PERFORMING PULSE WAVE VELOCITY MEASUREMENTS USING THE DOPPLER FLOW VELOCITY SYSTEM

Procedure:

The purpose of this document is to provide Doppler Flow Velocity System (DFVS) users with an overview of how to perform Pulse Wave Velocity (PWV) measurements.

Pulse Wave Velocity is used as in indices for arterial stiffness, which can for example be determined in the aorta. Increased arterial stiffness is considered to be an early biomarker of atherosclerotic and structural changes which may occur due to progression of a cardiovascular disease, exposure to a specific compound or drug, or as a result of aging.

PWV is most accurately assessed with simultaneous Doppler velocity spectrogram acquisitions at two different locations. This requires that two probes, having the same output frequency, be placed at a given distance from one another along the same vessel. The example shown here will be from the aorta of a mouse, with velocity spectrogram acquisition completed at the aortic arch and the abdominal aorta. If two probes are not available for simultaneous acquisition, sequential acquisition may be completed with the Doppler velocity spectrogram taken at either the aortic arch and then the abdominal aorta, or vice versa. During sequential acquisition, it is crucial that the ECG signal be recorded to allow the spectrograms to be aligned during analysis.

For additional information on how to operate the DFVS please refer to the Doppler Signal Processing Workstation User Manual.

Suggested Equipment:

In addition to the DFVS, we suggest that you confirm the availability of the following equipment:

- Two (2) probes having the same frequency, i.e. for mice two (2) 20MHz probes, for rats two (2) 10MHz probes if necessary based on size of rat.
- One (1) micro-positioner. * *two (2) micro-positioners may be required if prolonged PWV measurements are required to follow an acute response*
- Calipers or ruler to measure distance between the tips of the probes

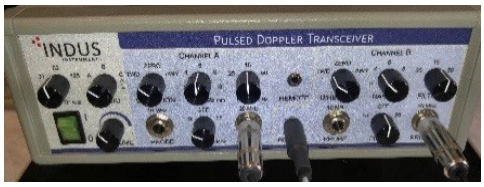
Procedure:

Hardware and Software Set-up for Simultaneous PWV Measurement

To most accurately assess PWV two (2) probes having the same frequency should be used; in this example 20MHz probes will be used to assess the PWV in the aorta of a mouse.

1. Connect the two (2) probes into the front of the transceiver box, one into each Channel (A & B).

   Ensure that no other probes are connected to the transceiver box. The Remote depth control may be plugged into either Channel, whichever probe will be used first.

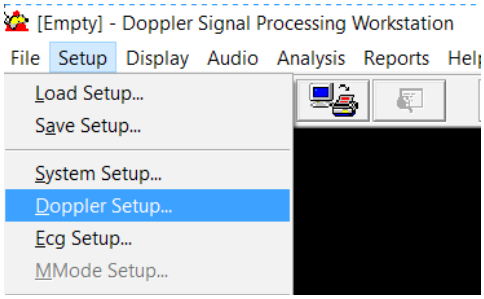


2. Connect one of the BNC connectors to each of the In-Phase connectors for Channel A and B on the back of the transceiver box.

   Ensure the same color connectors are connected to Ch 1 and Ch2 on the front of the digitizer box.

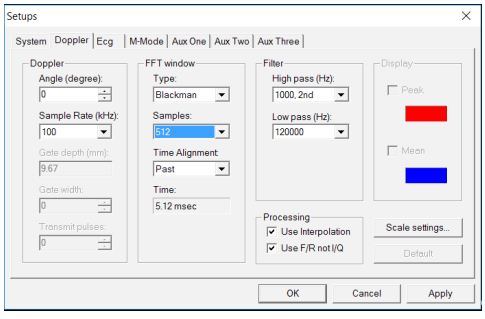
3. Within the Doppler Signal Processing Workstation, select Setup.

   Choose Doppler Setup.



4. Ensure the Processing check-box which states Use F/R not I/Q is checked.

   Choose Apply.



5. Check the system set up by beginning to acquire a spectrogram.

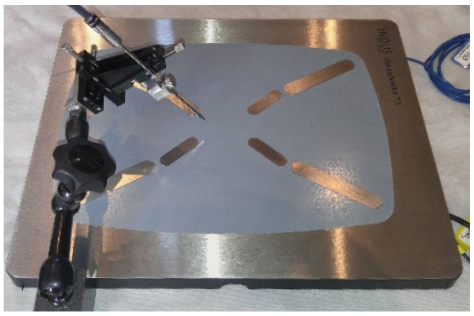
   Tap the end of one probe with a wet finger and observe the resulting signal, i.e. positive velocity scale only. Tap the end of the other probe with a wet finger and observe that the resulting signal appears in the opposite direction to the first probe, i.e. negative velocity scale only.

   Note: to hear the signal for either probe, confirm that the Radio knob on the front of the transceiver is turned to the appropriate channel.

Animal Set-up and Measurement of Simultaneous Doppler Flow Velocity Spectrograms

1. Set up the animal on the heated surgical platform as you normally would for Doppler flow velocity assessment.

2. Secure the micro-positioner to the heated surgical platform.

   Typically, the micro-positioner is used to hold the probe used to obtain the Doppler flow velocity spectrogram from the abdominal aorta.

   Please note: Make sure that the probe on the abdominal site is perpendicular to the aorta. This will ensure the most accurate distance measurement made in Step 7. Unlike typical velocity measurements, where the probe should be at a very shallow angle to the blood flow, timing of the pulse is key in this case and velocity is not calculated.

   If a second micro-positioner is used, it will hold the probe used to obtain the spectrogram from the aortic arch.

3. Obtain the Doppler flow velocity spectrogram on the abdominal aorta.

   Ensure the Remote depth control is plugged into the appropriate channel AND the Radio button is turned to the appropriate channel to hear the Doppler signal.

   Note: try not to have anything other than the probe touching the animal to obtain this spectrogram.

4. Prior to obtaining the Doppler flow velocity spectrogram on the aortic arch the Remote depth control will need to be moved to the second channel.

   Prior to unplugging the Remote, **ensure that the Channel Range on the front of the transceiver box matches that on the Remote.**

   Plug the Remote into the Channel being used to obtain the second spectrogram.

5. Obtain the Doppler flow velocity spectrogram on the **aortic arch.**

   Ensure the Radio button is turned to the appropriate channel to hear the Doppler signal.

   Confirm that the previously obtained spectrogram on the abdominal aorta is still optimized.

   Note: try not to have anything other than the probe touch the animal to obtain the spectrogram.

6. **Save** the desired spectrogram once the signal from both the abdominal aorta and aortic arch are optimized.

   Note: In this measurement, the time axis is most important, so the start of flow in each spectrogram must be clearly visible.

7. Prior to removing either probe from the animal, **use calipers (or ruler) to measure the distance from probe tip to probe tip.** Take note of this Distance measurement, as it will be needed to calculate the PWV.

NOTE: Make sure you restore the connections described in step 2 and step 4 to switch from dual probe (PWV) mode to single probe (regular) mode.

Analysis of Simultaneous Doppler Flow Velocity Spectrograms and Calculation of PWV

If required please consult the Doppler Flow Velocity System Analysis Overview Guide for additional information on the detailed steps required to work through the steps of the Analysis Control Window.

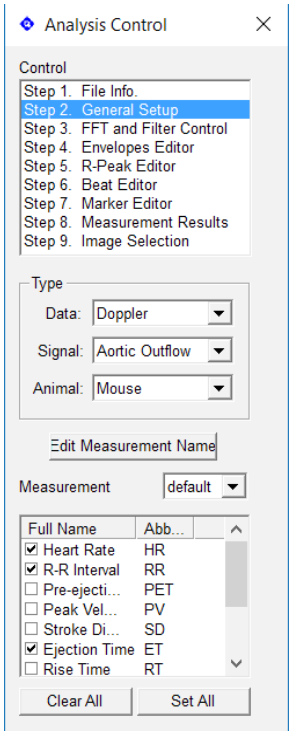
1. Open the desired spectrogram into the Doppler Signal Processing Workstation.

2. In **Step 2** of the Analysis Control Window, select the Aortic Outflow from the Signal drop-down list.

   Ensure that only the Heart Rate, R-R Interval, and Ejection Time are selected in the Measurement list.

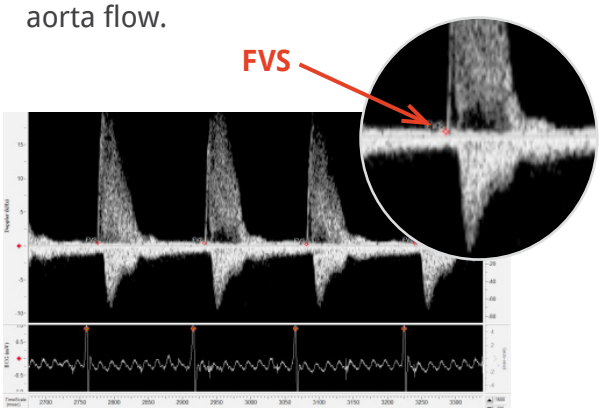
   Note: There is an option under the Signal dropdown list for Pulse Wave Velocity, these measurements are for spectrograms acquired sequentially, first on the aortic arch second on the abdominal aorta.

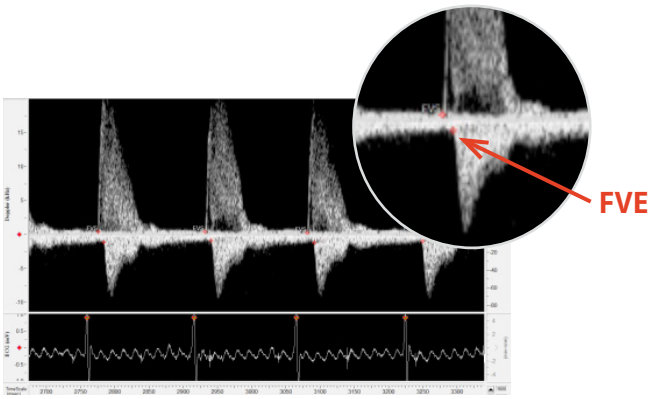
   The more accurate way to measure PWV is simultaneously as described in this example.



3. In **Step 7** of the Analysis Control Window, start with the **FVS (Flow Velocity Start)** Marker, and place this at the start of the aortic arch flow.

   Next, with the **FVE (Flow Velocity End)** Marker, place this at the start of the abdominal aorta flow.





4. In **Step 8** of the Analysis Control Window, the Ejection Time Measurement Result will be the Time measurement necessary to calculate the PWV.

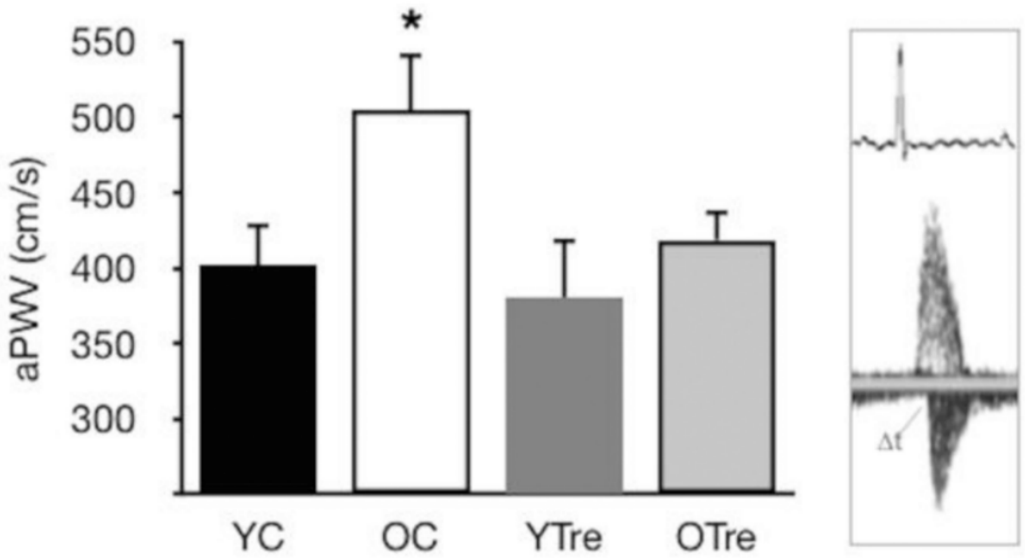
5. Calculate the PWV using the Distance and Time measurements noted previously.

$$PWV\ (m/s) = \frac{Distance\ (mm)}{Time\ (ms)}$$

Examples from Literature:

Mitochondrial Quality Control and Age-Associated Arterial Stiffening (LaRocca, TJ, et al. Exp Gerontol. 2014)

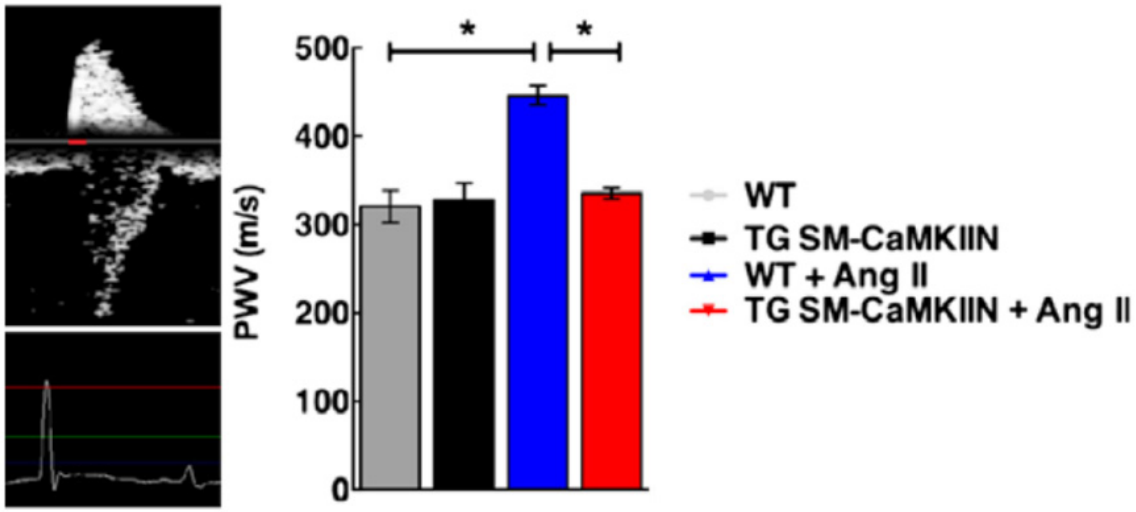
In this paper, LaRocca et al. undertook a study to determine the underlying mechanism of age related stiffening of elastic arteries. Trehalose was used to enhance mitochondrial quality control, and was found to reduce age associated arterial stiffening.



Above: aortic pulse wave velocity (aPWV) in young and old control (YC and OC) and young and old trehalose treated (YTre and OTre) mice. Representative Doppler flows at right, taken from the aortic arch and abdominal aorta.

Calcium/Calmodulin-Dependent Kinase II Inhibition in Smooth Muscle Reduces Angiotensin II-Induced Hypertension by Controlling Aortic Remodeling and Baroreceptor Function
(Prasad, AM, et al. J Am Heart Assoc. 2015)

In this paper, Prasad et al. examined the effect of the multifunctional calcium/calmodulin-dependent kinase II (CaMKII) in vascular smooth muscle cells (VSMC). Working with an Angiotensin II (Ang II) model of induced hypertension in mice, they studied the effect of inhibiting the action of CaMKII. This group found that inhibition of VSMC CaMKII protects against aortic stiffening in Ang II induced hypertension in mice.



Above: aortic pulse wave velocity (PWV) in wild type (WT), transgenic (TG), and Angiotensin II (Ang II) models. Representative Doppler flows at left, taken from the aortic arch and abdominal aorta.