Guide to Small Animal Intermediate Cardiac Imaging using Vevo Ultrasound Systems

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Introduction to Intermediate Cardiac Imaging

This document builds upon basic echocardiography views and techniques to provide more comprehensive insights into cardiac function and associated vasculature in rodents. The use of Vevo systems high-resolution ultrasound provide detailed anatomical and functional information on the rodent cardiovascular system. These techniques aim to enhance understanding and characterization of cardiac structure and function in rodent models, which are valuable in research areas such as cardiovascular diseases, assessing therapeutic interventions, or exploring genetic factors affecting cardiac function.

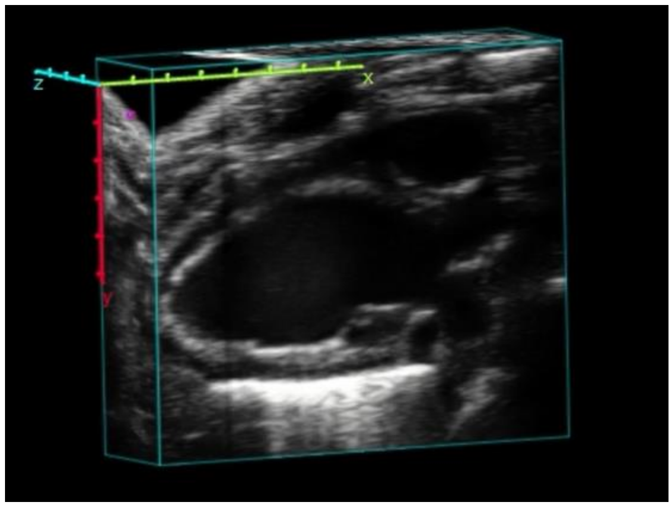

Figure 1: 4D Image of the mouse heart acquired with the Vevo F2 system.

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

4D Acquisition

If the disease phenotype results in non-uniform cardiac remodelling, 4D imaging should be considered to accurately assess cardiac systolic function. 4D image analysis makes no geometric assumptions about the shape or dynamics of the heart. 4D image acquisition is suitable for all disease models to accurately assess systolic function, however it is recommended for non-uniform cardiac remodelling (such as myocardial infarct/LAD ligation) disease models.

4D imaging requires the Vevo 3D motor and 4D imaging software (3D, ECG gating, and EKV (ECG-gated Kilohertz Visualization))

One of the basic echocardiography views can be used for image acquisition:

1. PSLAX (parasternal long axis view of LV)
2. SAX (short axis view of LV)

Within the 4D acquisition panel the transducer automatically determines the optimal step size

- Do not decrease the step size as this results in over-acquisition.
- Increasing the step size will reduce overall acquisition time.

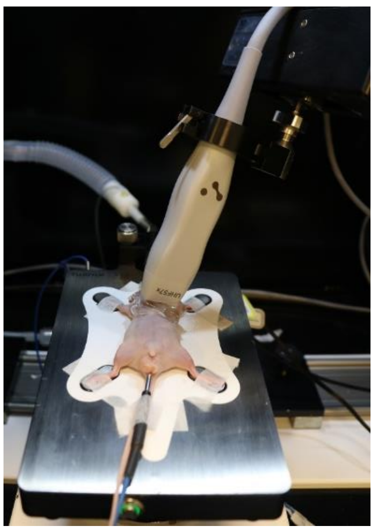

Figure 2: Animal and transducer positioning for acquiring a 4D image from the SAX view; ensure the transducer is perpendicular to the 3D motor.

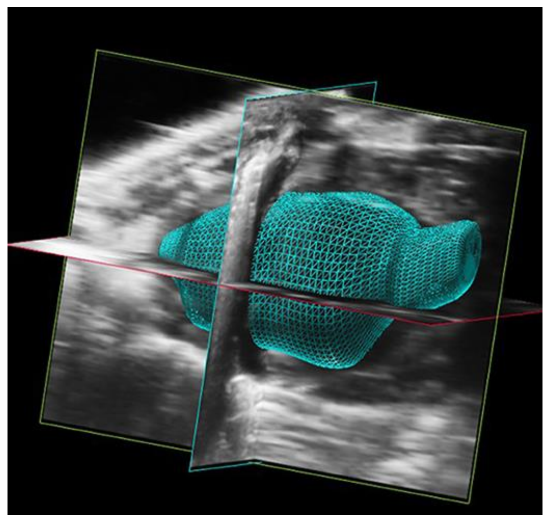

Figure 3: Processed 4D image of the left ventricle using Vevo LAB software.

4D Color Imaging

Color Doppler can be acquired simultaneously with a 4D acquisition. This mode allows visualization of dynamic, directional blood flow in the entire heart.

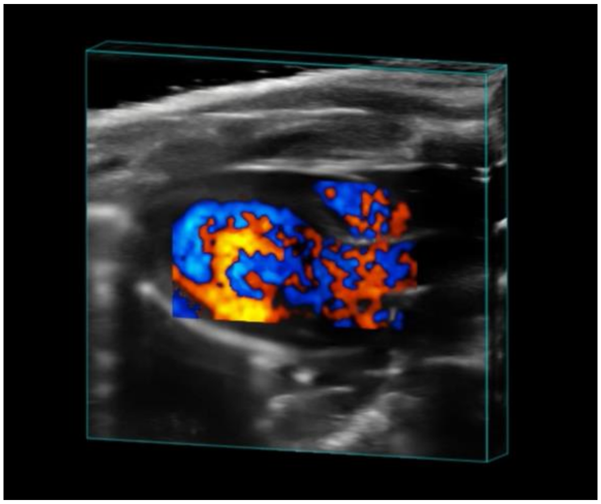

Figure 4: 4D Color image of heart (4D Color is not available on Vevo ultrasound systems released before the Vevo F2)

Simpson's Method

If 4D mode is unavailable, the Simpson's method (known as the Simpson's biplane method in the clinic) is an alternative analysis package used to quantify cardiac systolic parameters. The Simpson's method involves the quantification of cardiac chamber volumes and function by analyzing **FOUR** two-dimensional echocardiographic images from different views of the mouse heart.

Image Acquisition (4 cardiac views):

1. **PSLAX**

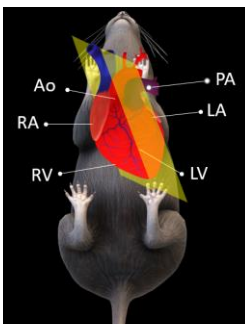

Figure 5a: Schematic illustration of ultrasound imaging plane (yellow) for PSLAX view.*

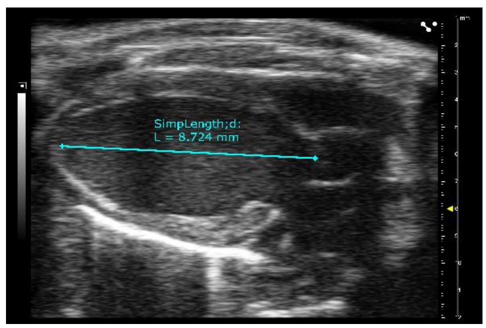

Figure 5b: B-mode image of PSLAX. Endocardial length is measured at systole and diastole for Simpson's quantification.

2. SAX (Mid)

- This is the typical SAX view with both papillary muscles in view. This image axis is in the middle of the heart.

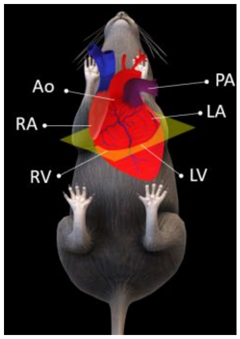

Figure 6a: Schematic illustration of ultrasound imaging plane (yellow) for SAX (mid) view.*

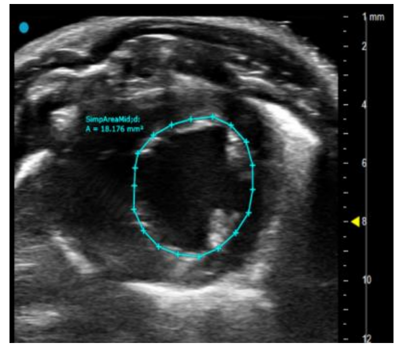

Figure 6b: B-mode image of SAX (mid). Endocardial area is measured at systole and diastole for Simpson's quantification.

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3. Proximal SAX

- This view is proximal to the SAX mid axis, meaning towards the base of the heart.

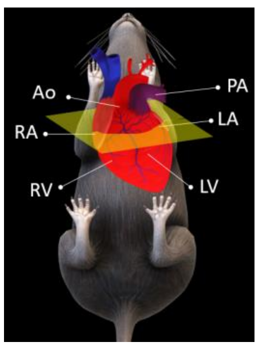

Figure 7a: Schematic illustration of ultrasound imaging plane (yellow) for proximal SAX view.*

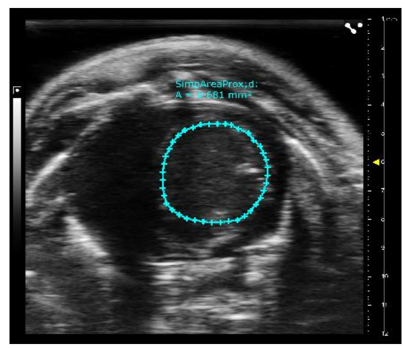

Figure 7b: B-mode image of proximal SAX. Endocardial area is measured at systole and diastole for Simpson's quantification.

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4. Distal SAX

- This view is distal to the SAX mid axis, meaning towards the apex of the heart.

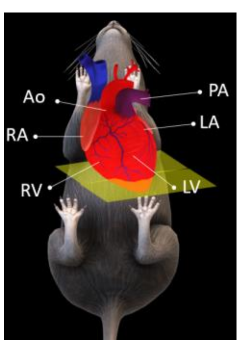

Figure 8a: Schematic illustration of ultrasound imaging plane (yellow) for distal SAX view.*

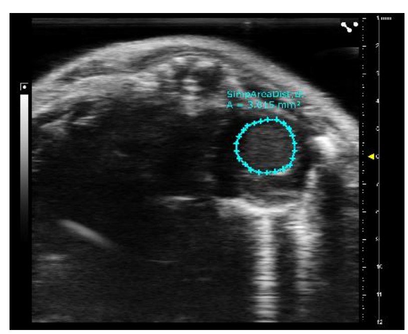

Figure 8b: B-mode image of distal SAX. Endocardial area is measured at systole and diastole for Simpson's quantification.

*** *Schematic illustrations courtesy of Dr. María Villalba Orero, CNIC, Madrid, Spain.***

Left Atrium Imaging

The following views can be acquired to measure the left atrium area at maximal extension:

- Apical 4 chamber view
  - The mitral valve must be closed to ensure maximal extension of the LA.
- PSLAX
  - A slightly modified PSLAX view may be necessary to visualize the LA; do this by scanning lateral on the chest of the animal. A steeper transducer angle/tilt will likely be necessary as well.
  - The LV must be in full systole to ensure maximal extension/area of the LA.

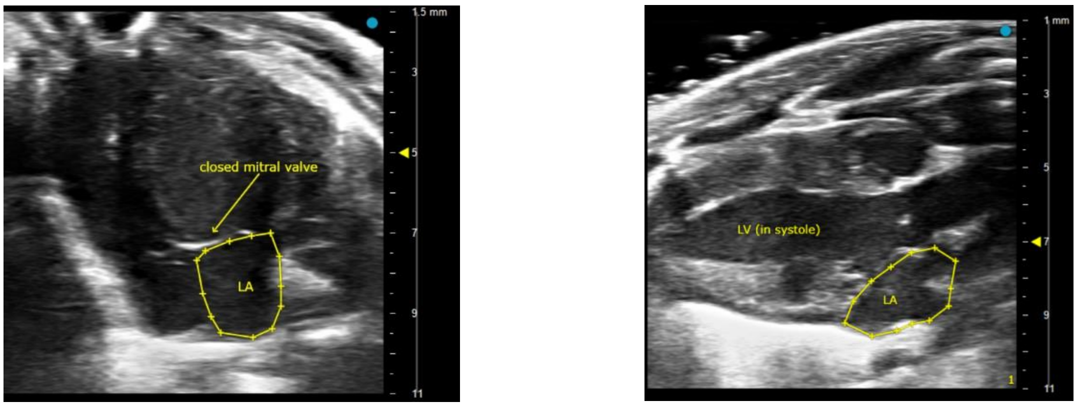

Figure 9: Left atrium area outline in apical 4 chamber view (left) and PSLAX view (right).

Vascular Views

Aortic Arch

Transducer and Animal Positioning:

Tilt the platform to the right (to expose the right side of the animal's chest while in supine position) and elevate the animals head (optional). The aortic arch view is obtained from a modified right parasternal view. The transducer should be parallel to the sternum of the animal at an angle (~15-45 degrees; animal dependent). Rotating the transducer slightly clockwise may be necessary to visualize the entire arch.

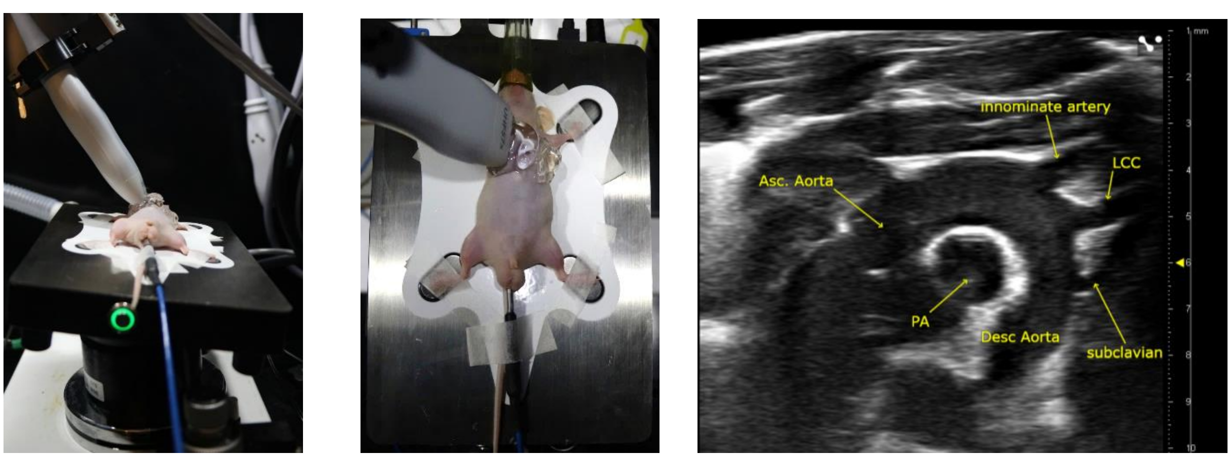

Figure 10: Aortic arch transducer and animal positioning (left/middle) and B-mode image of the aortic arch (right).

Aortic Arch in Color Doppler Mode and PW Doppler

Directional blood flow change is evident using Color Doppler in the aortic arch view. Blood moving into the ascending aorta from the LV is displayed as red (flow moving towards the transducer), then shifts to blue color as the blood flows to the transverse and descending aorta and is moving away from the transducer.

To measure blood flow velocity, place the PW Doppler gate in the region of highest blood flow e.g. where aliasing occurs in Color Doppler. Adjust the Doppler angle in the direction of the blood flow; the Doppler angle should not be greater than 60 degrees; any angle >60 degrees will yield inaccuracies in the blood flow velocity calculation*. An angle >60 is indicated by a color change in the PW Doppler angle. A PW Doppler angle as low as possible (try for <45 degrees) is recommended for the ascending aorta, however if this cannot be achieved, beam steering can be used to reduce the Doppler angle by 15^{o}.

**More information regarding Doppler imaging can be found in VisualSonics "Principles of Doppler Imaging" guide.*

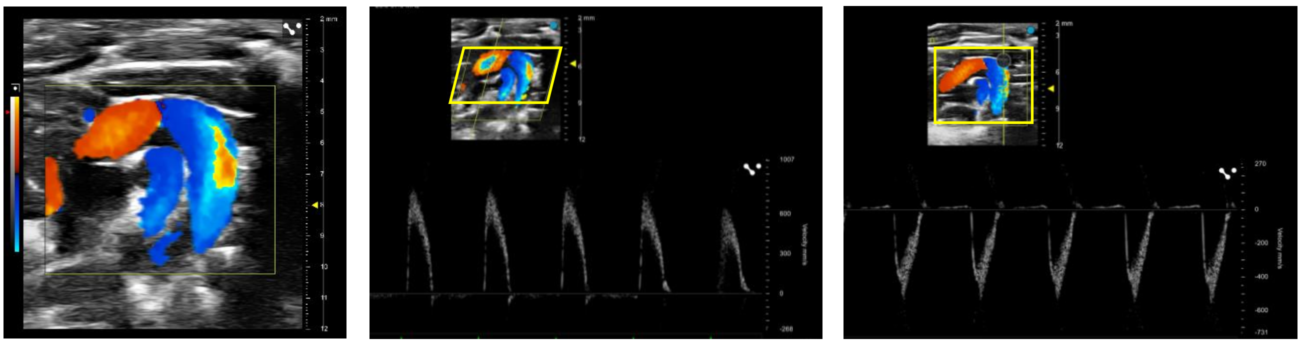

Figure 11: Color Doppler image of aortic arch (left), and PW Doppler of the ascending aorta blood flow velocity (middle) and descending aorta blood flow velocity (right). Beam steering has been implemented for the ascending aorta acquisition (indicated by the shift in color box shape) to reduce the Doppler angle by 15 degrees.

Suprasternal View

The suprasternal view can be used if the aortic arch (ascending aorta) view is unable to give PW Doppler angle less than 60 degrees to yield accurate blood flow velocity.

Transducer and Animal Positioning:

Adjust the platform so that the animals head is elevated greater than 45 degrees and tilt the platform slightly to the left. Place the transducer on the midline of the mouse at the level of the thoracic inlet and angle the base of the probe towards the animal's head by approximately 50 degrees. This position is very similar to the PSLAX view, however the angle between the animal and transducer is very sharp. To obtain aortic PW Doppler Mode, place the Doppler sample volume just above the aortic valve leaflets.

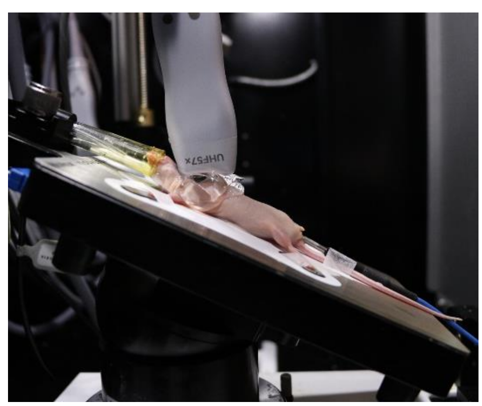

Figure 12: Animal and transducer positioning for the suprasternal view.

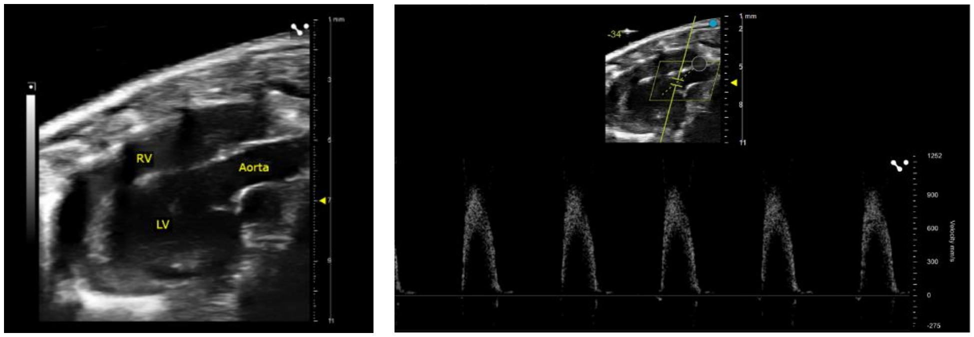

Figure 13: B-mode of ascending aorta (left) and PW Doppler waveform of aortic valve outflow (right) via the suprasternal view.

Pulmonary Artery

Measuring the pulmonary artery (PA) is important in cardiovascular research and clinical practice because it provides valuable information about the right sided heart function and the pulmonary circulation.

Pulmonary artery function can be assessed from either the sagittal or transverse view. The PA measurements and calculations are comparable for each view.

- **Sagittal View:** To obtain the sagittal view start in the PSLAX orientation, then scan slowly towards the head of the animal until you see the pulmonary artery. Color Doppler can also be a useful tool to help identify the PA.

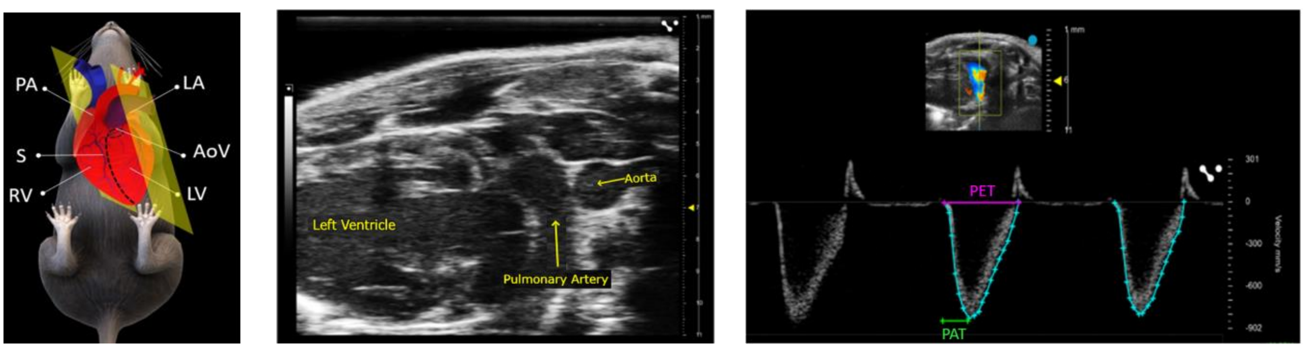

Figure 14: Pulmonary Artery in sagittal view. (Left) Schematic illustration of the imaging plane. (Middle) B-mode image. (Right) PW Doppler waveform showing the measurements to assess PA function.*

- **Transverse View:** To obtain the transverse view start in the SAX orientation, then scan towards the head of the animal until you see the pulmonary artery at the level of the bifurcation. The aorta will also be visible as a circular structure in the transverse plane.

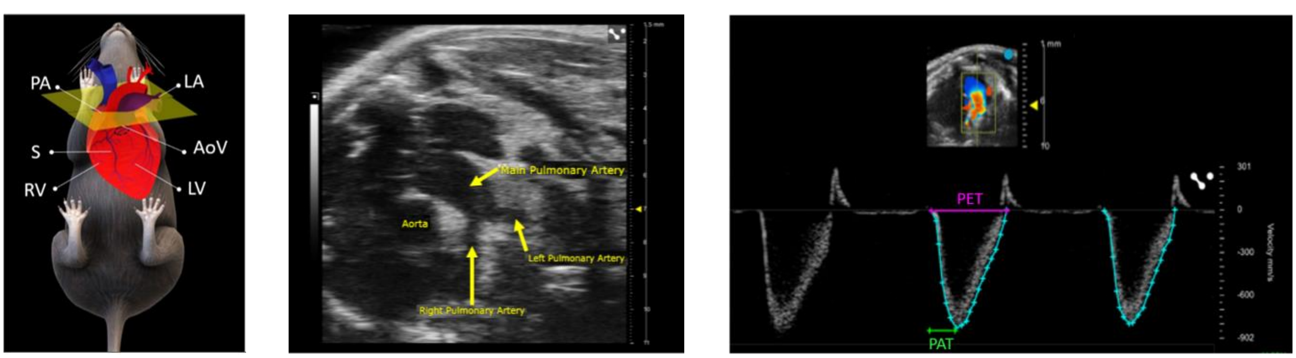

Figure 15: Pulmonary Artery in transverse view. (Left) Schematic illustration of the imaging plane. (Middle) B-mode image. (Right) PW Doppler waveform showing the measurements to assess PA function.*

**Schematic illustrations courtesy of Dr. María Villalba Orero, CNIC, Madrid, Spain.*

The following measurements and calculations can be quantified using the PA imaging views:

- Peak velocity
- Pulmonary Acceleration Time (PAT)
- Pulmonary Ejection Time (PET)
- PAT/PET
- Mean pulmonary artery pressure
- Pulmonary artery CO and SV (must measure diameter of PA in B-mode and VTI in PW Doppler)

Pulmonary Vein

The pulmonary vein can be visualized using the slightly modified PSLAX view for viewing the left atrium. Color Doppler is recommended to confirm the pulmonary vein is visible.

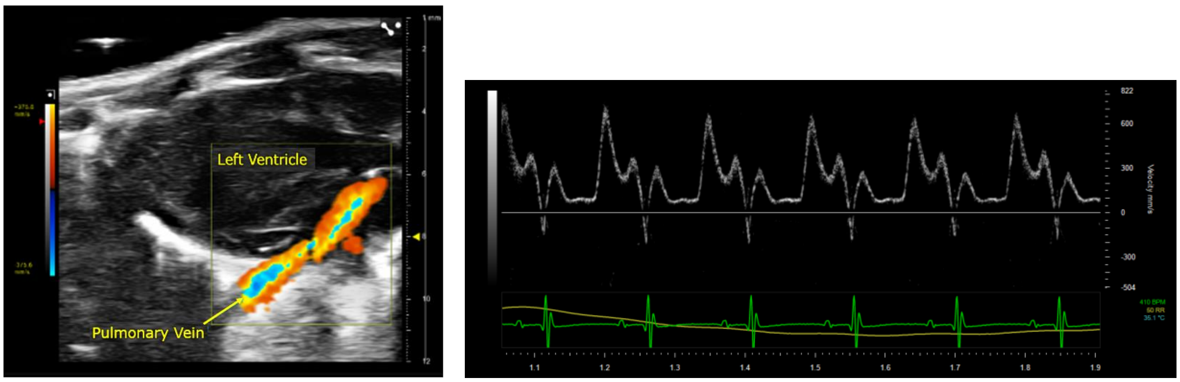

Figure 16: Pulmonary vein flow entering the left atrium shown using Color Doppler (left) and PW Doppler waveform of the PV (right).

Coronary Artery

When in PSLAX view the coronary artery can be visualized using Color Doppler. Beam steering can be implemented to better visualize the blood flow if needed. Once visualized the gate size of the PW Doppler can be reduced to the size of the lumen of the coronary artery to avoid any background interference from the neighboring pulmonary artery blood flow.

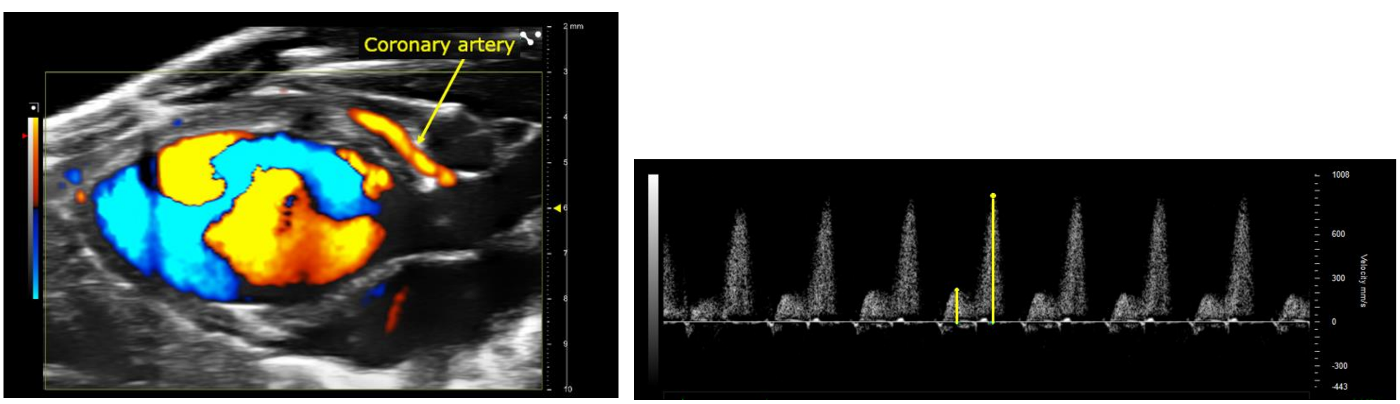

Figure 17: Color EKV of the coronary artery (left) and PW Doppler waveform of the coronary artery (right).

Right Ventricular Assessment

RV Free Wall

Animal and transducer positioning for the right ventricle is similar to the aortic arch view, but the transducer face should be parallel to the animal's sternum. Once positioned, slide the transducer in the x-axis direction to view the RV free wall.

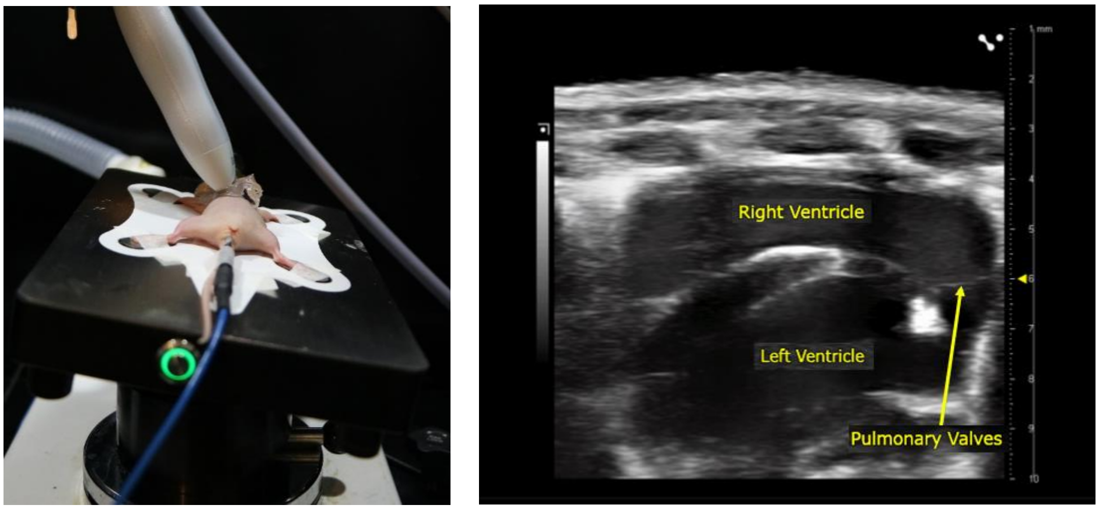

Figure 18: Animal and transducer positioning for RV free wall (left) and representative B-mode image of the RV (right).

The RV wall thickness and internal chamber diameter can be measured using an M-mode image. To standardize, ensure the M-mode sample gate is consistently placed along the RV free wall between animals.

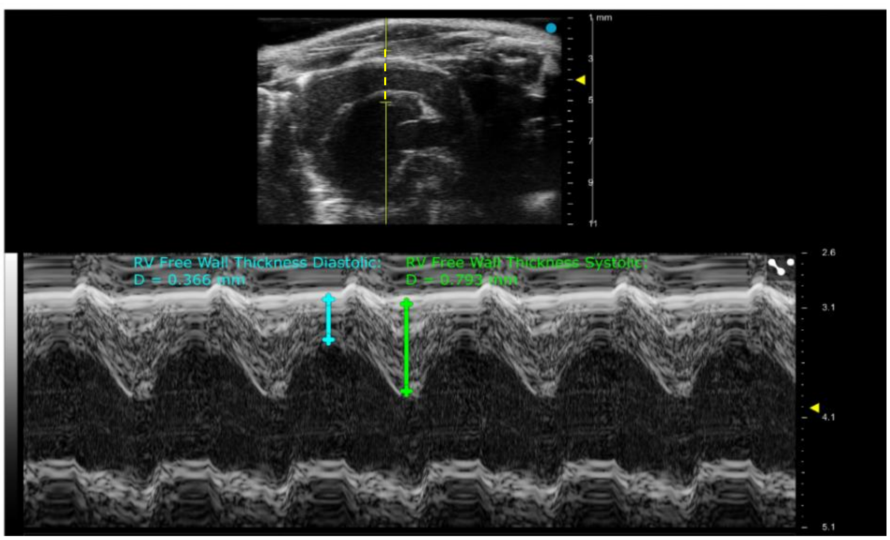

Figure 19: M-mode image showing measurements of RV free wall thickness.

RV SAX: Fractional Area Change (FAC)

RV FAC refers to the percent area change of the right ventricle. It is a measurement used to assess the contractility and systolic function of the right ventricle. Like left ventricular FAC, RV FAC is calculated from area measurements of the SAX view (mid-papillary level) at both end-diastolic and end-systolic phases.

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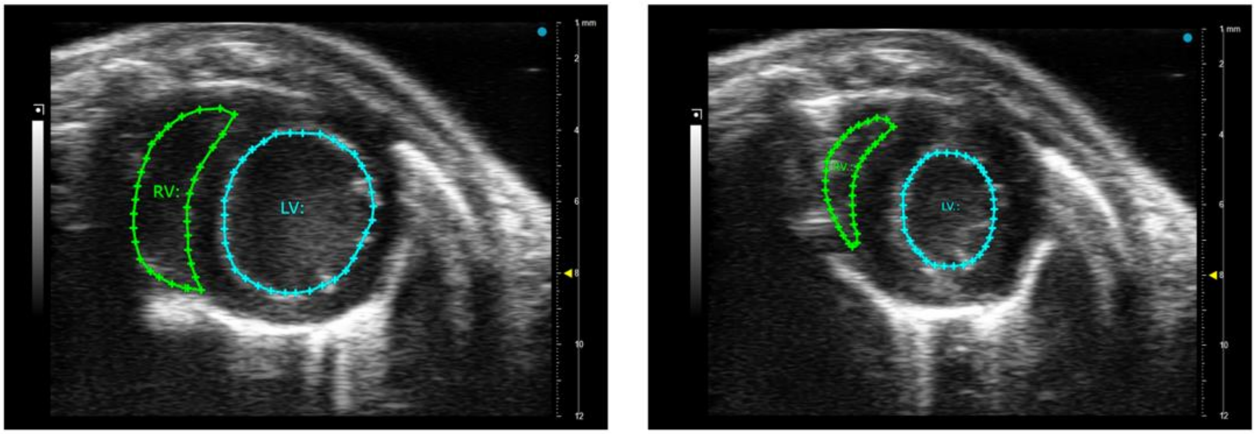

Figure 20: RV endocardial area trace in diastole (left) and systole (right).

Tricuspid Flow

Assessing the blood flow through the tricuspid valve is important for evaluating the function of the right side of the heart. The apical 4 chamber is the standard echocardiographic view used to visualize the RV and tricuspid valve. Use Color Doppler to locate the tricuspid blood flow and place the PW Doppler sample volume within the area of highest blood flow velocity to display the appropriate waveform.

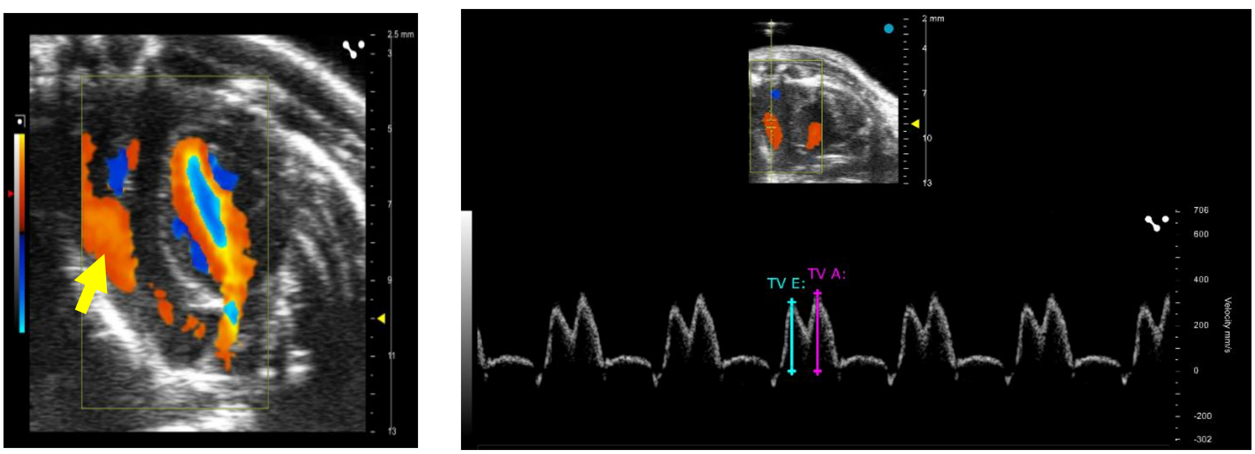

Figure 21: Apical 4 chamber Color Doppler view of the rodent heart with tricuspid flow indicated by arrow (left) and PW Doppler waveform of tricuspid blood flow with E and A peaks indicated.

TAPSE (Tricuspid Annular Plane Systolic Excursion)

TAPSE is a measurement of the longitudinal displacement of the lateral tricuspid annulus towards the apex during contraction of the right ventricle. This measurement is obtained using a combination of the apical 4 chamber view and M-mode. The tricuspid valve should be visible and M-mode sample volume positioned perpendicular to the tricuspid annulus at the point where it meets the RV free wall. The M-mode line should also be directed toward the apex of the heart to ensure longitudinal motion of the tricuspid annulus is accurately captured. In cases where the apex is not in line with the annulus, AM-mode (Angle Manipulation mode) can be used to manipulate the M-mode angle, ensuring proper alignment for TAPSE measurement.

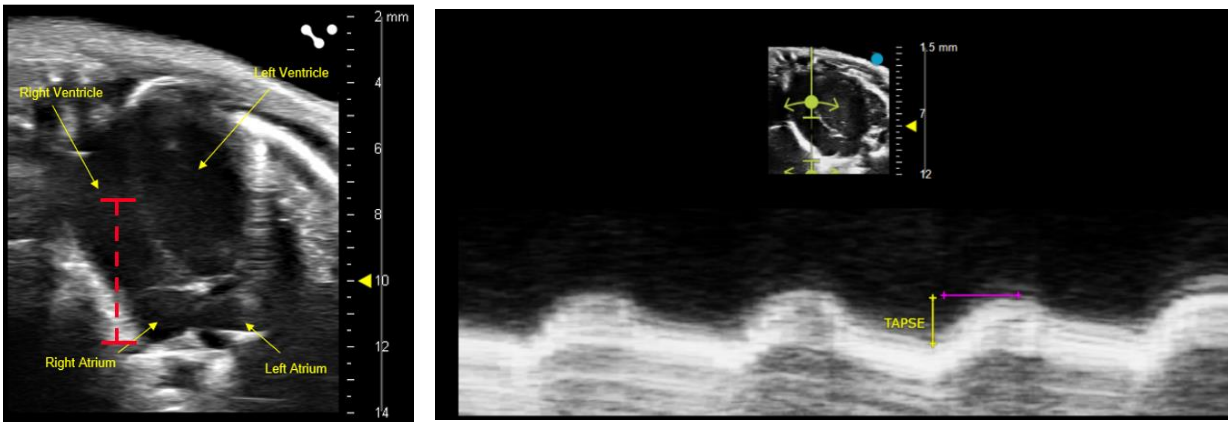

Figure 22: Apical 4 chamber view of the rodent heart showing correct placement of the M-mode line for TAPSE measurement (left) and M-mode image showing TAPSE measurement (right).

RV Strain

Clinically, RV strain has been used to evaluate various cardiopulmonary pathologies such as pulmonary embolism, heart failure, arrhythmogenic RV cardiomyopathy, acute respiratory distress syndrome, aortic coarctation, pulmonary hypertension, congenital heart defects, and even COVID-19 pathologies.

VisualSonics Vevo Strain software can be used for speckle-tracking analysis of the RV free wall.

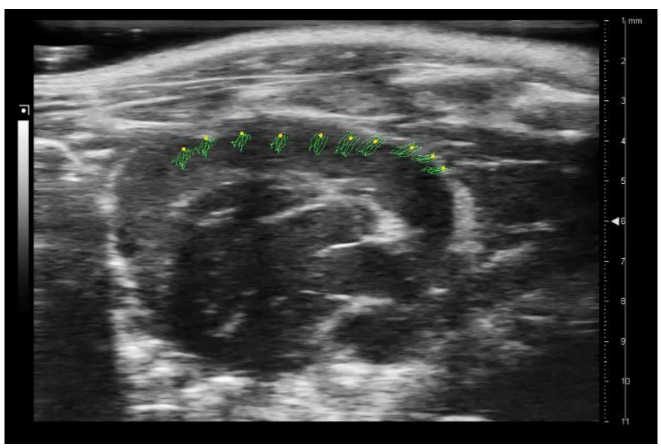

Figure 23: Orbital vector lines placed along the RV free wall for speckle tracking using Vevo Strain software.

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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