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Guide to Small Animal Basic Echocardiography using the Vevo Ultrasound Systems

Basic Echocardiography Introduction

To perform basic echocardiography using Vevo ultrasound systems, you must master the fundamental ultrasound cardiovascular views. These are the parasternal long axis (PSLAX), parasternal short axis (SAX), and the apical 4 chamber view. Generally, the rodent heart has an ellipsoidal shape and is positioned at an angle towards the left side of the chest adjacent to the sternum. The optimum imaging window is located parasternally on the left side of the chest during transthoracic exams. This document will provide step by step guidance to you help achieve optimal views for accurate analysis of both systolic and diastolic function of the rodent heart.

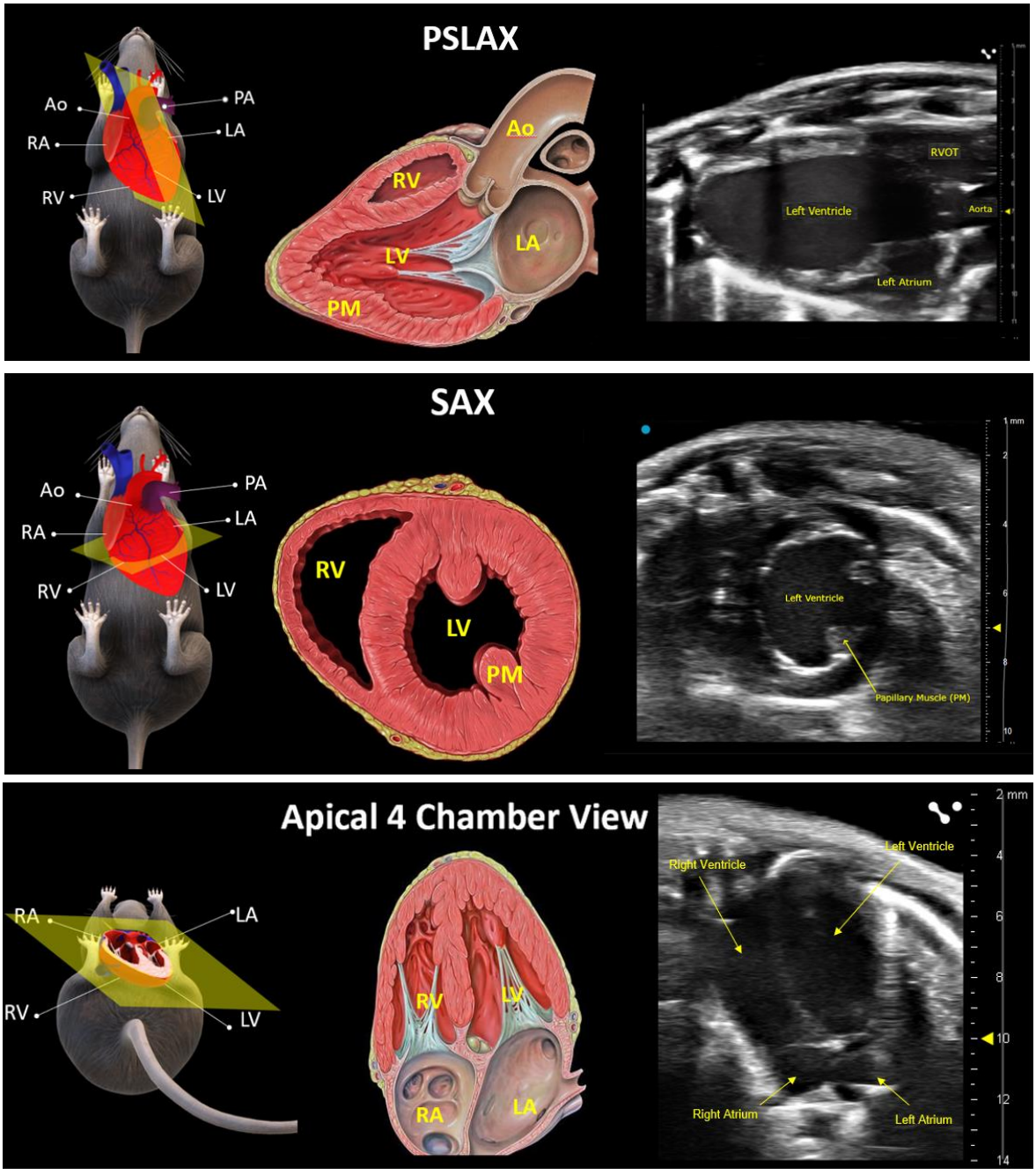


Figure 1: Basic cardiac views showing schematic illustration of the imaging plane (left) in a rodent, gross anatomy illustrations of the heart (middle), and Vevo ultrasound images (right). Schematic illustrations courtesy of Dr. Maria Villalba Oreo, CNIC, Madrid, Spain. Gross anatomy illustrations: https://commons.wikimedia.org/wiki/Category:Medical_illustrations_by_Patrick_Lynch

Selecting the Appropriate Transducer

The following transducers are recommended for rodent echocardiography using the Vevo F2*:

- For young mice and neonates: UHF71x (71MHz) transducer.
- For adult mice: UHF57x (57MHz) transducer or UHF46x (46MHz)
- For overweight mice >35g: UHF46x (46MHz)
- For rats: the UHF29x (29MHz) transducer or the UHF22x (22MHz) if >350g

*If using another Vevo ultrasound product, see technical specifications to select the appropriate transducer.

Animal Preparation – Animal preparation should adhere to approved institutional animal use protocols.

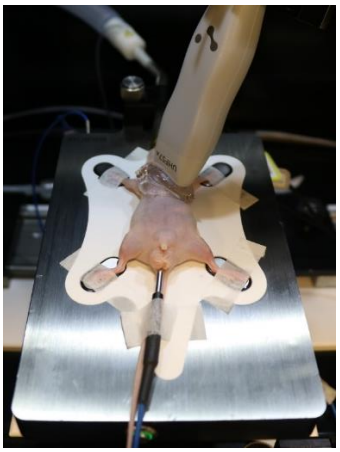
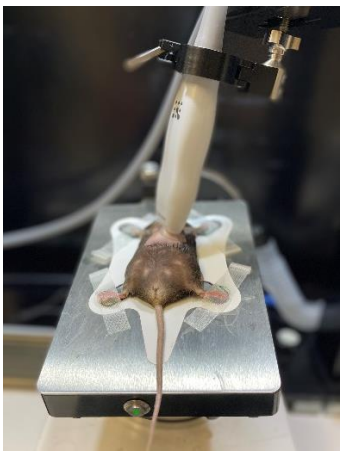
Left Ventricular Assessment: Systolic Function

Parasternal Long Axis (PSLAX) View

Transducer and Animal Positioning

The heart is located towards the left side of the chest and (in rodents) the apex is typically shifted upward towards the chest wall. To correctly position the animal, you can use one of the following approaches:

1. Manipulate the transducer position; animal platform will remain horizontal.
2. Manipulate both the transducer and animal platform.

Approach 1: Platform remains horizontal	**Approach 2:** Manipulate both transducer and animal platform
1. Keep the animal platform horizontal (avoid tilting as this can cause changes in the hemodynamics). 2. Place the transducer over the sternum. 3. Rotate the transducer to the right shoulder of the mouse. 4. Tilt the transducer to the left to avoid the shadow from the sternum.	1. Tilt the animal platform up so that the animal's head is elevated. 2. Tilt the platform to the left to expose the left side of the animal. 3. The transducer should be angled parallel to the animal's sternum, then slightly rotated (approximately 15-45° depending on individual anatomy) counter-clockwise towards the right shoulder of the animal.
 *Figure 2: Transducer positioning for PSLAX imaging where the animal platform remains horizontal*	 *Figure 3: PSLAX imaging where the transducer and animal platform are manipulated*

The standardized PSLAX cardiac view will have:

- aorta open
- apex pointed (not closing inwards or rounded)
- aorta and apex in a horizontal line
- visible myocardial walls

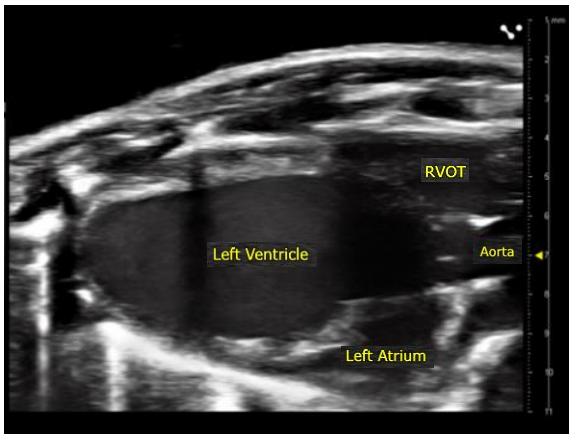


Figure 4: Representative PSLAX image

Parasternal Long Axis View in M-Mode

To obtain an optimal M-mode image, the LV must be horizontal in orientation (aorta and apex in-line with each other). Place the M-Mode axis in the midsection of the LV at the largest diameter.

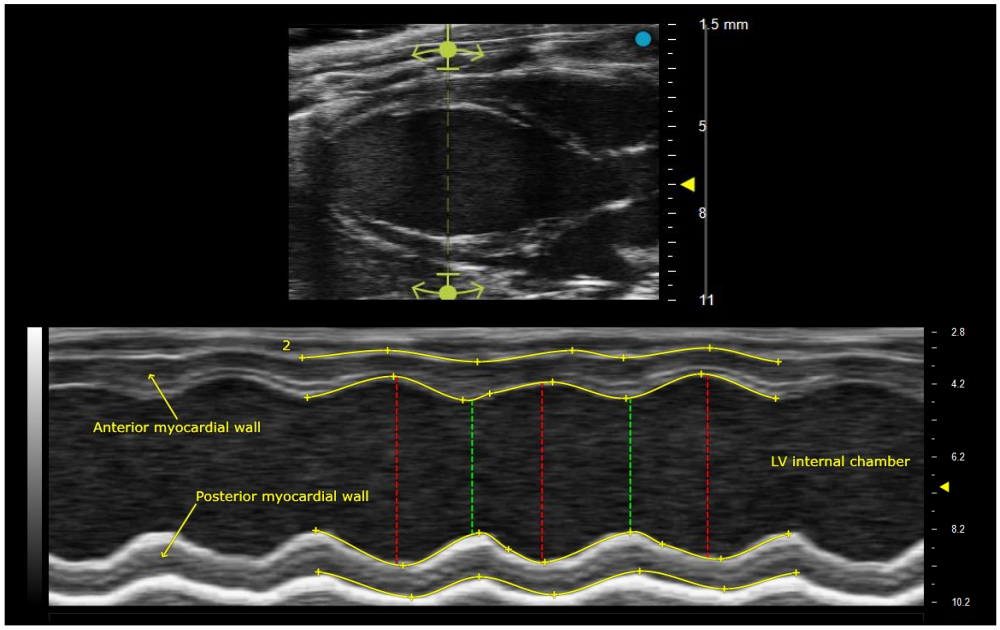


Figure 5: M-mode image of PSLAX view displaying motion of the anterior and posterior walls over time.

Note: If the apex is angled, and not in-line with the aorta, M-mode measurements can be overestimated. Use the Anatomical M-mode (AM-mode (purchasable feature)) to adjust the M-mode line to the correct position.

Parasternal Long Axis View in Color Doppler

Color Doppler mode is a tool to visualize directional blood flow (blood movement towards or away from the transducer) and provides semi-quantitative blood flow velocity. Color Doppler can help users to identify important vascular landmarks associated with the cardiac system, including the **aortic root, pulmonary artery, aortic valve, and pulmonary veins**.

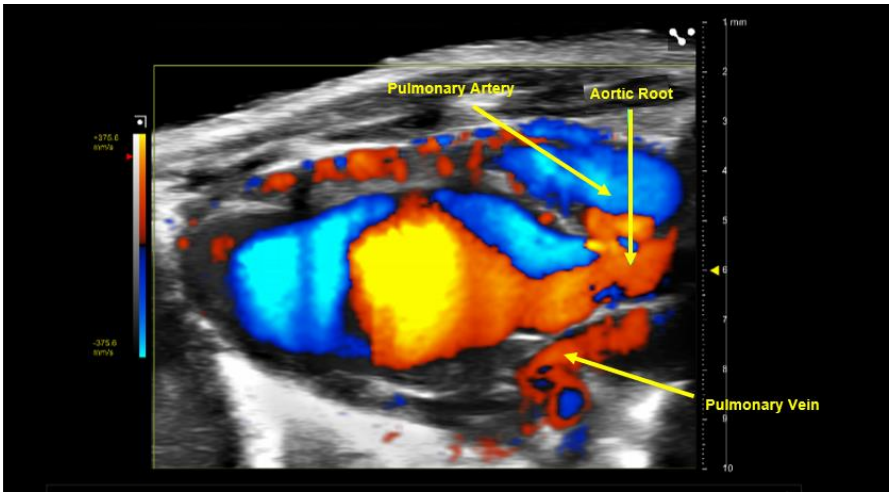


Figure 6: Aortic root in Color EKV Doppler (Color EKV is not available on Vevo ultrasound systems released before the Vevo F2)

Parasternal Short Axis (SAX) View

Transducer and Animal Positioning

The platform should remain in the same position used for the PSLAX view (horizontal platform or animals head elevated with platform tilted to the left).

- Start with the transducer positioned for the PSLAX view and ensure that the LV is in the middle of the screen.
- Rotate the transducer 90° clockwise.
- The heart will be displayed in the parasternal short axis view and the main landmarks are the **papillary muscles** (at 2 and 4 o'clock).
- To have the papillary muscles in the view, slight Y-axis translations (head to tail) might be necessary.

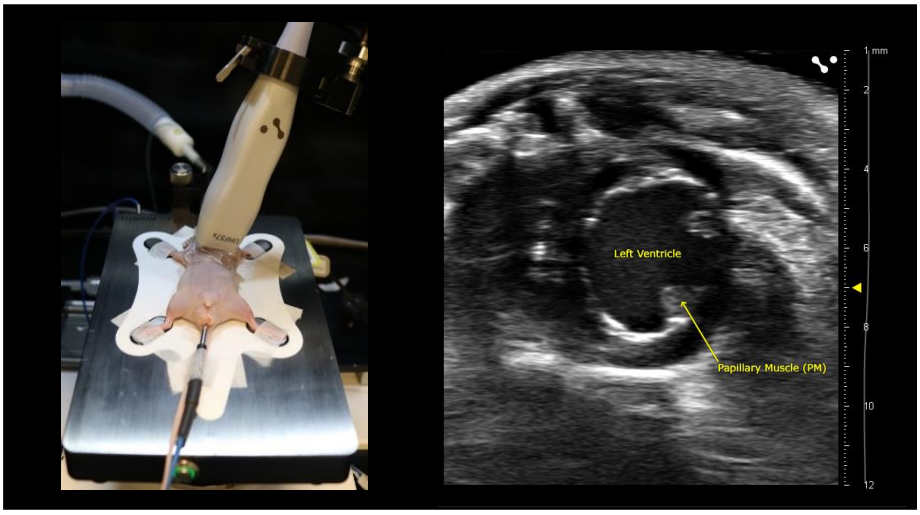


Figure 7: SAX position. (Left) Animal and transducer positioning for SAX view; transducer is rotated 90 degrees clockwise from the PSLAX position. (Right) Representative B-Mode image of the LV in short axis (SAX) with the papillary muscles (PM) in view.

Parasternal Short Axis (SAX) View in M-Mode

M-mode axis should be placed at the mid-level of the LV cavity, just medial of the papillary muscles.

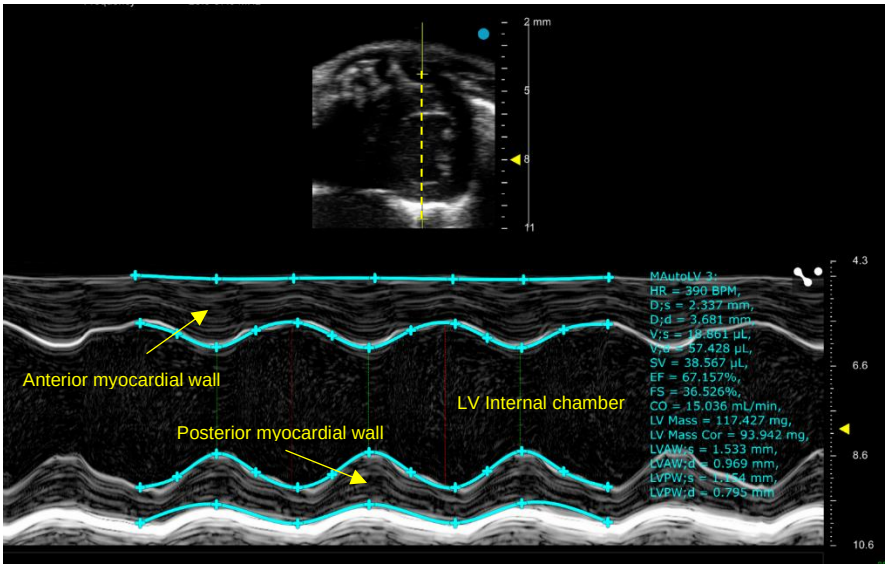


Figure 8: M-mode image of the LV in short axis (SAX). Heart walls are traced using Auto LV feature showing calculation of various cardiac parameters.

Measurements and Calculations Available for PSLAX and SAX*

- Wall thickness
- LV internal diameter
- LV volume
- Ejection Fraction
- Stroke Volume
- Fractional Area Change
- LV mass
- Cardiac Output

*Note: This is only a subset of available measurements and calculations. Please refer to the user manual and VisualSonics cardiac analysis guide for a complete list. Measurements and calculations vary by imaging mode.

Left Ventricular Assessment: Diastolic Function

Apical Four Chamber View

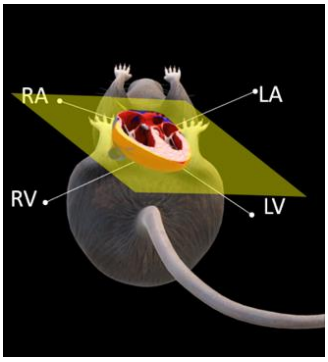


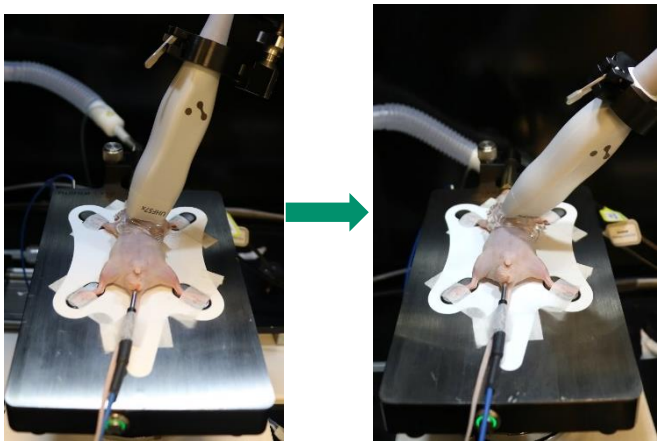
Figure 9: Schematic illustration of the apical four chamber imaging plane.

Transducer and Animal Positioning

The apical four chamber view is used to visualize all 4 chambers of the heart. This imaging technique requires the transducer face to be positioned at a steep angle near the bottom of the rib cage. The 4-chamber view will show ventricles at the top of the image and the atria at the bottom of the image.

To correctly position the animal, you can use one of the following approaches:

1. Manipulate the transducer position; animal platform will remain horizontal.
2. Manipulate both the transducer and animal platform.

Approach 1: Platform remains horizontal	**Approach 2:** Manipulate both transducer and animal platform
1. Start with the transducer positioned for the short-axis view. 2. Tilt the transducer toward the back wall (~45º angle), whilst maintaining the same angle as the SAX view. 3. Begin imaging just below the rib cage at the apex of the heart, scanning towards the head of the animal to visualize all 4 chambers. 4. The ventricles will appear at the top of the image, and the atria will be at the bottom of the image. 5. Ensure mitral flow is present continuously from the LA to LV using Color Doppler mode.	1. Adjust the animal platform so that the animals head is down (<45º angle), then slightly tilt the platform to the left. 2. Start with the transducer positioned for the SAX view, then adjust the aim of the transducer to a steep angle (45º angle or more) towards the animal's right shoulder or ear. 3. Begin imaging just below the rib cage at the apex of the heart, scanning towards the head of the animal to visualize all 4 chambers. 4. The ventricles will appear at the top of the image, and the atria will be at the bottom of the image. 5. Ensure mitral flow is present continuously from the LA to LV using Color Doppler mode.
 *Figure 10,11: Transducer positioning for SAX view (left). Transducer positioning for apical 4 chamber view (right).*	 *Figure 12: Apical 4 chamber imaging where the transducer and animal platform are manipulated.*

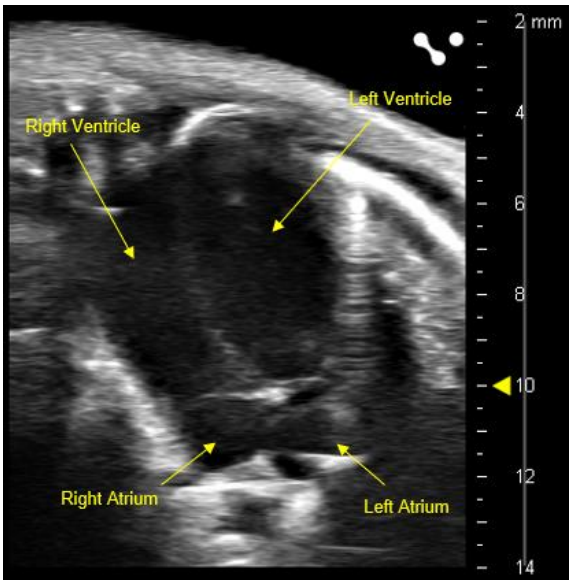


Figure 13: Representative apical 4 chamber view

Apical Four Chamber View in Color Doppler

The 4-chamber view with Color Doppler overlayed is used to identify mitral blood flow. In an optimal 4-chamber view showing mitral blood flow, the Color Doppler signal should begin in the LA and move continuously into the LV. Mitral flow will be red in color (blood flow moving towards the transducer) with high velocity aliasing (color change to blue hues) within the LV chamber.

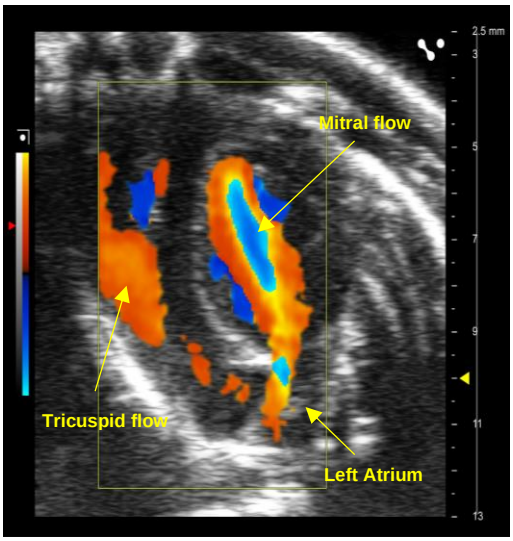


Figure 14: Apical 4 chamber view with Color Doppler of mitral blood flow (and tricuspid flow)

Apical Four Chamber View: Mitral blood flow velocity using Pulse Wave (PW) Doppler

The apical four chamber plane is optimal for mitral valve flow assessment. With the aid of Color Doppler, the mitral blood flow is identified, and PW Doppler mode allows real-time display of blood flow velocity waveforms. In rodents, the PW Doppler sample volume should be placed in the area of highest blood flow velocity indicated by aliasing (color change to blue hues; see Figure 14) within the LV chamber.

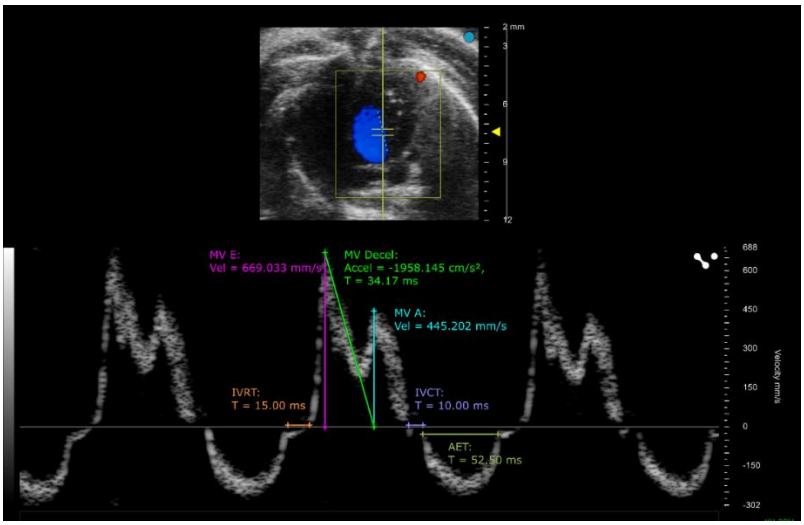


Figure 15: PW Doppler Mode waveform (and measurements) of mitral valve blood flow in the apical four chamber view.

Apical Four Chamber View: Mitral Valve Tissue Doppler

For mitral valve Tissue Doppler, the sample should be taken at the annulus of the valve on the septal wall side. In rodents, the MV-free wall is typically not visible.

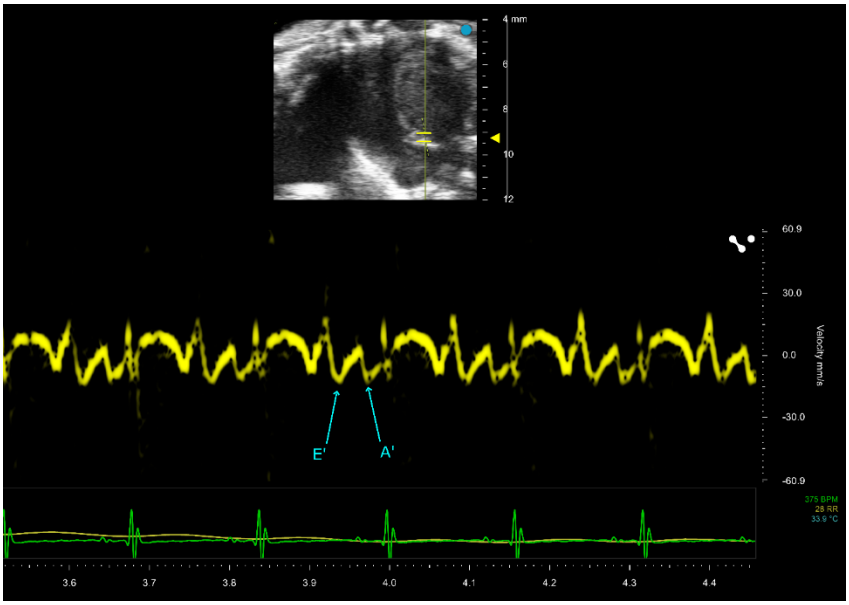


Figure 16: Mitral Valve tissue doppler waveform

Basic Echocardiography Systolic Values in Rodents

Note: These values are only guidelines as they can vary greatly according to animal strain, size, sex, age, and health.

Parameter	Mice	Rats
Ejection Fraction	55-85%	55-80%
Fractional Shortening	30-50%	30-50%
Left Ventricle Mass	65-90 mg	600-1000 mg
Stroke Volume	40-70 µL	100-135 µL
Cardiac Output	20-35 mL/min	40-55 mL/min

Echocardiography Standardization Reference Publications

1. O'Riordan CE, Trochet P, Steiner M, Fuchs D. Standardisation and future of preclinical echocardiography. Mamm Genome. 2023 Jun;34(2):123-155. doi: 10.1007/s00335-023-09981-4. Epub 2023 May 9. PMID: 37160810.
2. Zacchigna S, Paldino A, Falcão-Pires I, Daskalopoulos EP, Dal Ferro M, et al. Towards standardization of echocardiography for the evaluation of left ventricular function in adult rodents: a position paper of the ESC Working Group on Myocardial Function. Cardiovasc Res. 2021 Jan 1;117(1):43-59. doi: 10.1093/cvr/cvaa110. PMID: 32365197.

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