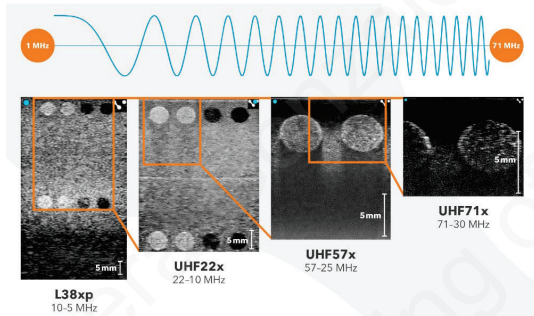


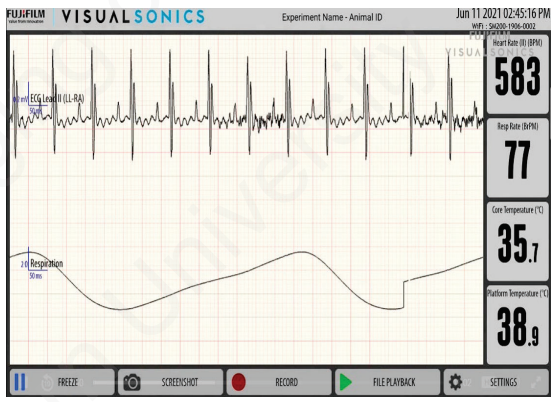
ULTRASOUNDS BASICS

1. Start-up protocol

- **Materials:** Surgical tape, US gel, lubricant, ECG gel, cue tips, tape, gauze
- **Machine start-up**
  - **Power:** switch (only if not used for ~1w), green button (after each use)
  - **Sign in:** Study browser > New study > series > image
  - **Select transducer probes:** (Note: tumor detection only [possible if 1mm, MAYBE 0.5mm])
    - UHF57x: cardiac, kidney (NOT for NLC), 40 MHz center, ~40um axial res, 20 mm depth
    - UHF29x: OK for big mice & rats, also guided injections & high PW [25 MHz C, ~60 um AR, 30 mm D]
    - P10: ok for large animals (resolution tradeoff)



- **Initialize (2x).** Make sure top right blue notch in screen matches the one in the probe...
- **Application:** more controls > general > application...
- **Animal Set-up. Steps**
  1. Plug and turn on animal system, turn on US gel warmer, get anesthesia ready (e.g., iso), place animal in induction chamber (check airflow), start delivery (dose must follow IACUC guidelines)
  2. Prepare ECG gel in platform (at ~39C), cut tape, place mouse once asleep, put eye ointment, tape paws
    - If using ECG leads (e.g., if animal is too big for platform): Select “external” electrode set (vs “pads” when using the integrated platform), place electrodes like AHA code



3. Remove hair in chest (cream or razor), insert lubricated rectal probe and tape to platform
  4. Take gel from warmer, apply a lot focused on one spot to avoid bubbles, then position probe on top
- **Export:** either via USB or shared network > Vevolab > DICOM/png/mp4...
  - **Cleaning:** wipe probe & mouse from US gel, kill the app in the screen (disconnect the black connection behind platform), close series in Vevo (automatic when logging out or starting a new series), turn green button OFF.

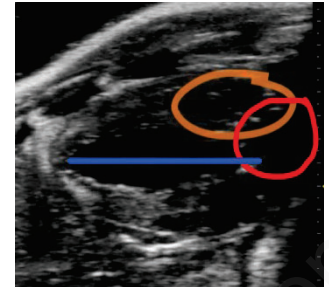
**2. Cardiac Imaging**

- Use 57x (notch towards wall!) HR ~400-500 bpm (best >450bpm), RR ~50-60bpm min

**Parasternal Long Axis (PSLAX)**

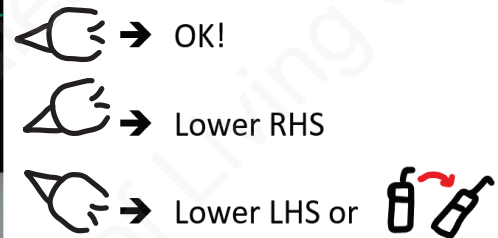
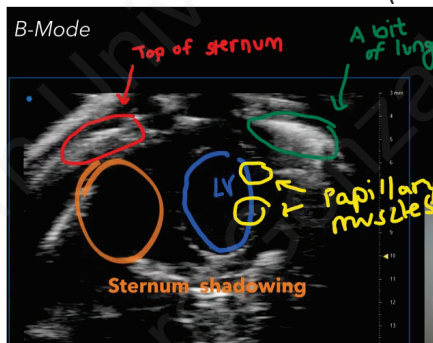


- **Steps:**
  - Position probe: flip metal up, align notch, align lines in holes, lock metal to avoid rotation (fully lock)
  - **Tip:** position blue notch at the right in screen so R=head, L=tail (match probe, notch should be facing sagittal)
  - Transducer and platform need to be tilted to avoid rib/lung artifacts & account for the position of the heart within the animal. The heart sits diagonally in the chest with apex facing upwards (see image).
  - Tilt table by moving the lever of the table, then tilting left, then towards the feet. Point transducer towards center of the animal & towards yourself; then rotate 45 counterclockwise
  - Apply warm gel to animal chest & lower transducer with wheel thing until making contact. Gross adjustment done manually, finer adjustments with XY micromanipulator
- **PSLAX characteristics:**
  - Aorta & apex on same plane (blue line)- prevents SAX overestimation
  - Visible LV (football shape) & RVOT (orange) & open aortic root (red)
  - Tip: ensure separation between LV & LA, ideally find capillary muscles
- **Image optimization:** decrease width, depth, depth offset; align focal zone with posterior wall. Once satisfied, "Save clip" or "cine store"

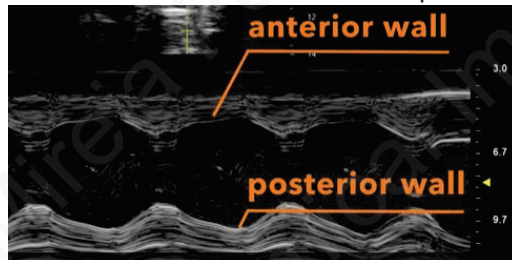
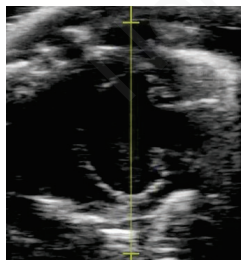


**Parasternal Short Axis (PSSAX)**

- From LA: turn transducer 90 clockwise, perpendicular to heart axes. Focal zone still aligned with posterior wall
- **B-mode PSSAX:** RV hidden behind sternum, 2 papillary muscles at 2 & 4 o'clock
  - **Image optimization:** Reduce width to increase frame rate (narrow). Then "save clip" or "cine store"



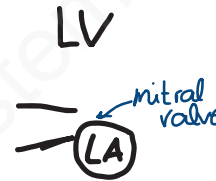
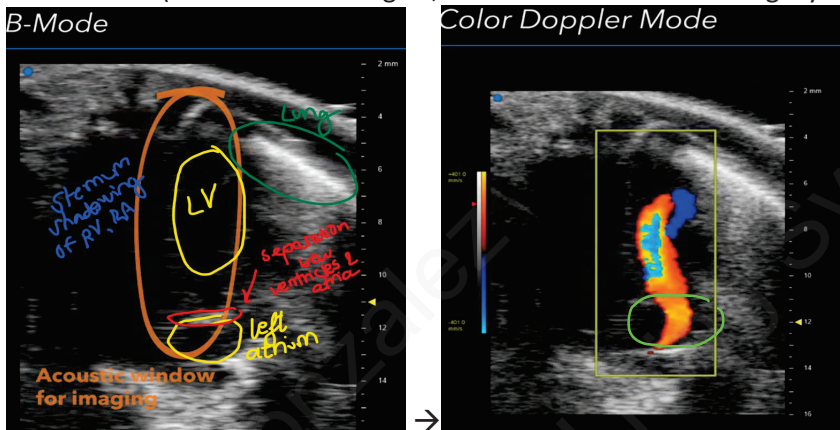
- **M-mode PSSAX:** Position yellow line at widest portion of LV, parallel to capillary muscles. Ensure horizontal edges are outside LV (left of capillaries, max distance to cover LV). Press "Start" or "Update" to show heart motion over time. Ensure A&P wall can be seen before "save clip" or "cine store" (saves <=5s). Can analyze EF%!



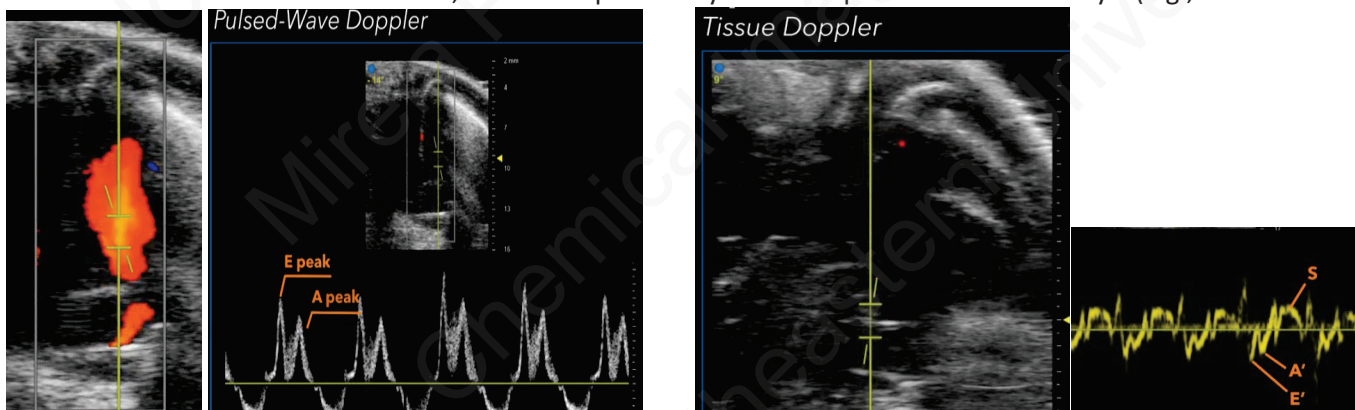
**Apical four-chamber (most challenging)** use 29x probe if we cannot get enough mitral flow signal

- Loosen lever to tilt platform backward, aim transducer towards right shoulder/ear, then lower until contact with gel. Check for the bright line to ensure it's the apical orientation. Then use the X/Y microregulator
- Press "Color": Red = blood coming towards the transducer; Blue = blood going away from the transducer
  - Adjust window to see "shooting into ventricle", adjust velocity scale with Pulse Repetition Frequency (PRF) until some aliasing is observed (only 1 area!!). Make sure the animal is not being crushed.

- **Aliasing:** visual indicator of point of highest velocity, needs to be adjusted depending on pathology/area! If it wraps around too many times, we don't know well the limit. GOAL: place gate in yellow area, angle parallel to blue (blue flow is leaving LV, red is from atria contracting... yellow is highest velocity point!)



- **PW doppler (Pulsed-wave):** to sample velocity
  - Move lines towards the center of aliasing, open sample volume gate width to max (with fingers), then correct PW angle to match direction of flow with direction from LA to LV... Then press START. PW doppler mode will show velocity over time
  - Positive velocity = towards transducer (towards ventricle); Negative velocity = going away from transducer
  - If only 1 peak, use microregulators to aim for better definition of peaks... if they are too close to each other, tissue doppler would help obtain E' & A' because we may not be able to get the E/A...
- **Apical 4C to look at mitral flow:** E (early = ventricle relaxes, passive filling) & A (atrial = atria filling ventricle) peaks can be confirmed comparing with ECG - happening before & after (A) the P wave. **Analysis:** E, A, E/A (= how much atria works to fill ventricle), myocardial performance index (Tei index). "SAVE" for later
- **Tissue doppler (TD):** to evaluate tissue velocity, commonly for small displacement but high-speed tissues (e.g. valve motion) because they'd not appear in B-mode or M-mode.... Can also serve for diastolic function (E', A')
  - Press TD to obtain a line like PWV, place volume gate at mitral annulus (where septal wall reaches mitral valve) & make it as wide as possible (max); then correct angle to point directly towards the apex. We want to sample mitral valve where it meets the septal wall. **Differences with PWV:** Negative peaks = diastole → Dim peak = isovolumetric relaxation; Two strong peaks = E' & A' (prime because we're in tissue); Dim peak again = isovolumetric contraction ... Positive peaks = systole (broad positive peak)...
  - These can be confirmed with the ECG → P wave = atrial contraction = sit between E' and A'
- Sometimes, 4 peaks: isovolumetric times and E' & A' (we will only be able to identify the E', A' with the ECG)
- **Analysis:** E', A', E'/A', E/E' (comparison of pulse doppler to tissue doppler). Once satisfied, press "save clip"
  - **Note:** if ECG = too low, the E' & A' peaks may be more spread out than usually... (e.g., ~85 ms for 290 bpm)



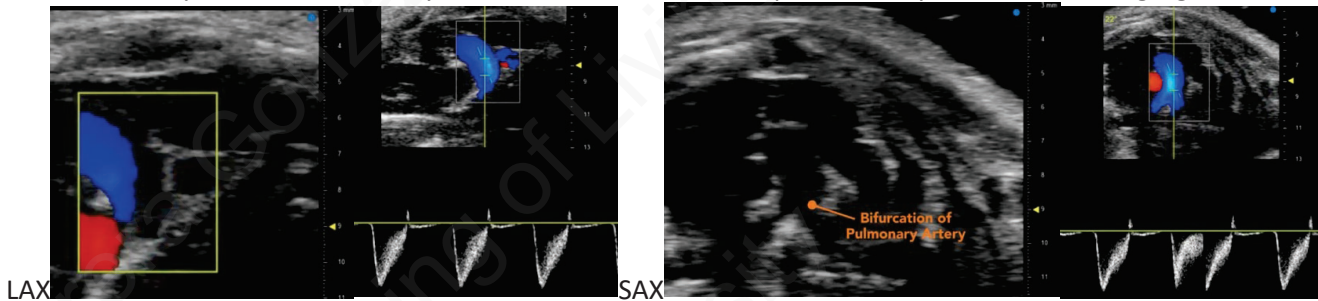
**3. Intermediate cardiac imaging**

**Pulmonary artery (long axis)**

- Start with PSLAX, then rotate probe more clockwise to be more in line with the body. Pulmonary artery (PA) will appear in right side of screen, next to RV. Use micromanipulators to center the PA
  - Save image to later analyze diameter PA, diameter RV output tract (RVOT = where it flows into artery)
- Use CD to optimize direction of flow: adjust window size & PRF/frequency until some aliasing (area of fastest flow), turn PWD on, place box on area of interest & adjust CD angle to match blood flow direction. Click start to see PWD signal. Save if satisfied, to later measure: PA acceleration time, peak velocity of PA flow, PA regurgitation.

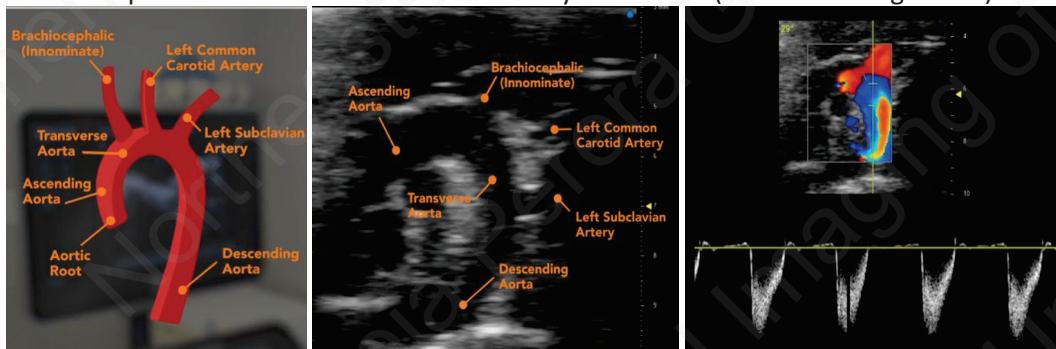
**Pulmonary artery (short axis):** From LAX, rotate probe 90 clockwise. Move platform towards user (probe moves towards animal head), use PA bifurcation as anatomical landmark... Save B-mode for diameter measurements.

- Turn CD on, place window on top of the bifurcation, adjust PWD on aliasing region, and adjust the angle. Click start when ready. Save for later analysis of: PA acceleration time, peak velocity of PA flow, PA regurgitation.



**Aortic arch**

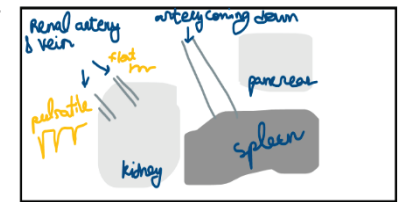
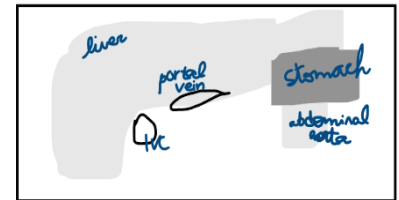
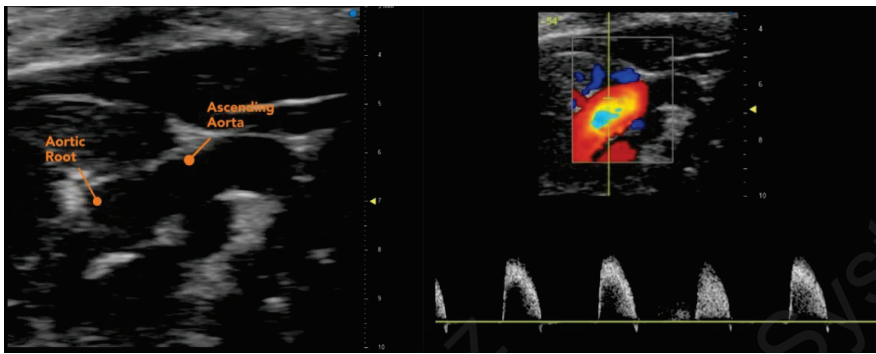
- Platform: lower bottom right corner (almost mirror from PSLAX). Move transducer to angle it coming from animal right shoulder towards midline. Then lower probe with micromanipulators until you see aortic arch in the screen
- **Applications:** TAC models to visualize suture between innominate and LCCA, atherosclerotic models to visualize plaques (high color encoded regions in the screen)... Turn CD on see flow in aortic arch, place it distal to a ligation to observe the effects. Press start to observe signal evolution. If peak velocity is not captured within the scale for the TAC model, use a lower frequency transducer to capture full range of signal. Click save when satisfied
- To move towards RV view, push away from you. RV will be covered by sternum (use R/L microregulators to see it). Some probe counterclockwise rotation may be needed (towards the right wall)



- **Aortic arch:** PWV at proximal & distal + B-mode to calculate distance (d=5-7 mm) with same image settings!
  - Another (better) way: from abdominal aorta. Switch to bigger probe (29x?) for longer distance, bring probe at an angle... start from beginning of abdominal aorta, end before bifurcation (d~20 mm!)
  - Going to aortic arch again (with 29x), flow will be A LOT because lower frequencies have a lot of sensitivity! (not much is lost from bone etc)... but make sure we can see distances & peaks in PWV!!
    - Could also do Tissue D (at proximal & distal), then B-mode to calculate the distance in Vevo
  - At the beginning we can find LVOT = closure of aortic valve (as a flip coming down in PWV readings)

**Ascending aorta: Applications** in aortic insufficiency models, aortic valve prolapse...

- Rotate probe counterclockwise to see AA in the middle, SAVE. Turn CD ON, adjust PWD & angle, START



**Abdomen imaging**

- Move to heart 1<sup>st</sup>, start going down until you see liver, then duodenum and kidney...
  - When focusing on kidney, turn respiratory gating ON...
  - To run 3D, turn gating OFF, then adjust 3D, then turn gating ON again!
- Needs some pressure! It will tell us if it's a vein (will collapse under pressure)
  - Adreno gland will be at the top of the kidney, will look like a horse show...
  - Bladder looks super dark, ribcage looks like stripes

**3D imaging**

- From kidney: 2<sup>nd</sup> icon bottom left, we can turn color/B-mode off, change B&C...
  - Top left 3D region: trace with left clicks, close contour with right click. Draw volume for all slices, then explore rendering options (e.g., sculpting volume)
- On cardiac, need to start with 4D: 3<sup>rd</sup> icon > CLOSED contour ("open" only for irregular tumor delineation) > draw every 6<sup>th</sup> or so, and write down distance of the slice where capillaries appear (where we'll stop!) so that we can use the same distance for ED. Repeat this for different timepoints as software requires

**4D imaging = 3D with triggered**

- Not as important to do everything perfect in terms of animal positioning (capillaries, 2 & 4 o'clock..) because we'll get the measurements from the entire volume!
- From SAX, rotate probe to be perpendicular to motor. Then release actuator and turn it left (down to me on the closer side as well). Don't touch skin with probe to avoid rippling effect! Leave 3-4 mm to be safe
- Start & stop: adjust to clear apex, then reach pulmonary (in blue with CD, has bifurcation)
- Change step size as needed, collapse 3D menu and expand 4D...

**Carotid artery (CA)**

- Have probe perpendicular and look for salivary glands & vessels (locate them in the center of the screen).
- Rotate probe 90 so it's in line with the body of the mouse and use R/L microregulators to find carotid bifurcation.
- **Analysis:** peak velocity (negative), resistive index (d{peak, end of diastole}) & pulsatility, PSV, EDV = end-diastolic velocity... from tracing, used to derive areas

**Cardiovascular (CB)**

- **PWV of aorta** (any view)... 4D advantage: no assumptions considered as in SA/LA.
  - Come from the back, start with cardiac (57x) then switch to back
  - From LA → turn probe 90 for 4D: move motor to cover start (apex) and end (base)
  - Increase frame rate by narrowing windows, turn EKG for full volume calculations, leave 3-4 mm distance at the top so probe isn't touching skin directly (could create artifacts)
- **Diastolic function** = 4CH view: tilt head back, swing probe towards front wall, push mouse all the way forward
- **Mitral flow:** go higher = farther from valve to try and distinguish two peaks
  - Analysis: MV I, MV A, AET (aortic ejection time), Isovolumetric (IV-RT = end of aortic ejection to beginning of next curves, before next peak; IV-CT = contraction time from end of A wave to AET)
  - Automatically-derived: E ratio, MPI... can be exported in CSV file!
- **Strain analysis:** commonly from apex to 80% base, common to measure ability to expand

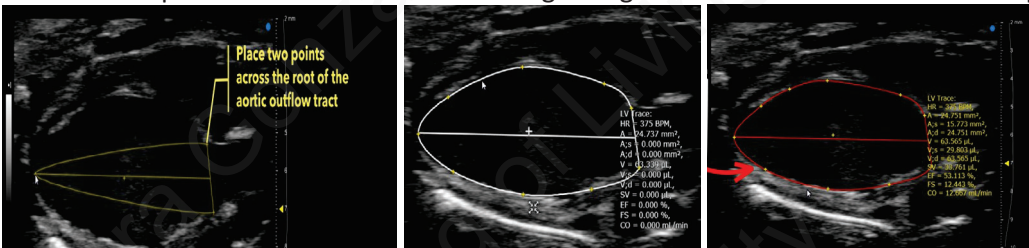
4. Basic Cardiac Analysis

• **VEVO SOFTWARE**

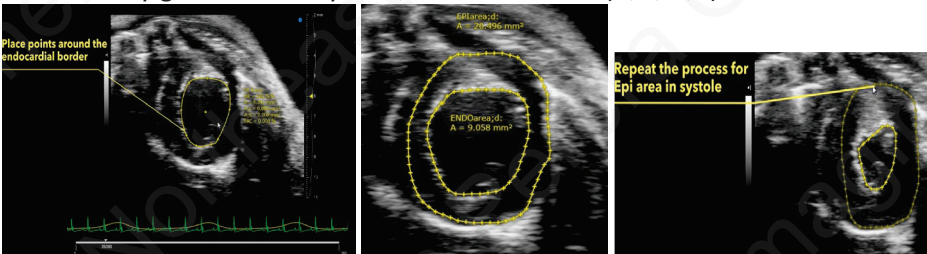
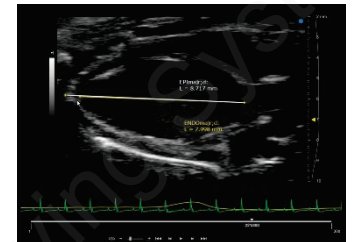
- For sequence development: we can change loops, pulses...
- Import ("COPY FROM") > Drive 10 > ... files will appear as a checkbox
- Question mark (Right) to check license type etc

• **LA B-mode LV trace:** Can be started at either full systole or full diastole (no difference)

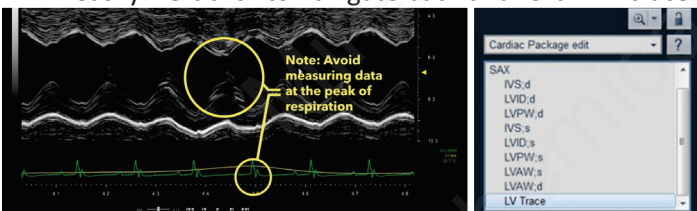
- Place 2 points across the root of the aortic outflow tract, and a 3<sup>rd</sup> to anchor at the apex, then adjust the width. Then place a few points across the anterior and posterior walls. Once satisfied, right click to fix the trace and observe the results. Analysis method uses ASE consensus rules
- Green/red will indicate smallest/largest trace, respectively
- Data presented is based on the average... Right click on the info to see more options!



- **Auto LV analysis (only on LAX):** Find fullest diastole, select "AutoLV". It will look at the starting point and look at the library of existing traces... and generate the automatic LV tracing
- **LA B-mode endo-epi:** ENDOmajor/EPImajor- Requires a single LA to place the ENDOmajr & EPImajr (same origin, different ending point)... need to be repeated until next systole
- **SA LV trace:** Place points around the endocardial border. Most researchers include papillary muscles in them- important: be consistent. Repeat the process for full systole
- **SA B-mode endo-epi:** Similar but ENDOarea; d/EPIarea; D → Trace area around endo/epicardium border. Repeat process for end systole. TIP: press "Esc" to cancel measurement. Be consistent about capillary muscles & interventricular border- most important!
- Only gives area at systole, area at diastole (A; s, A;d) and Fractional Area Change (FAC)...

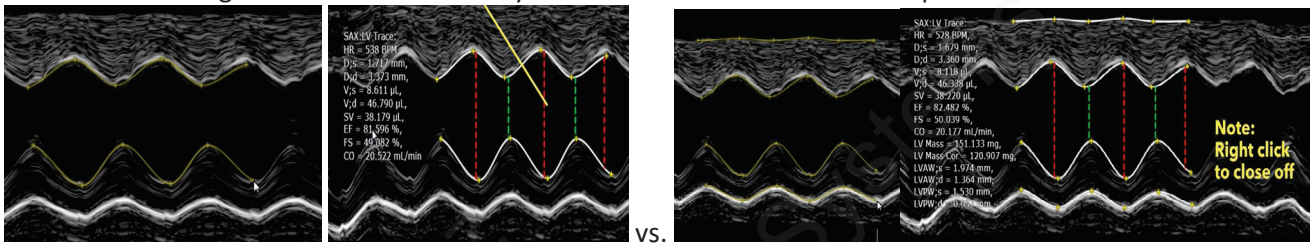


- **SA M-mode LV trace:** Load M-mode image, play with the frame speed as needed. Inspiration effects can be seen easily. Left click to navigate back and forth. LV trace can be used in short axes

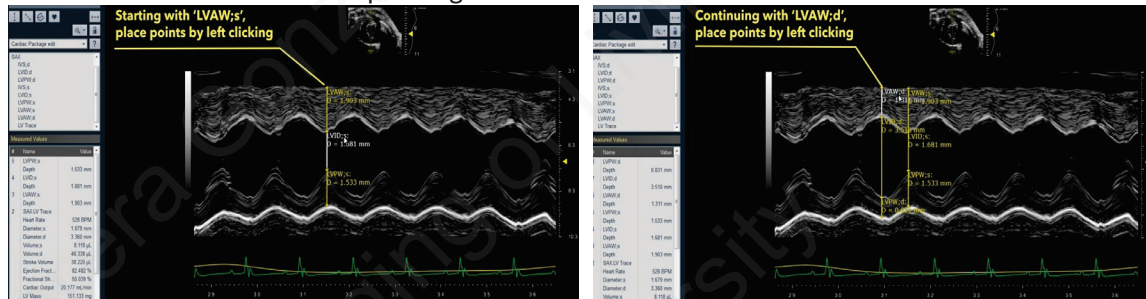


- Icon series: can control B&C, select cardiac package > short axes > LV trace (also 3<sup>rd</sup> icon bottom left SA)
  - Turn on image guides: >400 bpm for 60-80 % EF... at 300bpm 50% EF should be OK
  - Run LV trace in SA first, then in LA (we'll want to find peak ED, then do ES).
- Place points starting at the anterior cardiac border and collect at full systole and full diastole along the border
- Left click to place points, right click to close points (no need to align between anterior and posterior walls)
- If only interested in interior measurements, right click to stop trace & obtain ONLY functional measurements

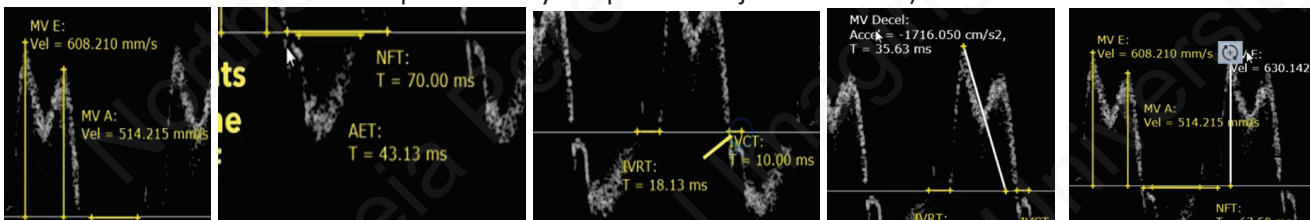
- To also measure epicardium: place points in borders that **DO** match the interior walls
  - Green /red will indicate systolic /diastolic areas. Least amount of motion = anterior epicardium
  - Right click to close off and you'll be able to obtain additional parameters



- SA M-mode string method** (If the LV trace is not available): to measure anterior & posterior wall via LV measurements can be acquired with LVAW; s for systole, LVAW; d for diastole. Left click for each wall to run these measurements. NOTE: report is generated in demand



- REPORT TAB:** When clicking on a measurement, particular image thumbnail appears on the right side. Multiple measurements are aggregated when repeated and mean/std is calculated. Inherently, the LV trace method is an average (so the more points, the better the accuracy of the measurement)
- Apical 4CX Color Doppler:** Almost purely qualitative guide. Color doppler can augment imaging by providing a guide for placement of PV Doppler sample volume gate
- PW Doppler of Mitral Valve (to look at MV flow):** Select E and A, AET: area ejection time where flow is being ejected
  - Leading edge/trailing edge **MUST BE** consistent! Common: leading to trailing
- NFT: non flow time** (convention... point where E peak starts to go up)
- Myocardium performance index given by NFT & AET; Other parameters are IVRT/IVCT
- MV deceleration: follow slope of the peak
- Many researchers place 3 measurements for averaging (easier to just click on the blue thing with the + and rotating arrows which allow to duplicate easily the parameter just measured)

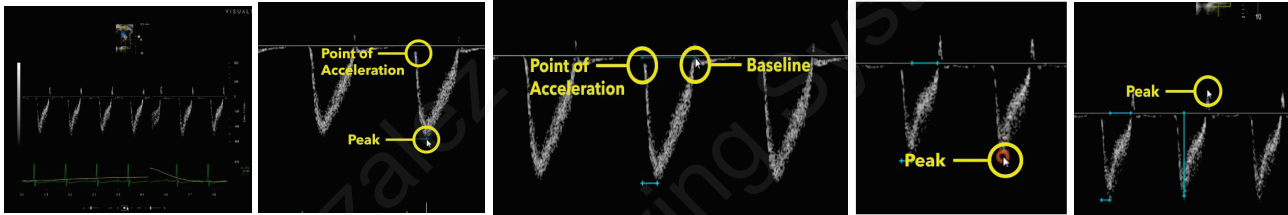


Tips: Use mouse scroll wheel to navigate through screen

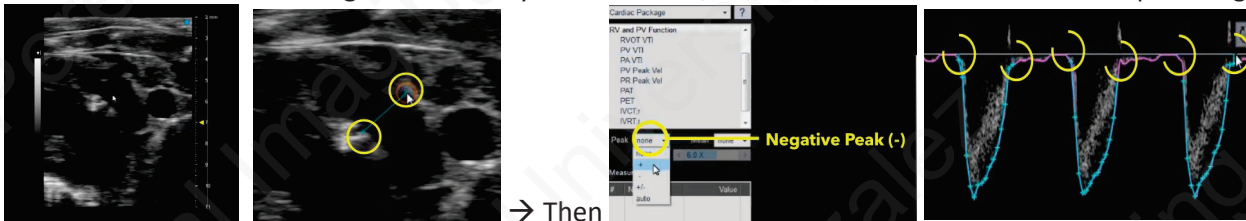
5. Intermediate Cardiac Analysis

- **Pulmonary artery, RVOT flow and diameter:** From data already loaded into VEVO lab, select pulmonary axis PWD from SAX. Avoid areas where mouse is inspiring by scrolling right/left

- **PA acceleration time (PAAT):** start from acceleration point to negative peak
- **PA ejection time (PET):** start at acceleration, end at baseline
- **Peak PA velocity (PVPV):** start at highest peak
- **Peak PA regurgitation (PRPV):** one click at positive peak



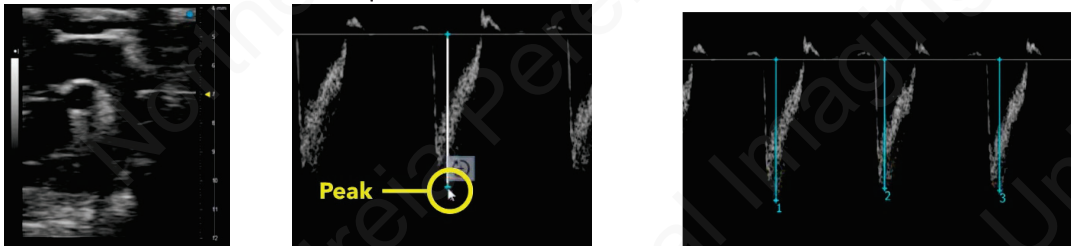
- Results will be populated under the cardiac package. If interested in RV function, we may want to take a picture at the level of the pulmonary valve or RVOT to see valve in B-mode. B-mode & PD will be required
  - If closer to RV, we'd need to use RVOT measurements in the panel (then RV VTI). If pulmonary valve is seen easily, use PV diam and select both sides of the vessel
- Find PWD sample volume taken at similar location. Avoid inspiration & select PV VTI (because of PV diam).
- Select **negative** peak because trace is going down from baseline. Sensitivity can be changed but should be consistent throughout the study. Select PV VTI, and left click at start & end of each peak.. right click to finish



- **Analysis:** VTI, mean/peak velocity and gradient, as well as cycles..
- In the report: RV & PV function menu, calculations now include PV CO, PAT/PET...

- **Aortic arch, diameter, and flow:** Start with B-mode aortic arch image

- **Applications:** view banding in TAC model (can be measured with line/length measurement)
- **Descending Aorta velocity** (select the peak). The report will be automatically populated with the measurements acquired and will include the mean and std.



- **LV CO** (needs 1 B-mode, 1 PWD): Start with Aortic Valve Flow (AoV flow). If we are at the level of the valve, use AoV Diam, and select both sides of the vessel
- **Ascending Aorta Velocity Time interval (AoV VTI):** Select direction of the peaks, start at end of the peak. New area in report for calculations of AoV CO and SV.

