast agents. Contrast agents are used to enhance the visualization of blood flow in very small blood vessels, as small as capillaries. The software not only allows for enhanced visualization, but also provides quantification tools so that useful hemodynamic parameters can be determined.

Overview of the Parameters to be optimized

B-Mode Parameters:

There are numerous parameters which can be set to optimize a B-Mode image; outlined below are a few key parameters. For a full list of parameters and how they affect B-Mode imaging, please consult our Vevo 2100 Operators Manual.

Display Maps

Display maps are used to assign grayscale colors to detected ultrasound signal intensities; this allows optimized grayscale differentiation of specific anatomical targets. The Vevo 2100 imaging system comes with numerous factory settings which can be selected from to provide the best quality images.

Dynamic Range

Dynamic range determines the range of grayscale intensities which can be displayed on an image. Together with the display maps, dynamic range can be adjusted to provide the best quality images.

Focal Zones

The Vevo 2100 imaging platform can have one, two or three focal zones, with the spacing of the two and three focal zones being narrow or wide. Using additional focal zones provides an image which is focused over a broader depth, however increasing the focal zones will decrease the maximum frame rate. The number of focal zones and their placement should be selected based on the anatomical structures to be imaged.

Line Density

Line density allows the user to change the space between lines on the ultrasound image. Increasing the line density will increase the resolution, texture and overall will increase the quality of the image. However, it will decrease the maximum frame rate at which the system can image.

M-Mode Parameters:

There are various parameters which can be set to optimize M-Mode imaging. The B-Mode image should be optimized prior to M-Mode imaging to give the best quality spectrum. The sample volume is adjusted to suite the size of the anatomical target and the sweep speed is adjusted to the user's preference.

Pulsed-Wave and Color Doppler Mode Parameters:

There are various parameters which can be set to optimize Doppler imaging in PW and Color Doppler imaging modes. Please see our Quick-Start Guide: *Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes* for an explanation of these parameters and how they will affect the image quality.

Selecting the Appropriate Probe for Imaging

The selection of the probe for imaging becomes important when considering which images are required and how large the animal is and how large the heart being scanned is.
When acquiring B-Mode images on a mouse the ideal probe would be the MS400, but good images can also be obtained with an MS550D.
When imaging a rat B-Mode images should be acquired with the MS250 or the MS200 probes depending on the size of the animal.

Software Measurements

The Vevo 2100 imaging software allows the user to make several measurements and calculations.
In B-Mode imaging general size measurements can be made, including area and volume measurements. In Doppler and Contrast imaging modes an assessment of vascularization can be completed.

Animal Preparation

The animal should be anaesthetized so as to prevent movement during the imaging session. Typically isofluorane is used, at a concentration of 1.5-2%. Once the animal is anaesthetized in a knock down box it should be positioned on the platform ventral side up. Eye lubricant should be placed on each eye to prevent drying of the area. A small amount of ECG gel should be placed on the copper leads on the platforms and the paws taped to them, this will provide the ECG and respiratory physiology. A rectal probe should be inserted to monitor the temperature of the animal during the imaging session.
The hair around the area to be imaged should be removed using a depilatory cream, such as Nair and rinsed with water prior to imaging.

Imaging Guide Outline

I. Parasternal Long Axis View (PLAX)
     A. Long axis view in B-Mode
     B. Long axis view in M-Mode
     C. Left ventricular outflow tract measurement
     D. Pulmonary outflow
     E. Superior (cranial) vena cava flow
     F. Pulmonary veins

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 parasternal short axis view

III. The Apical Four Chamber View
     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

VII. Tissue Doppler Mode

VIII. 3D Cardiac Imaging
     A. 3D Cardiac imaging in diastole
     B. 3D Cardiac imaging in systole
     C. Measurements and calculations

IX. Simpson Measurement

X. Left Ventricle (LV) Mass

XII. Color Doppler Mode Imaging

XIII. Pressure Volume Loops

XIV. Normal Values

I. Parasternal Long Axis (PLAX) View

Visible anatomy in PLAX view:

- Left ventricle (LV)
- Right ventricle
- Left atrium
- LV Anterior wall
- LV Posterior wall
- Intraventricular septum

- Aorta
- Pulmonary artery
- Aortic valve
- Mitral valve
- Pulmonary veins
- Papillary muscles

A. Long axis view in B-Mode

Scanhead position:

- Place the transducer in vertical fashion, with the notch pointing towards the animal's head. Then rotate the transducer approximately 35° counter-clockwise.

Figure 2. Image showing the positioning of the MS400 scanhead on a mouse to obtain a parasternal long axis view (L). B-Mode image of the left ventricle in the long axis view (R).

Available Measurements and Calculations:

- LV area trace
- LVOT

LV Area Long Axis Trace

To activate this trace tool, press [icon] in the measurement tool box. Then locate diastole in the long axis loop, trace the endocardial area. Then repeat for systole. Once these measurements are complete, the following data will be available in the calculations menu:

- Diastolic and systolic areas
- Stroke volume
- Cardiac output

- Diastolic and systolic volumes
- Ejection fraction
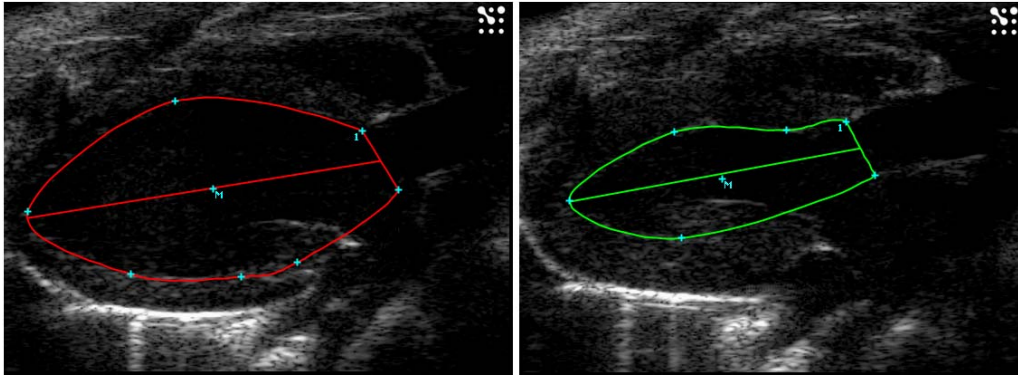
Figure 3. Long axis image showing the LV trace in diastole (L). Long axis image showing the LV trace in systole (R).

B. Long axis view in M-Mode

To obtain a correct M-Mode, the LV must be horizontal in orientation. Place the M-Mode axis immediately under the papillary muscle.
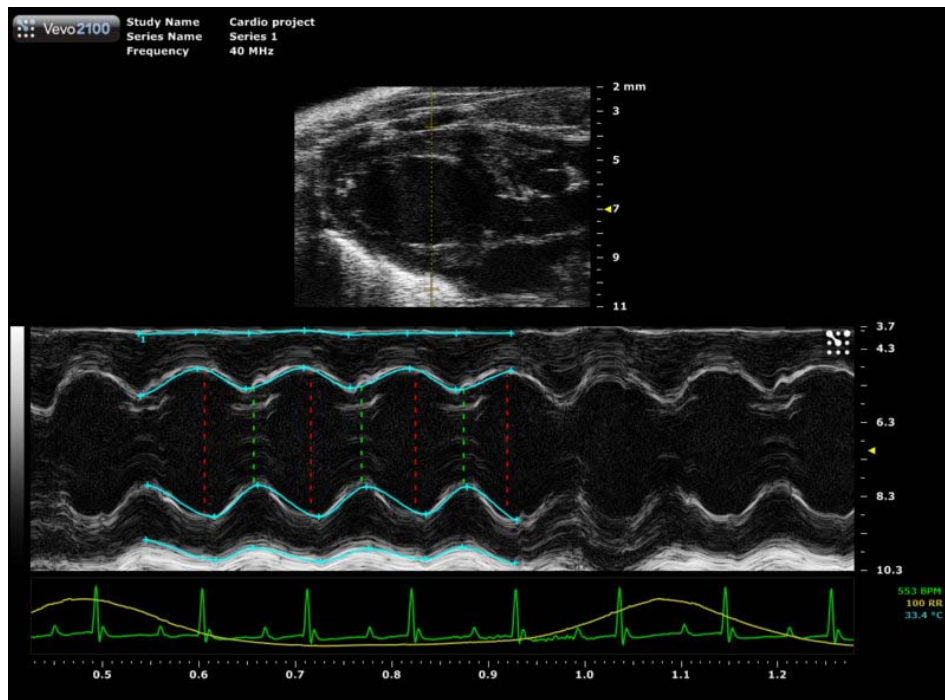
Figure 4. M-Mode image of parasternal long axis view showing placement of M-Mode sample volume and tracing of the anterior and posterior walls.

Available Measurements and Calculations:

- LV anterior wall thickness in diastole and systole (LVAWd, LVAWs)
- LV internal diameter in diastole and systole (LVIDd, LVIDs)
- LV M-Mode trace

- LV posterior wall thickness in diastole and systole (LVPWd, LVPWs)
- intraventricular septum thickness in diastole and systole (IVSd, IVSs)

LV M-Mode Trace

This trace can be performed in the long and short axis view. To activate this trace tool, press [icon] in the measurement tool box in an M-Mode window. Proceed by tracing the anterior endocardial wall border, followed by the posterior wall border for 3 cardiac cycles. Once these measurements are complete, the following data will be available:

- Diastolic and systolic areas
- Stroke volume
- Cardiac output

- Diastolic and systolic volumes
- Ejection fraction

Note: The calculations obtained from this measurement tool are the same as the LV B-Mode long axis trace. However the equations used to derive these calculations are not the same. For these equations, please refer to the help [?] section in the Vevo 2100 imaging software.

C. Left Ventricular Outflow Tract (LVOT) Measurement

The LVOT can be measured by placing markers from leading edge to leading edge at the level of the aortic valve leaflets. Alternatively, M-Mode imaging can also be performed at this level.

Scanhead position:
- Same as PLAX view

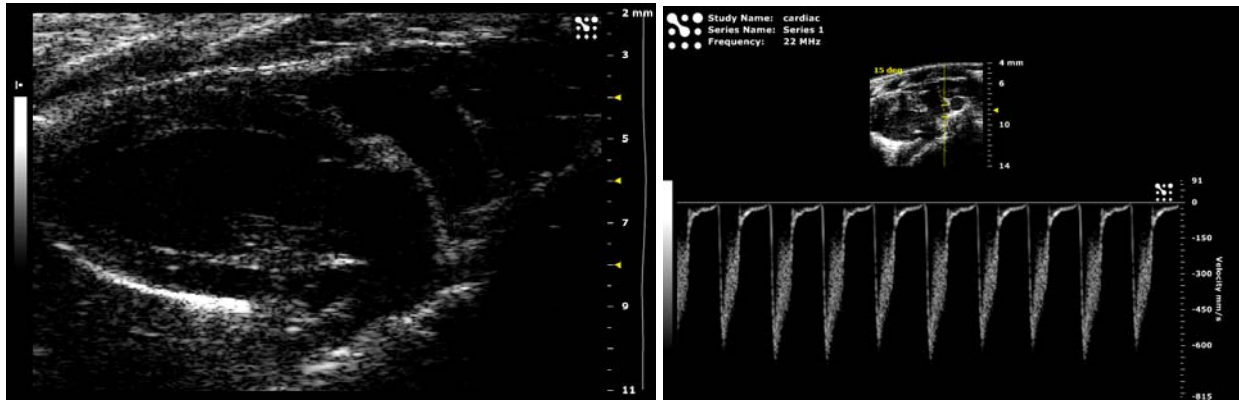**Figure 5.** LVOT view showing placement of measurement caliper.

D. **Pulmonary Outflow**

Pulmonary artery flow can be assessed using PW Doppler Mode. Peak velocity and VTI can be measured.

Scanhead position:
- From the PLAX view, the pulmonary artery should come into view when the probe is shifted slightly to the animal's left.

Figure 6. Parasternal long axis view (PLAX) showing the pulmonary artery (L). PW Doppler Mode waveform of the pulmonary artery (R).

Available Measurements and Calculations:
- Pulmonary velocity time integral (PV VTI)
- Peak velocity (PV peak V)
- Acceleration time (PAT)
- Ejection time (PET)

E. **Superior (Cranial) Vena Cava Flow**

The right and left superior vena cava generally run parallel to the aorta and are located slightly to the right and left relative to the aorta. Their flow can be assessed by Doppler.

Scanhead position:
- From the suprasternal view, the right and left superior vena cava should come into view when the probe is shifted slightly to the animal's right and left respectively.
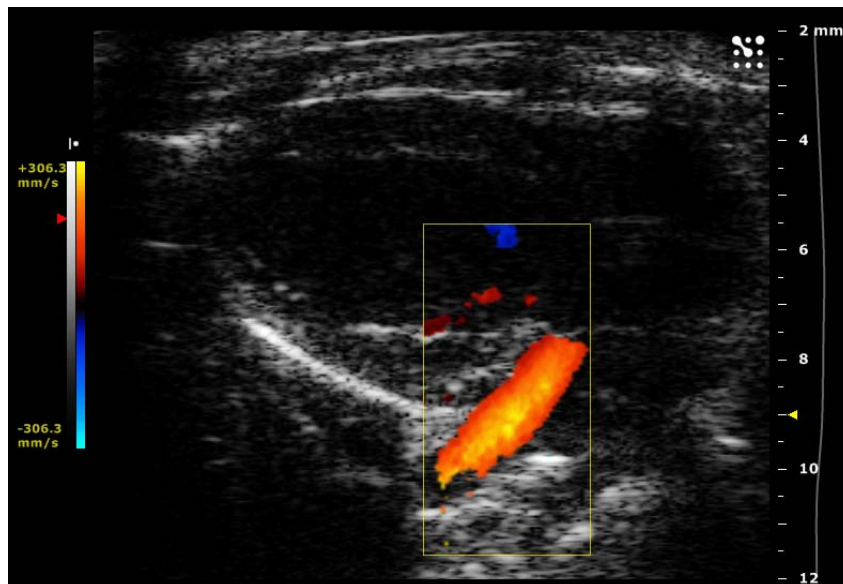
Figure 7. PW Doppler Mode waveform of flow in the superior vena cava.

F. Pulmonary Vein

Pulmonary veins run into the left atrium. They can be visualized and assessed in a standard parasternal long axis view.

Scanhead position:
- From the parasternal long axis view, adjust the x-axis slightly until the left atrium and pulmonary vein come into view.

Figure 8. Color Doppler Mode image of the pulmonary vein entering the left atrium. Please see section XI for more details about Color Doppler Mode imaging.

II. Parasternal Short Axis View (PSAX)

Visible anatomy in PSAX view:

- Left ventricle
- Anterior wall
- Intraventricular septum

- Right ventricle
- Posterior wall
- Papillary muscles

A. <u>B-Mode Image of the Parasternal Short Axis View</u>

Scanhead position:

- From the PLAX view, parasternal short axis view can be obtained by rotating the scanhead 90° clockwise. Following this 90° rotation, the Y-axis may need further adjustment to obtain the proper view.
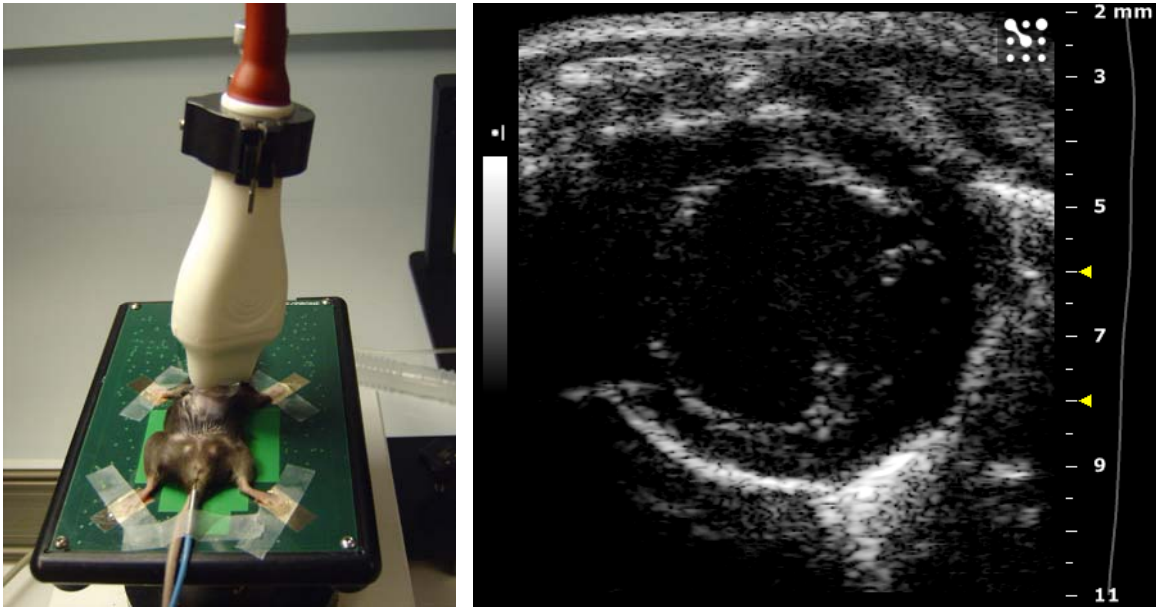
Figure 9. Image showing the positioning of the MS400 scanhead on a mouse to obtain a parasternal short axis view (L). B-Mode image of the short axis view of left ventricle (R).

Available Measurements and Calculations:

- Epicardial and Endocardial trace (please see section X)

B. <u>M-Mode Image of the Parasternal Short Axis View</u>

M-Mode axis should be placed at the mid level of the LV, usually just medial of the papillary muscle. Measurements obtained here should correlate closely to measurements obtained in the long axis view.

Scanhead position:

- Same as the parasternal short axis view.

Available Measurements and Calculations:

- LV anterior wall thickness in diastole and systole (LVAWd, LVAWs)

- LV internal diameter in diastole and systole (LVIDd, LVIDs)

- LV M-Mode trace (please see section I; same as in PLAX view)

- LV posterior wall thickness in diastole and systole (LVPWd, LVPWs)

- Intraventricular septum thickness in diastole and systole (IVSd, IVSs)

C. Other Anatomy Visualized from a Modified Short Axis View

From the standard PSAX view, by shifting the transducer upwards to the base of the aorta, the aortic valve will appear in the middle. The tricuspid valve is on the animal's right (left side of the screen) and the pulmonary valves will be on the animal's left (right side of the screen). Coronary arteries may also appear in this view.

Scanhead position:
- From the standard PSAX view, shift the transducer upwards towards the base of the aorta (towards the animal's head) until the structures can be visualized.
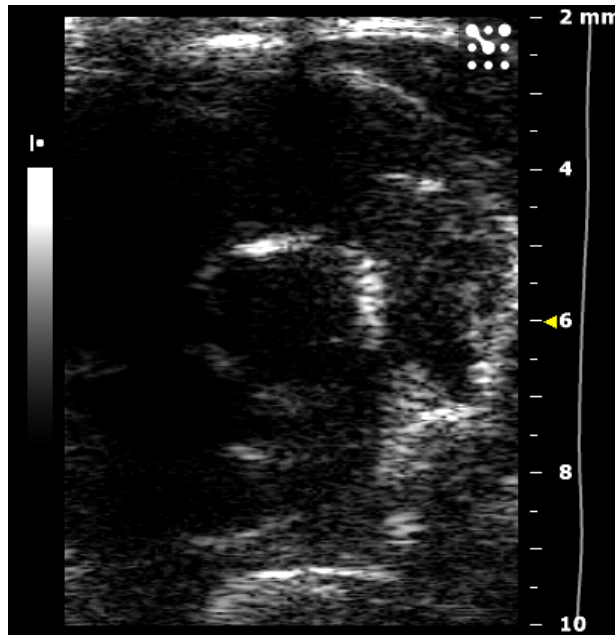
Figure 10. B-Mode image of the high short axis viewed at the aorta and pulmonary level.
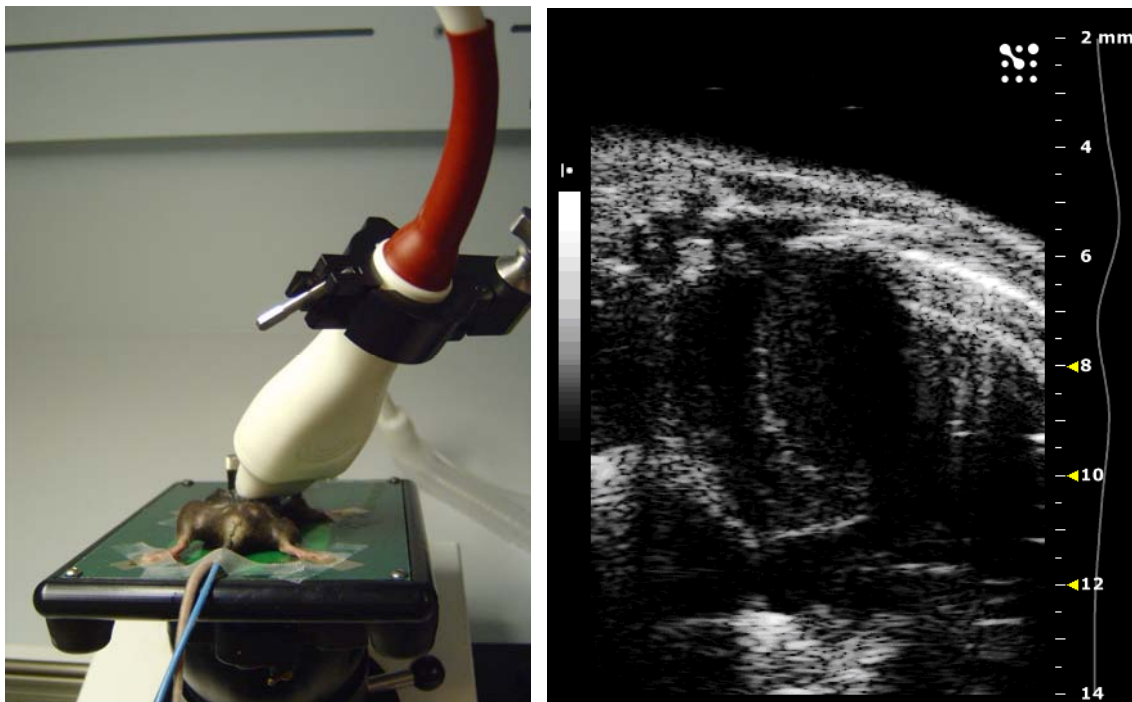
III. Apical Four Chamber View

Visible anatomy in the apical four chamber view:

- Left ventricle
- Right ventricle
- Mitral valve

- Left atria
- Right atria
- Tricuspid valve

The apical four chamber view is obtained to visualize the right and left ventricles, with the atria at the bottom of the screen. This view will provide an accurate angle for PW Doppler Mode of the mitral and tricuspid valves.

Scanhead position:
- From the short axis view, dramatically angle the animal's head down and also angle the base of the probe toward you. This is essentially to achieve a coronal view of the heart looking up towards the apex.

Figure 11. Image showing the positioning of the MS400 scanhead on a mouse to obtain an apical four chamber view (L). B-Mode image of the apical four chamber (R).

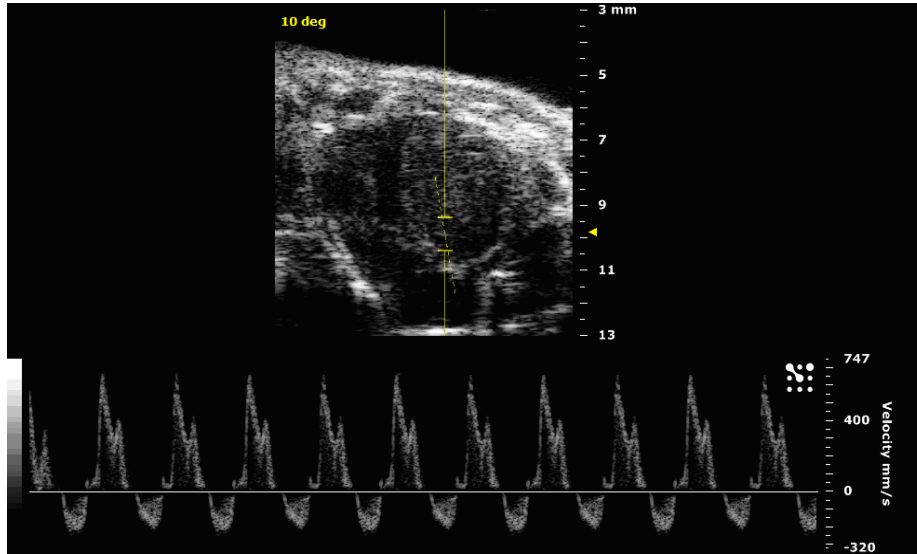## A. <u>Mitral flow velocity from the apical four chamber view</u>

Place the sample volume directly under the mitral valves.

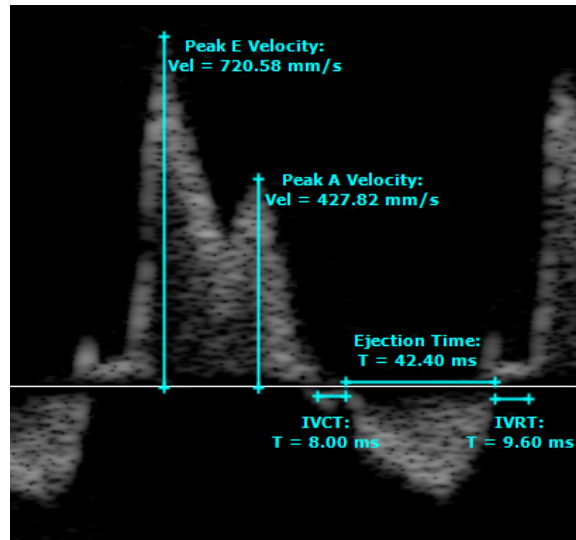**Scanhead position:**
- Same as the apical four chamber view.

Figure 12. PW Doppler Mode waveform of mitral valve flow in the apical four chamber view.

Available Measurements and Calculations:

- Mitral isovolumic relaxation and contract times (IVRT, IVCT)
- Mitral velocity time integral (VTI)
- Mitral deceleration time (MV Decel)
- Peak early and atrial filling (Peak E, Peak A)
- Aortic ejection time (AET)

Figure 13. PW Doppler Mode waveform of mitral valve inflow from apical four chamber view, showing relevant measurements.
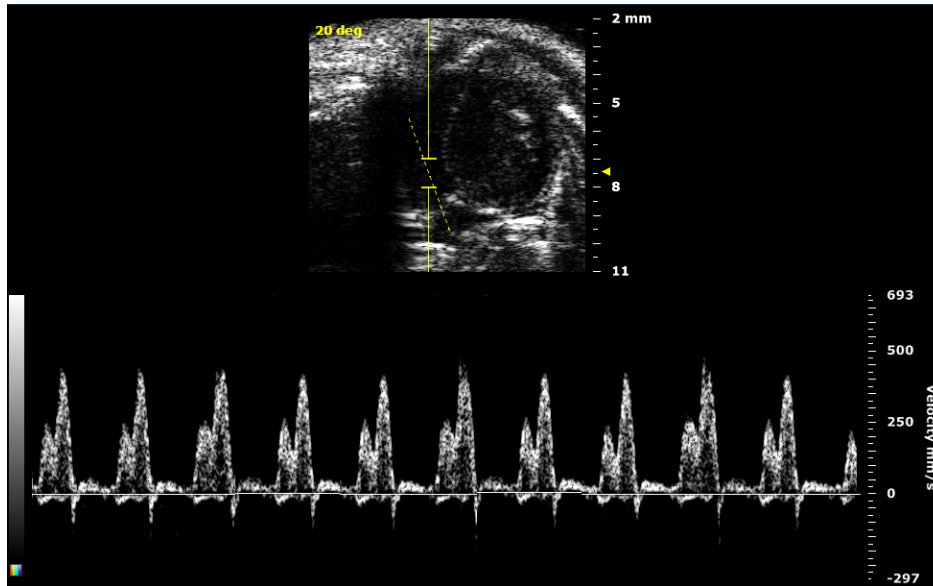
B. Tricuspid Inflow from the apical four chamber view

Place the sample volume directly under the tricuspid valves.

Scanhead position:
- Same as the apical four chamber view.

Figure 14. PW Doppler Mode waveform showing Tricuspid inflow in the apical four chamber view.

Available Measurements and Calculations:

- Tricuspid isovolumic relaxation and contract times (IVRT, IVCT)
- Tricuspid velocity time integral (VTI)
- Tricuspid deceleration time (TV Decel)
- Peak early and atrial filling (Peak E, Peak A)
- Aortic ejection time (AET)

IV. Aortic Arch View

Visible anatomy in the aortic arch view:

- Ascending aorta
- Innominate artery
- Left subclavian artery
- Descending aorta
- Left common carotid

The aortic arch view is obtained to visualize the aortic arch and the arterial branches in this region.

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 optimize the image.
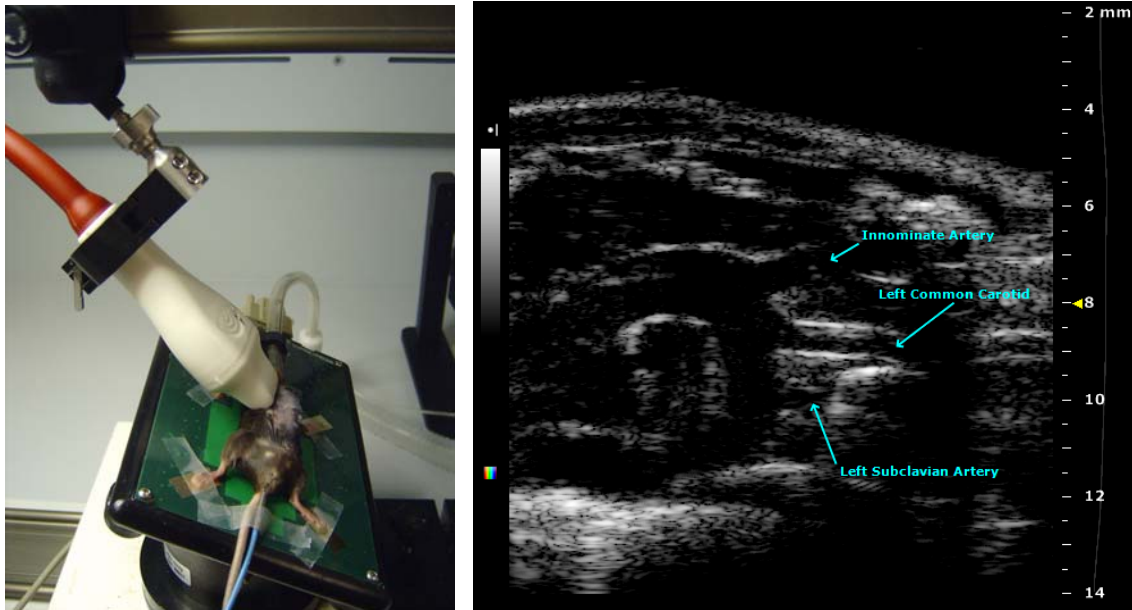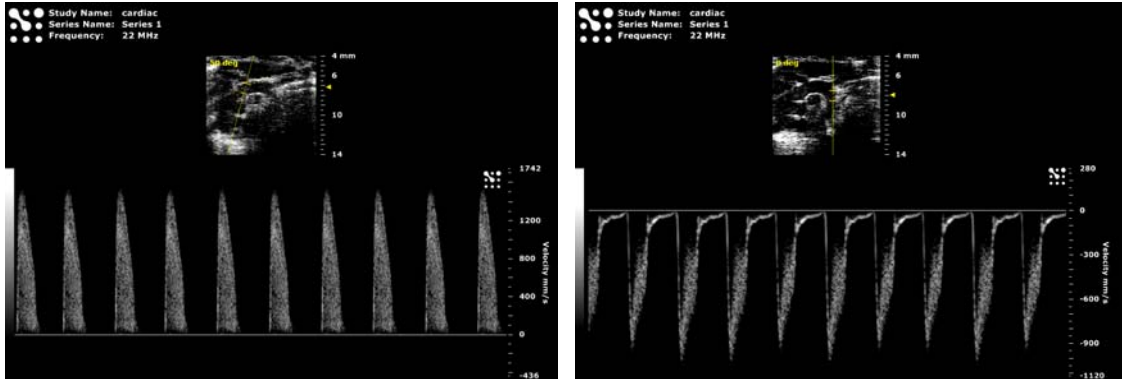

Figure 15. Image showing the positioning of the MS400 scanhead on a mouse to obtain an aortic arch view (L). B-Mode image of the aortic arch view showing the aortic arch (R).

A PW Doppler Mode sample volume can be placed in the ascending and descending aorta to measure peak aortic velocities. These waveforms may also be traced to obtain a VTI calculation.

Figure 16. PW Doppler Mode waveform of the ascending aorta from the aortic arch view (L). PW Doppler Mode waveform of the descending aorta from the aortic arch view.

V. Right Parasternal Long Axis View

The right parasternal long axis view is done to evaluate the right ventricle.

Scanhead position:
- From the parasternal long axis view, angle the base of the probe towards the animal's right by ~45°. The platform may need to be moved slightly to the animal's left to bring the right ventricle into the imaging plane.
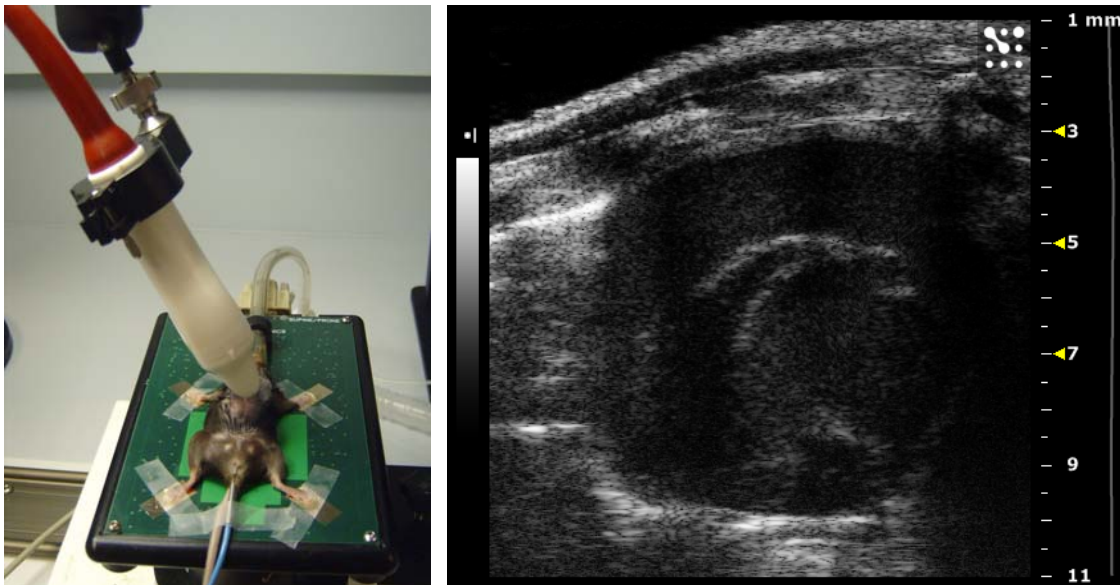
Figure 17. Image showing the positioning of the MS400 scanhead on a mouse to obtain a right parasternal long axis view (L). B-Mode image of the right parasternal long axis view (R).

VI. Suprasternal View

Visible anatomy in suprasternal view:

- Aorta
- Left ventricle
- Aortic valves

The aorta can be seen clearly in the parasternal long axis view. However, the angle of the aorta must be less than 60° relative to the vertical axis in order to generate a reliable Doppler velocity waveform. Therefore, the suprasternal view is performed to generate this angle in order to obtain blood velocities at the aortic valve and in the ascending aorta. With the advent of Doppler steering in the Vevo 2100 imaging system, one can steer the vertical axis up to 25°, the original angle of the aorta can be as high as 85° (60° + 25°). Therefore, if obtaining the suprasternal view is difficult, one can consider to obtain the aortic Doppler in a long axis view (with the aid of steering).

Note: Angle steering will lose detection sensitivity. Therefore, it should only be used when obtaining less than 60° is difficult or impossible.

Scanhead position:

- Place the probe on the midline of the mouse at the level of the thoracic inlet. Then angle the base of the probe towards the animal's head by ~50°. This should look like long axis view, but with the image rotated by 50° counter-clockwise. To obtain aortic PW Doppler Mode, place the Doppler sample volume just above the aortic leaflets.
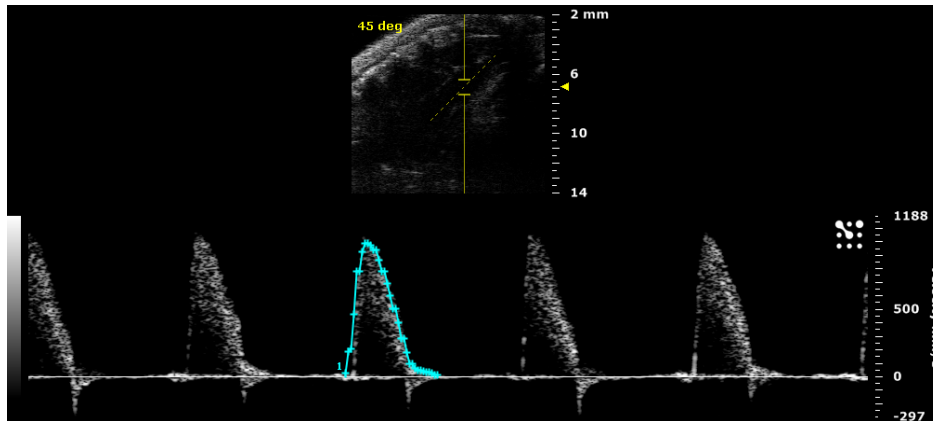
The velocity range of the PW Doppler Mode is 100-120 cm/sec, in a normal model.

Figure 18. Image showing the positioning of the MS400 scanhead on a mouse to obtain a suprasternal view (L). B-Mode image of the suprasternal view (R).

Figure 19. PW Doppler Mode waveform of the aortic valve outflow from a suprasternal view, showing a VTI trace.

Available Measurements and Calculations:
- Aortic Velocity time integral (Ao VTI)
- Peak velocity (Ao peak V)
- Acceleration time (AAT)
- Ejection time (AET)

<u>Aortic VTI Trace</u>
To perform the VTI trace, if the waveform is above the zero line, chose "+" on the "Peak" drop down menu. If below the zero line, chose "-".

VII. Tissue Doppler Mode

Tissue Doppler Mode measures the velocity of tissue instead of blood. This can be used to detect and assess the health of the myocardium wall of the left ventricle or the mitral annulus. A common view for this assessment is the apical four chamber view (please see section III).
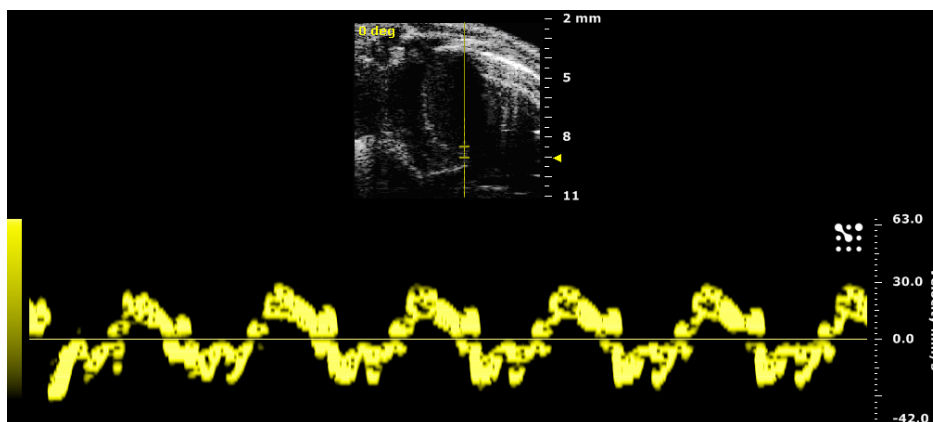

Figure 20. Tissue Doppler Mode image to assess mitral valve annulus from an apical four chamber view. In this example, the E' and A' waves can be measured and the ratio is calculated as an indication of myocardium health.

VIII. 3D Cardiac Imaging

Cardiac imaging on Vevo 2100 imaging system can be ECG-gated. Therefore 2D and 3D imaging can be done at a fixed position on the cardiac cycle. 3D cardiac imaging is a particularly powerful tool that enables precise volumetric measurements. Diastolic and systolic volumes can be measured in order to yield accurate stroke volume and cardiac output calculations.
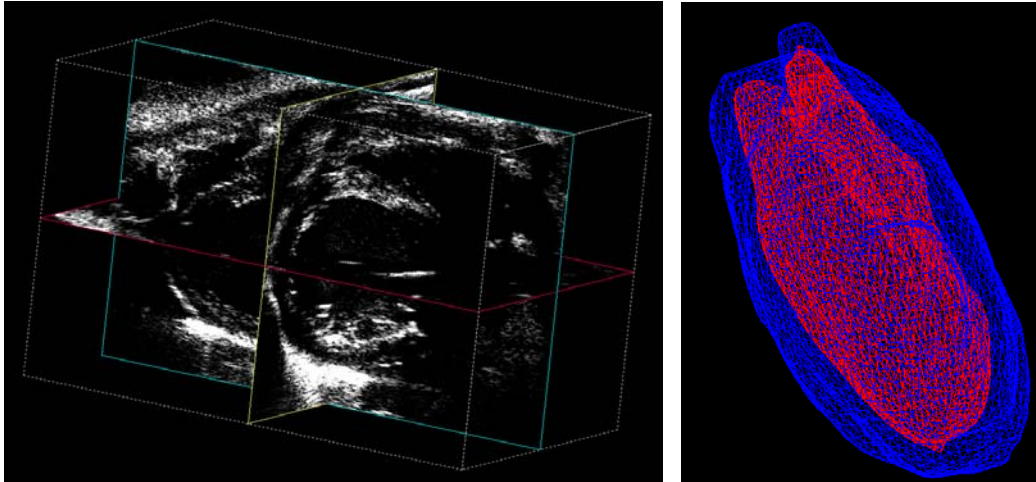
Figure 21. 3D image of the heart (L). 3D volumetric trace of the endo- and epicardial region (R).

A. 3D Cardiac Imaging in Diastole
Enable respiration and T1 ECG gating. Adjust T1 time delay until the maximum diameter of the LV is observed.

B. 3D Cardiac Imaging in Systole
Enable respiration and T2 ECG gating. Adjust T2 time delay until the minimum diameter of the LV is observed.

C. 3D Measurements and Calculations
Use the 3D volume trace tool to measure endo- and epicardial LV volumes in diastole and systole (please see the Vevo 2100 operator's manual for instructions on 3D volume tracing).
- Stroke volume = diastolic – systolic endocardiac volume
- Cardiac output = (diastolic – systolic endocardiac volume) x Heart rate
- LV mass = (epicardial – endocardial volume) x 1.06 g/μL

Note: Specific gravity of muscle = 1.06 g/μL

IX. Simpson Measurement

Simpson measurement measures the LV at multiple planes at diastole and systole and yields calculations such as LV diastolic and systolic and stroke volumes.
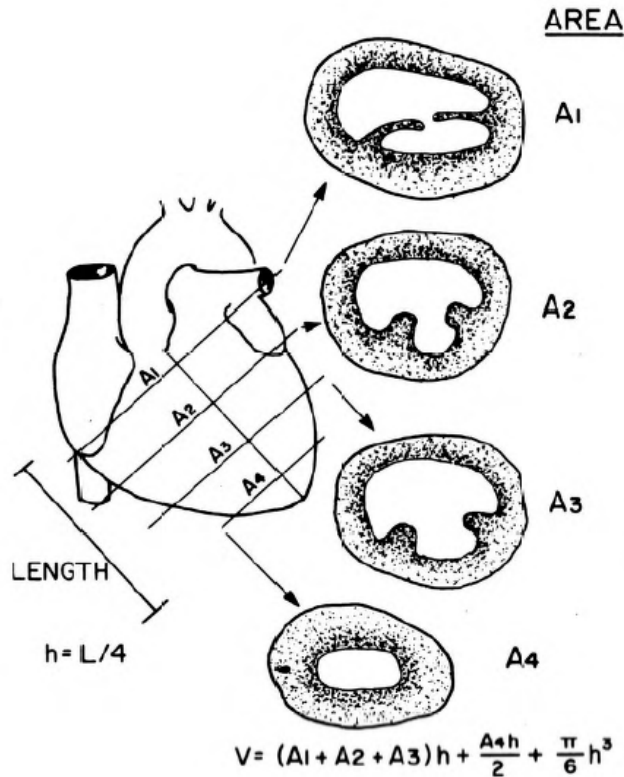
Figure 22. Schematic of views required to perform the Simpson measurement.

Workflow:

1. Acquire three short axis images at three different levels. E.g. one near the apex of the heart (SimpAreaDist), one at the mid section near the papillary muscle level (SimpAreaMid) and one just under the base of the aorta (SimpAreaProx). For each level, trace the endocardial border in diastole and systole.

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.

3. Once the measurements are made, the available calculations will be displayed in the calculations menu.

X. Left Ventricle Mass

Workflow:

1. Acquire a parasternal short axis view in diastole. Trace the left ventricle endocardium (ENDOarea;d,s). Trace the left ventricle epicardium (EPIarea;d,s).

2. Acquire a parasternal long axis in both diastole and systole. Measure the endo- and epicardial lengths in both diastole and systole (ENDOmajr;d,s, EPImajr;d,s).

3. Once the measurements are made, the available calculations will be displayed in the calculations menu.
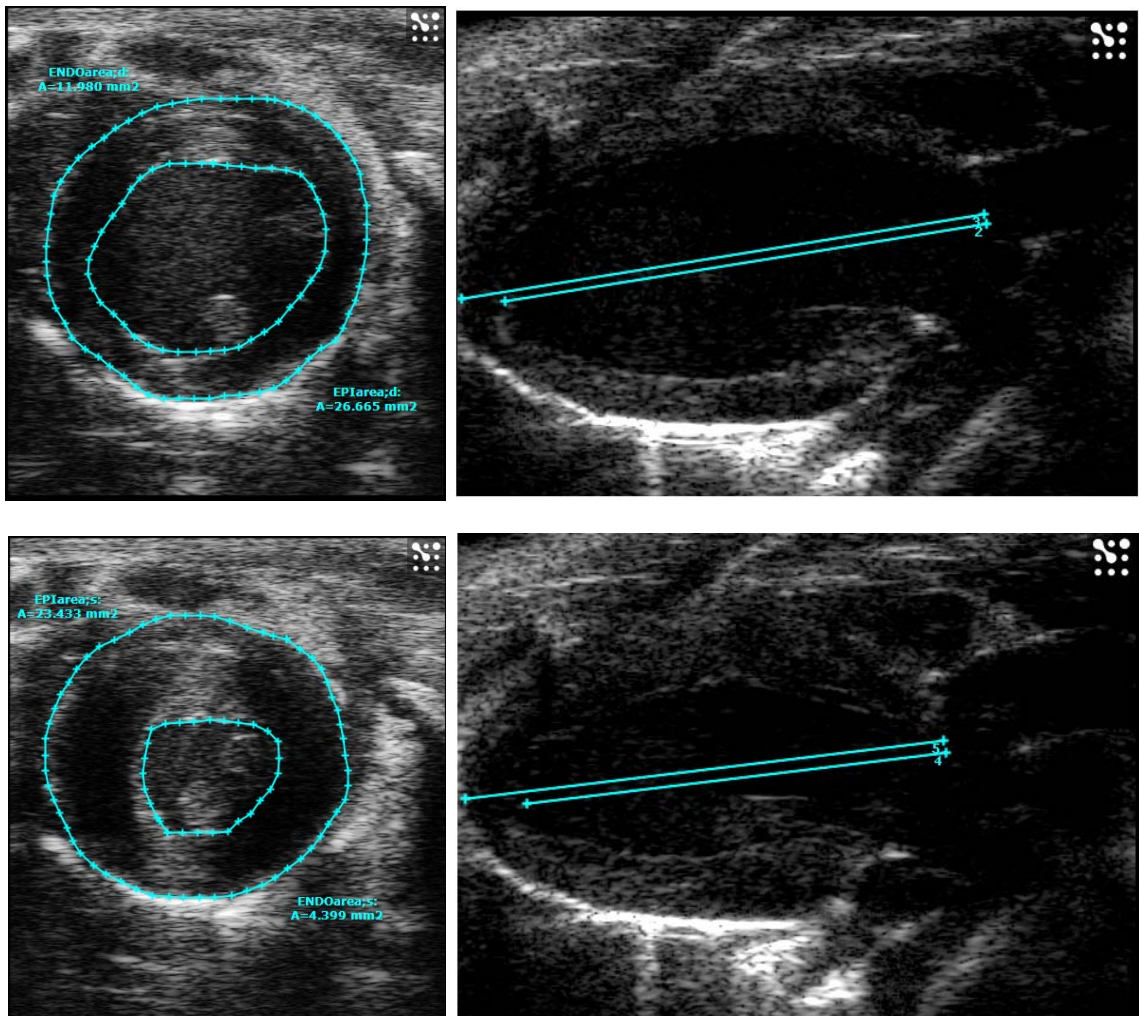

Figure 23. Endo- and epicardial area trace in diastole (top left). Endo- and epicardial length in diastole (top right). Endo- and epicardial area in systole (bottom left). Endo- and epicardial length in systole (bottom right).

XI. Color Doppler Mode Imaging

Color Doppler Mode imaging is a useful tool to assess flow patterns in an area of interest. Pathological conditions such as abnormally high flow velocities and regurgitations can be efficiently detected by unique characteristic color pattern.

For animals with normal physiology, optimal Color Doppler Mode settings can frequently be obtained simply by choosing the "fast flow" preset during Color Doppler Mode imaging. However, for abnormal animals, Color Doppler Mode settings may need to be adjusted manually to achieve optimal imaging quality (please refer to the Quick-Start Guide: *Imaging in Color Doppler, Power Doppler and Power Doppler 3D Modes*).
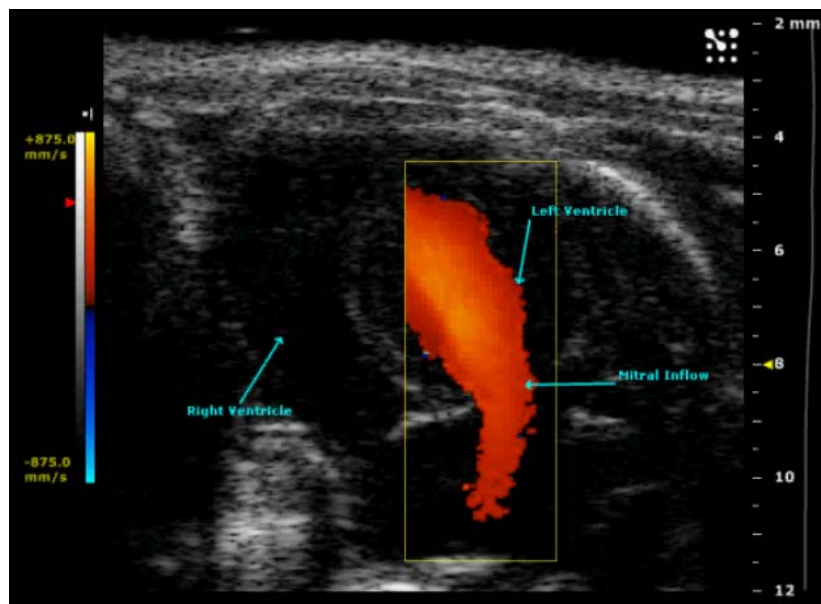
Figure 24. Color Doppler Mode image displaying the mitral inflow, from the left atrium to the left ventricle.

XII. Pressure–Volume Measurements

The Vevo 2100 system can integrate a blood pressure signal from a 3rd party continuous pressure monitoring system, such as:

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

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

- Pressure-volume analysis
- Vascular strain analysis
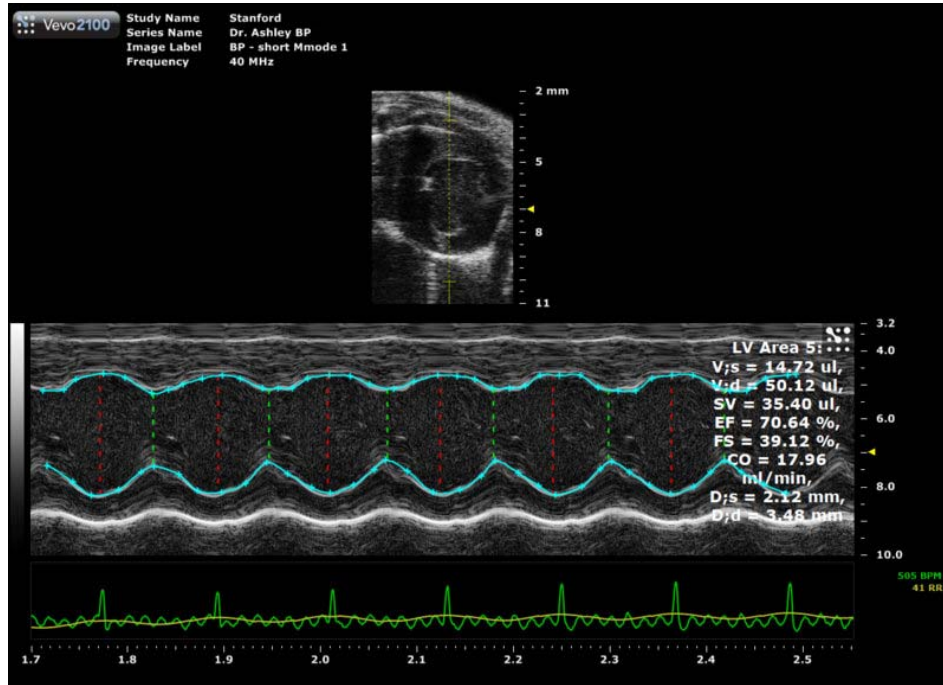
Figure 25. Example of a Pressure-Volume graph (top) generated from a blood pressure signal obtained in a short axis view (bottom).

XIII. Normal Values for Mice and Rats

Value	Mice	Rats
Ejection Fraction	55-85%	55-80%
Fractional Shortening	30-50%	30-50%
Left Ventricle Mass	65-90 mg	60-100 mg
Stroke Volume	40-70 µL	100-135 µL
Cardiac Output	20-35 mL/min	40-55 mL/min

These values vary according to animal strain, size, sex and health. This is only a guideline.

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