DSI Buxco® FinePointe™

Pulmonary Function Test

USER MANUAL

Publication 014181-001 Rev 03

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Essential Safety Notes

This section describes potential hazards which may exist in the operation of these units. A number of warning labels and symbols are affixed to your instrument. These symbols are used to inform you of potential dangers which may exist or where caution is required.

THE PROTECTION GIVEN BY THE EQUIPMENT MAY BE IMPAIRED IF USED IN A MANNER NOT SPECIFIED BY THE MANUFACTURER.

Environmental Conditions

- Indoor use
- Altitude up to 2000 m
- Storage and operation range: 4°C to 40°C; 10% - 80% Rh, Non-condensing
- Mains supply voltage fluctuations not to exceed ± 10% of the nominal voltage
- Supply voltage, voltage range: 12vdc, 4.0A
- Overvoltage Category 2
- Pollution degree 2

Hazards and Warnings

This instrument is subject to the following identified hazards:

CAUTION: The controller is heavy, to transport the controller use two people grasping either end of the controller and lift using proper techniques.

The AC/DC adapter requires connection to protective earthing (ground), only use with a power supply outlet and power supply cord that provides protective earthing.

Only use a detachable power cord (AC/DC adapter) that allows for protective earthing and is a minimum of 18 AWG. This cord will need to have appropriate agency approvals, such as UL, CSA.

DSI cannot guarantee the safety of this device if used other than intended or used by any procedures other than those described in this manual.

The AC/DC adapter requires connection to protective earthing (ground), only use with a power supply outlet and power supply cord that provides protective earthing.

Only use a detachable power cord that allows for protective earthing and is a minimum of 18 AWG. This cord will need to have appropriate agency approvals, such as UL, CSA.

Do Not Operate with Suspected Failures

If damage is suspected on or to the product, do not operate the product. Contact qualified service personnel to perform inspection.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to manage the disconnection device.

Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

Observe all Warning Labels on Product

Read all labels on product to ensure proper usage.

Before installing your new unit, please take time to familiarize yourself with these warnings and symbols:

Caution

Electronic Waste

Welcome

Congratulations on joining the community of users worldwide who rely on DSI's products to perform preclinical physiologic research. Thank you for your interest in DSI products. We are committed to providing you with quality products and services.

This manual will help you get to know your FinePointe Pulmonary Function Test system. The structure of the manual was designed to sequentially guide you through using your DSI system from set up to data acquisition.

What you will be learning

1. Understand how respiratory data is collected using the Pulmonary Function Test system.
    a. Hardware
    b. Software
2. How to setup your Pulmonary function Test system hardware.
3. How to use the FinePointe software to:
    a. Create a hardware configuration
    b. Create a study
    c. Calibrate hardware
    d. Acquire data
    e. Generate reports
    f. Review data

Application Overview

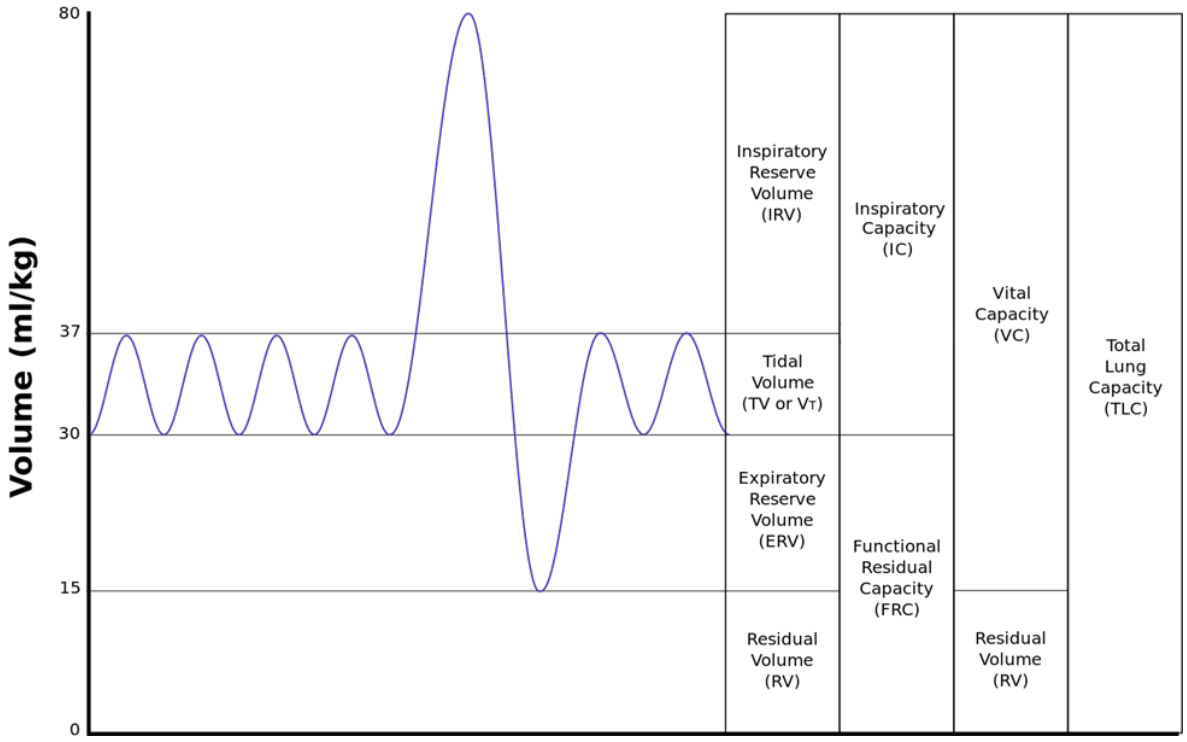
Pulmonary Function Testing (PFT)

This analyzer monitors a respiratory flow signal and any airway pressure signal during a succession of forced pulmonary tests from an anesthetized test subject. These tests offer a unique and comprehensive look at the function of the lung.

PFT is an acronym for Pulmonary Function Testing. This system is also known as Pulmonary Maneuvers. The PFT system automates a series of tests on an animal which simulate clinical pulmonary testing routinely performed on humans.

There are 3 tests that the PFT system can perform: FRC, PV, and FV. In addition, the system allows the researcher to acquire baseline resistance and compliance data.

This picture below illustrates all the lung volumes.



Lung Volumes

The FRC tests measures the FRC lung volume by using Boyle's Law. FRC lung volume is the Functional Residual Capacity. This is the volume of air that remains in the lung at the end of a normal expiration. This lung volume is important because by knowing this lung volume, it is easy to know all the other lung volumes.

See Section FRC for list of common parameters reported.

The PV test allows the researcher to know the static lung properties. Static lung properties are described by the pressure-volume relationship of the lung. The slope of the Volume-Pressure curve is the instantaneous compliance at a given pressure. See Section Quasistatic Pressure VolumeFRC Test for list of common parameters reported.

The FV test allows the researcher to know the dynamic lung properties. Dynamic lung properties are described by the flow-volume relationship of the lung. The Flow-Volume curve shows the flow at a given expired volume. Since the pressure across the lung is essentially constant during the acquisition of the flow-volume data, the flow value is proportional to the conductance of the limiting airways at a given expired volume. See Section Fast Flow Volume for list of common parameters reported.

See Section Resistance & Compliance for list of common parameters reported.

How It Works

The subject has a tracheotomy performed (or is intubated) and placed in the supine position on the bed within the plethysmograph. The trach tube is connected to the face plate where fresh air is provided either via bias flow or through ventilation. The face plate contains a manifold of valves which are actuated to perform the PFT tests. Also, supporting the manifold is a pressure panel which provides flows at safe pressures to the manifold. The pressure panel also contains preamplifiers necessary to condition the flow and pressure signals needed for the data analysis.

With the plethysmograph placed around the subject, the subject breathes through the wall of the face plate and the chest expands with the air entering and contracts as the air exits the lungs. This expansion forces an equal amount of air out of the plethysmograph through a screen pneumotach. The pneumotach resists the movement of air, and so, pressure in the chamber is created as a result of the air being forced through the pneumotach. This pressure is linearly related to flow of air passing through the pneumotach, and since that is the same as the volume inspired by the subject, the pressure is related to the flow in and out of the subject.

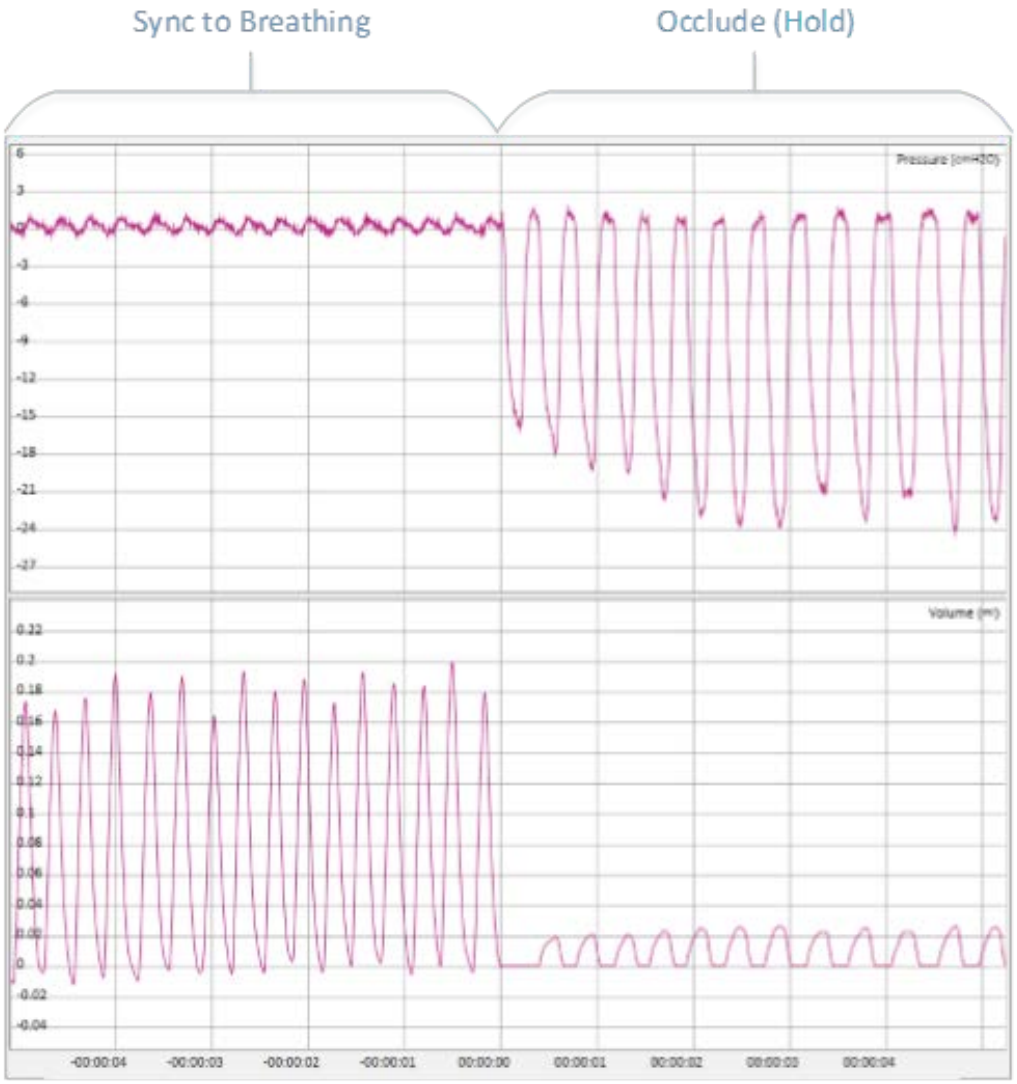
The lung pressure is measured in the tracheal line. Normally, the pressure is referenced to atmosphere, but it can optionally be referenced to the subject's esophagus or even to the intrapleural cavity using a needle.

PFT Test

The following summarizes the test automation:

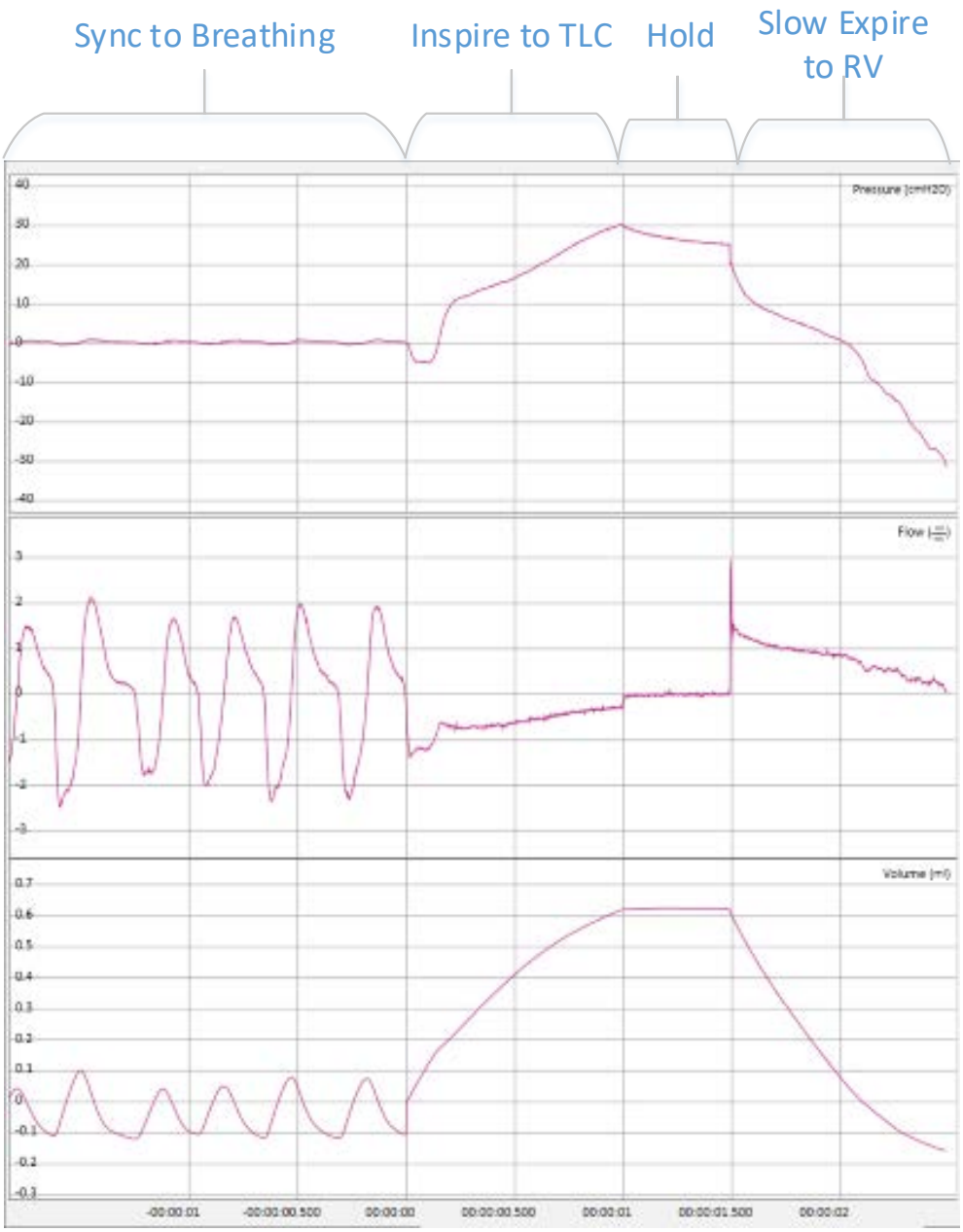
FRC (Functional Residual Capacity) Test Automation

- Uses Boyle's Law to determine FRC
- Synchronize to Breathing
- Occlude airway until the subject attempts to breathe on its own



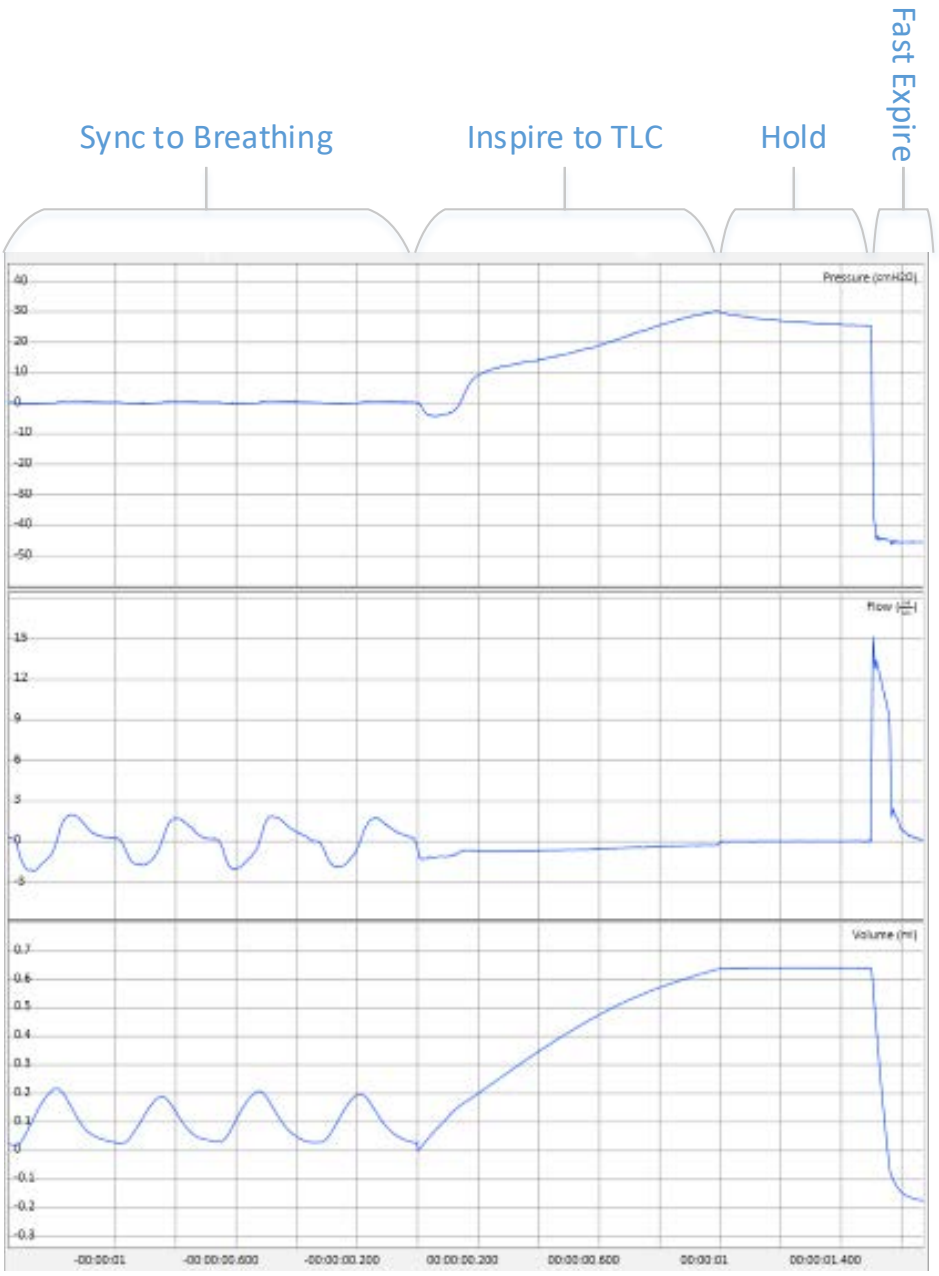
PV (Pressure Volume) Test Automation

- Synchronize to Breathing
- Inspire to TLC
- Hold
- Slow Expire to RV



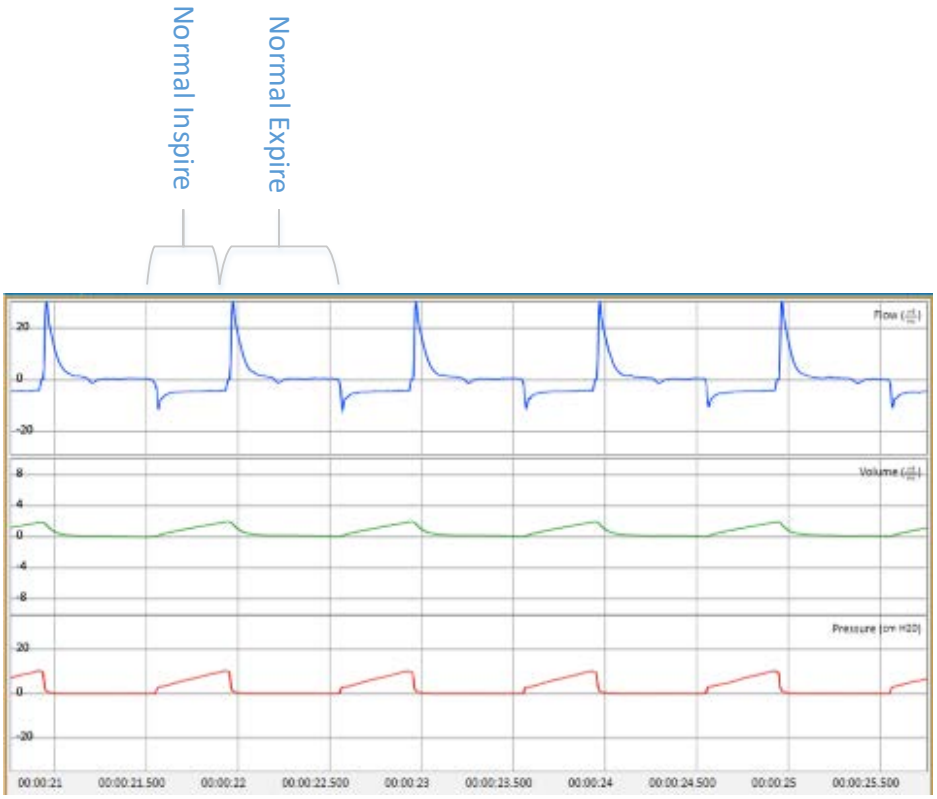
FV (Flow Volume) Test Automation

- Synchronize to Breathing
- Inspire to TLC
- Hold
- Fast Expire



Ventilator Automation

- Inspire
- Hold
- Passive Expire
- Repeat



Valve Sequence Automation

Sequence	Vfrc	Vinsp	Vexpire	Vff	How end is determined
Inspire	X	X	-	-	Determined at target pressure (e.g. 10cmH_2O) or timeout.
Inspire to TLC	X	X	-	-	Determined at target pressure (e.g. 30cmH_2O) or timeout.
Expire to RV	X	-	X	-	Determined at target pressure (e.g. -30cmH_2O) or timeout.
Fast Expire	X	-	-	X	Determined when flow is below a target or timeout.
Hold or Occlude	X	-	-	-	Determined by duration or when sufficient data is acquired
Passive Expire	-	X	-	-	Determined by duration
Idle	-	-	-	-	Indefinite

System Hardware

This chapter contains information on the hardware components of the system, including how to connect the hardware, and definitions and descriptions of the hardware.

PFT Controller Pressure Panel

PFT controller pressure panel

The PFT controller pressure panel consists of:

- A pump and pressure reservoirs, both internal and external.
- Regulators to charge the pressure in the reservoirs.
- Plumbing and associated actuators to control the breathing of the subject sufficiently to perform each test.
- Meters and controls which allow you to set flow rates suitable for the animals you are testing.
- Preamplifiers for flow and pressure transducers.
- A/D converter and USB interface for the PC to acquire data and control the tests.

Pressure Panel Front

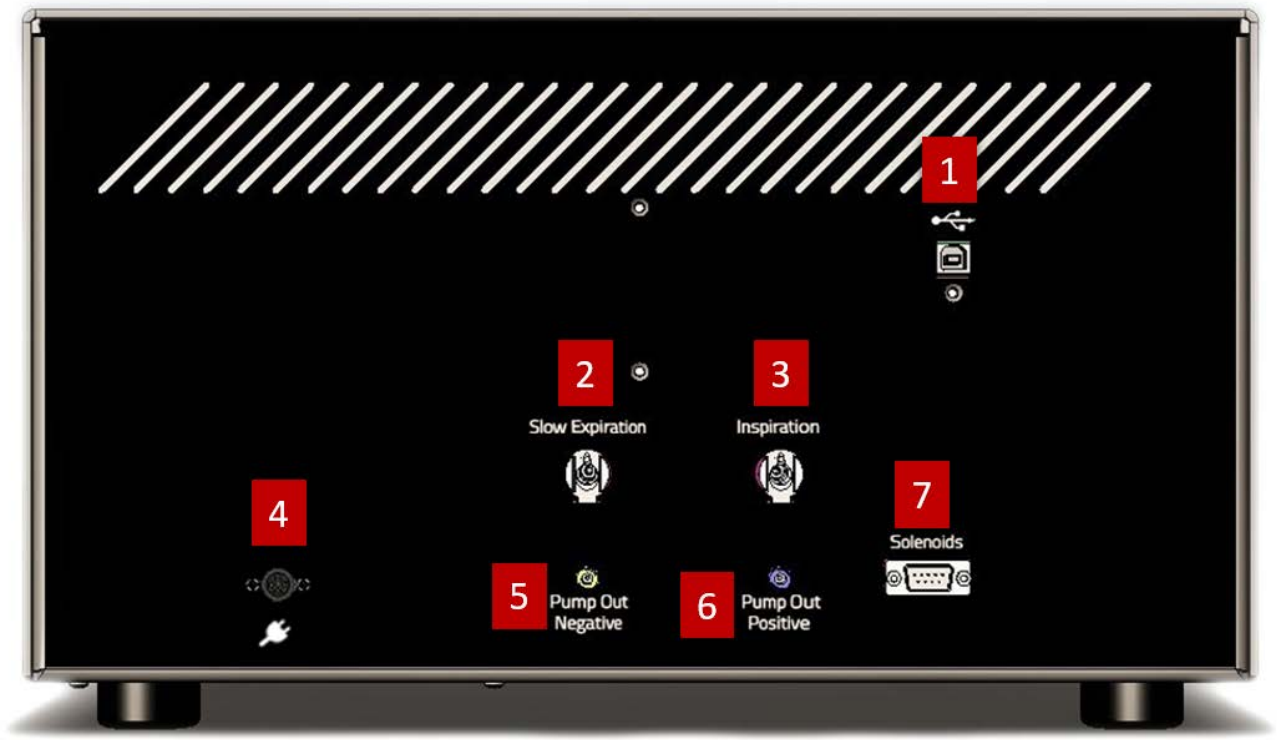
Pressure panel controls

The following are descriptions and operational definitions of controls and modules on the front of the Pressure Panel

1. **Preamplifier modules** (Flow, Mouth Pressure, Esophageal) - These modules provide signal amplification and conditioning for the flow, mouth pressure, and (optionally) esophageal pressure transducers.
2. **Valve Control module** - This module has two purposes:
   - The Positive Pressure and Negative Pressure adjustment knobs control the pressure that is maintained in the positive and negative pressure reservoirs. The pressure levels should be set at values that ensure the safety of the animal. **By default, set them to +50 cmH2O on the positive pressure reservoir and -50 cmH2O on the negative pressure reservoir.**
   - The valve control switches are used to set the valves to one of three states, On, Off, or Auto. On closes the valve. Off opens the valve. Auto allows the FinePointe software to control the valves. Valves are manually controlled when setting flow rates and when calibrating the lung pressure. Auto control is used when FinePointe is controlling the system during the test recording session.
3. **Signal Generator module** - This module provides pre-set computer-generated waveforms. This allows you time to learn how the system works without having to run with a live animal. When set to the **Live** position, the generated signals are disabled, and live signals from the transducers are used. **The control knob must be set to the Live position when collecting data.** The Volume and High Volume reset buttons are inactive and not used by the system.
4. **Power module** - This module has the pressure panel On/Off switch, and 2 LEDs in the top right corner. These two LEDs confirm that the internal power supply is functioning. If they are dim, your power supply might not be putting out the required voltage. If they are off, the Pressure panel has no power.

5. **Large Animal/Small Animal mode selection switch** – Toggles between the large animal and small animal flow vale adjustment control knobs. A green indicator light will illuminate above the selected valve control knobs. The large green knobs control the large animal flow rates when the PFT Pressure Panel is connected to the large animal reservoir. The small silver knobs control the small animal flow rates when connected to the small animal reservoir.
6. **Inspiration Flow Valve Adjustment knobs** – These knobs control the large animal or small animal inspiration flow rate that is used in the PV and FV tests during inspiration to 30 cm H20 (this is a positive flow).
7. **Slow Expiration valve** - These knobs control the large animal or small animal slow expiration flow rate that is used during the Pressure Volume (PV) test during expiation (this is a negative flow).
8. **Digital Flow and Pressure Displays** - These displays are used to set the flow rates and confirm the pressure in the positive and negative reservoirs. There are trim screws to allow you to easily adjust the zero should the sensors drift.

Pressure Panel Back



Connections on the back of the pressure panel

The following are descriptions of the connections on the back of the Pressure Panel

1. **USB Port** - The USB port accepts a standard non-powered USB cable which is then connected to the PC. All data acquired from the controller, and all control commands (from the PC) are sent through this connection.
2. **Slow Expiration Port** - This port is connected to the negative pressure section of the pressure reservoir.
3. **Inspiration Port** – This port is connected to the positive pressure section of the pressure reservoir.
4. **Power Connector** – The power transformer is connected to this port.
5. Pump Out Negative Port – This port is connected to the pump port on the negative section of the pressure reservoir.
6. **Pump Out Positive Port**- This port is connected to the pump port on the positive section of the pressure reservoir.
7. **Solenoids Port** – This 9-pin D shell connector is connected to the chamber manifold. It controls the chamber manifold valves.

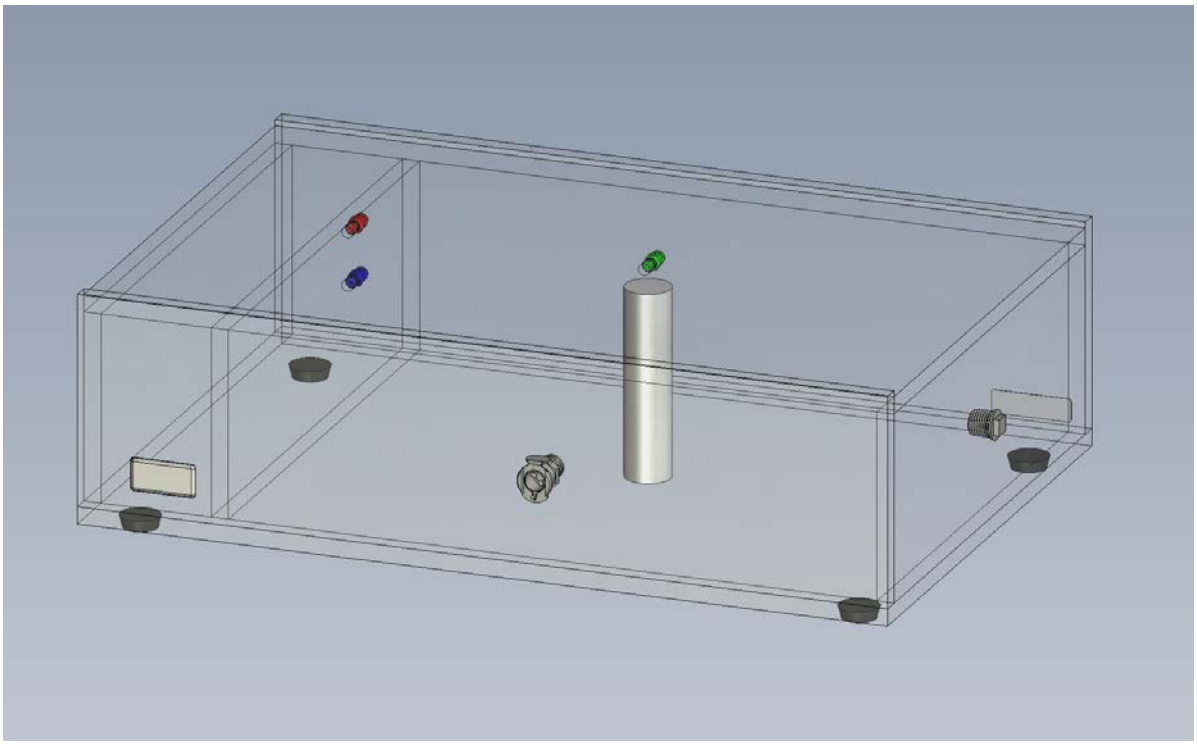
Pressure Reservoir

Small animal pressure reservoir

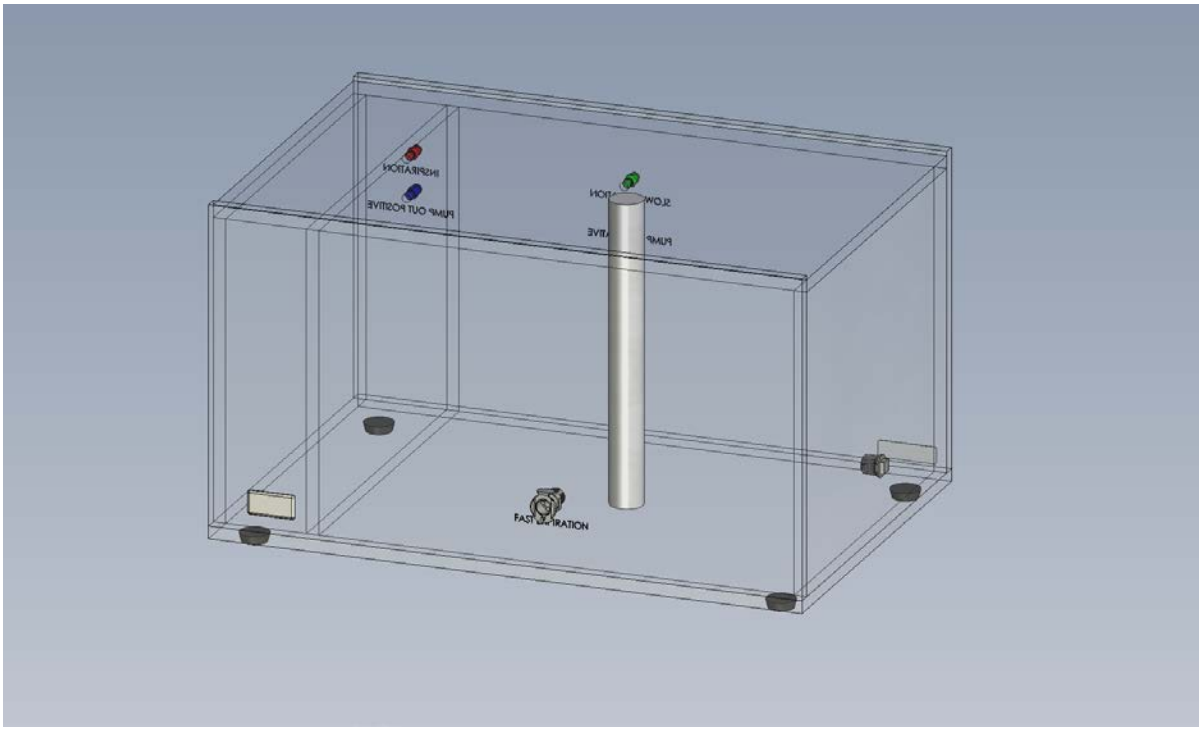
The pressure reservoir is divided into two sections, negative and positive. The positive reservoir is used to inflate the lungs at the configured inspiration flow rate. The negative reservoir section is used to slowly deflate the lungs at the configured slow expiration flow rate during PV tests, and to deflate the lungs as quickly as possible during FV tests.

The pressure in both reservoirs is set on the Valve Control Module.

The pressure reservoir is available in two sizes. Small animal (SA) and large animal (LA). The small animal model is used for mice, rats, and guinea pigs. The large animal model is used for rabbits, ferrets, and small primates.



Small animal pressure reservoir



Large animal pressure reservoir

Compressed Air Source

Every PFT chamber model other than the mouse chamber requires a compressed air source to articulate the pneumatic control valves on the manifold. These valves drive FRC cylinders and the Fast Expiration cylinders. This includes the Rat/GP, Rabbit, Ferret, and Primate chambers.

The compressed air source must deliver 40 to 50 PSI. If the pressure is higher than 60 PSI, the pneumatic valves will be damaged. The air sources include low noise air compressors with a pressure regulator, or a laboratory air source with a pressure regulator.

DSI offers a low noise air compressor for sale. The models have varied over the years, so model specific details are not included in this manual. If you purchased your compressor from DSI, please see the manufacturers product manual that was included with you unit. A compressor purchased from DSI will also include a kit that includes the tubing and fittings you will need to connect the compressor to the chamber.

Please contact your DSI sales representative or DSI technical support for more information.

Chamber Manifold Design

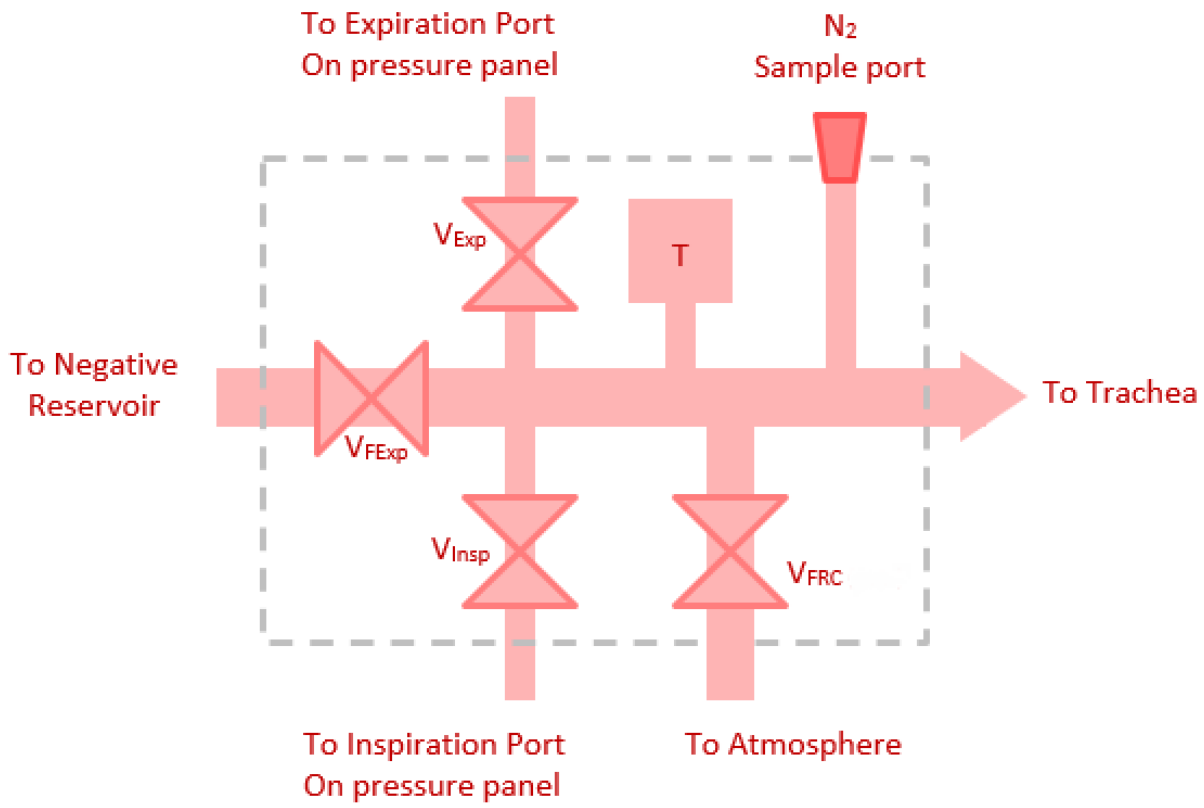
The manifold connects the trachea of the anesthetized animal to the valves that control each test. Although the manifolds for the mouse, rat or monkey do not look similar, they are identical in theory and operation.

Not all the PFT tests are "forced." For example, the FRC test operates from the driving pressure of the animal. All the PFT tests require tracheal connections. Gas flow paths must be switched to accommodate all the tests in rapid sequence. Most of the connection switching is performed automatically, under computer control.

The features of the manifold are described below:

Feature	Description
FRC Valve ($V_{FRC)}$	This valve is a low resistance normally open valve that the animal can breathe through when the test is not running. This valve is closed during each test.
Fast Expiration Valve (V_{FExp})	This valve is closed all the time except for expiration during the Fast FV test. This is a low resistance valve which (when open) exposes the trachea directly to the negative reservoir.
Slow Expiration Valve (V_{Exp})	This valve is closed all the time except for expiration during the Quasistatic PV test. This valve draws air from the trachea at a rate set by the adjustable valve on the Pressure Panel.
Inspiration Valve (V_{Insp})	This valve is open to either clear CO_2 build up in the dead-space of the manifold, or to force an inspiration.
N_2 Sample Port	This port is used to calibrate the pressure sensor.

The following is a schematic representation of the manifold (Note: T represents the mouth pressure transducer):

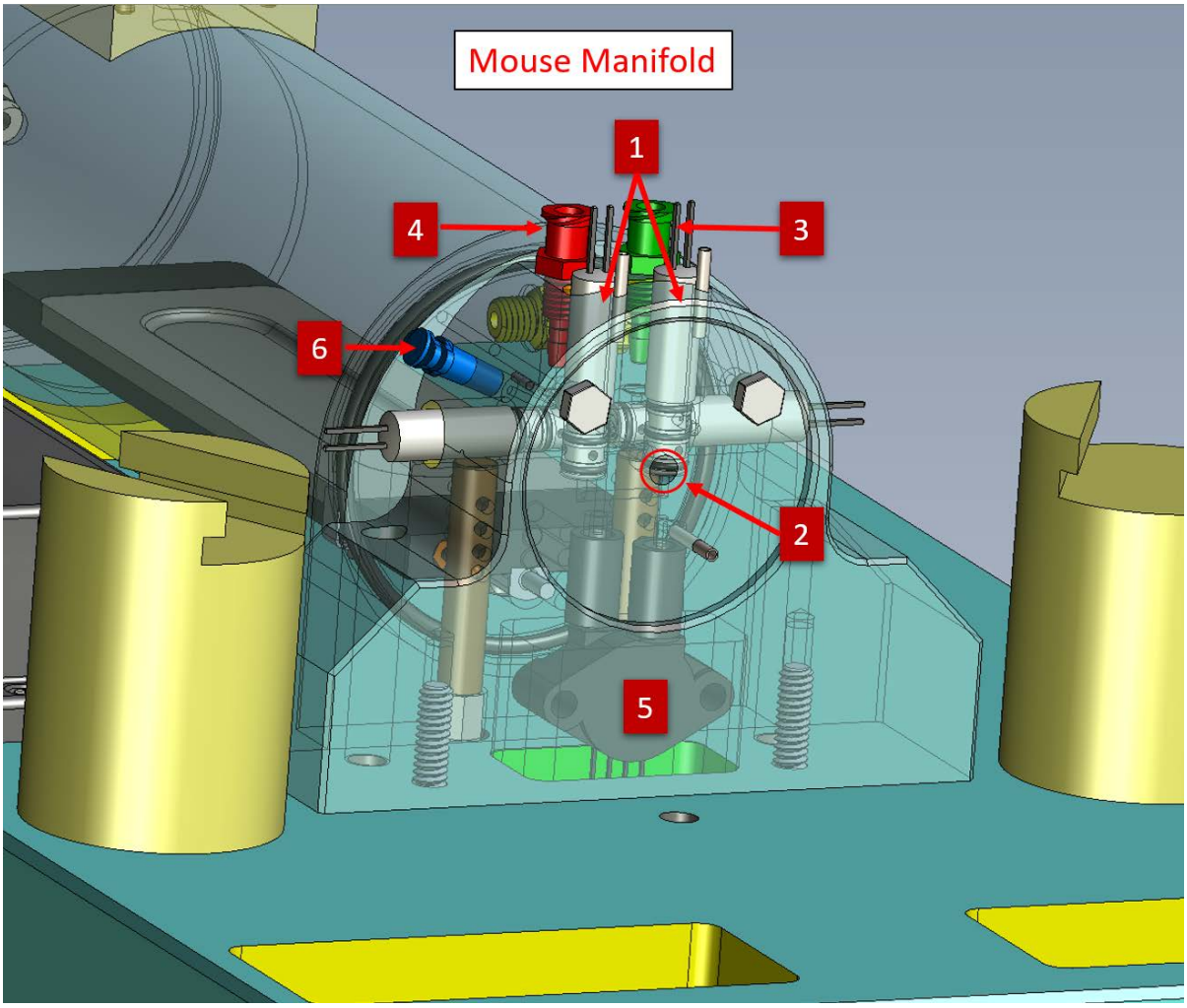


On the endplate of the animal chamber, there are two more ports on either side of the manifold. One port accommodates a cannula that connects the Pulmonary pressure transducer to the animal. The other port is used to monitor Blood Pressure, but this is not part of any maneuver; it's part of the Blood Pressure application, which uses its own separate software analyzer.

You can also use these two ports to run in any external line, for example, if you needed to continuously supply the animal with an anesthesia drip.

Manifold Types

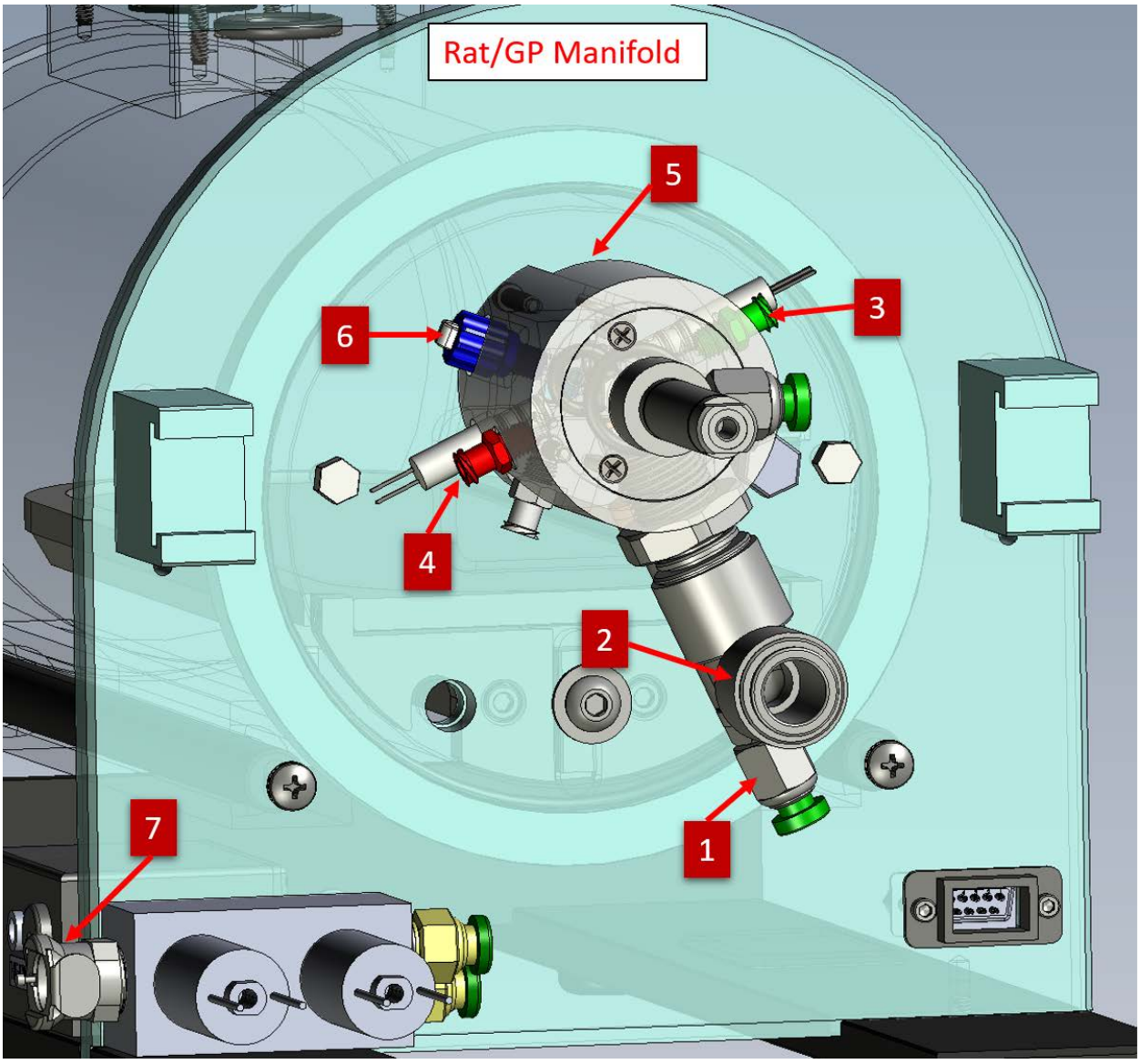
Mouse Manifold



The mouse manifold is used on mouse chambers only

1. FRC valve (the mouse manifold has two)
2. Fast expiration valve port (the valve is not mounted in the image above)
3. Slow expiration valve port
4. Inspiration valve port
5. Mouth pressure transducer port (the mouth pressure transducer is built into the manifold)
6. Inlet for sampling gases (N, O2, etc.). This port is also used to calibrate the mouth pressure signal.

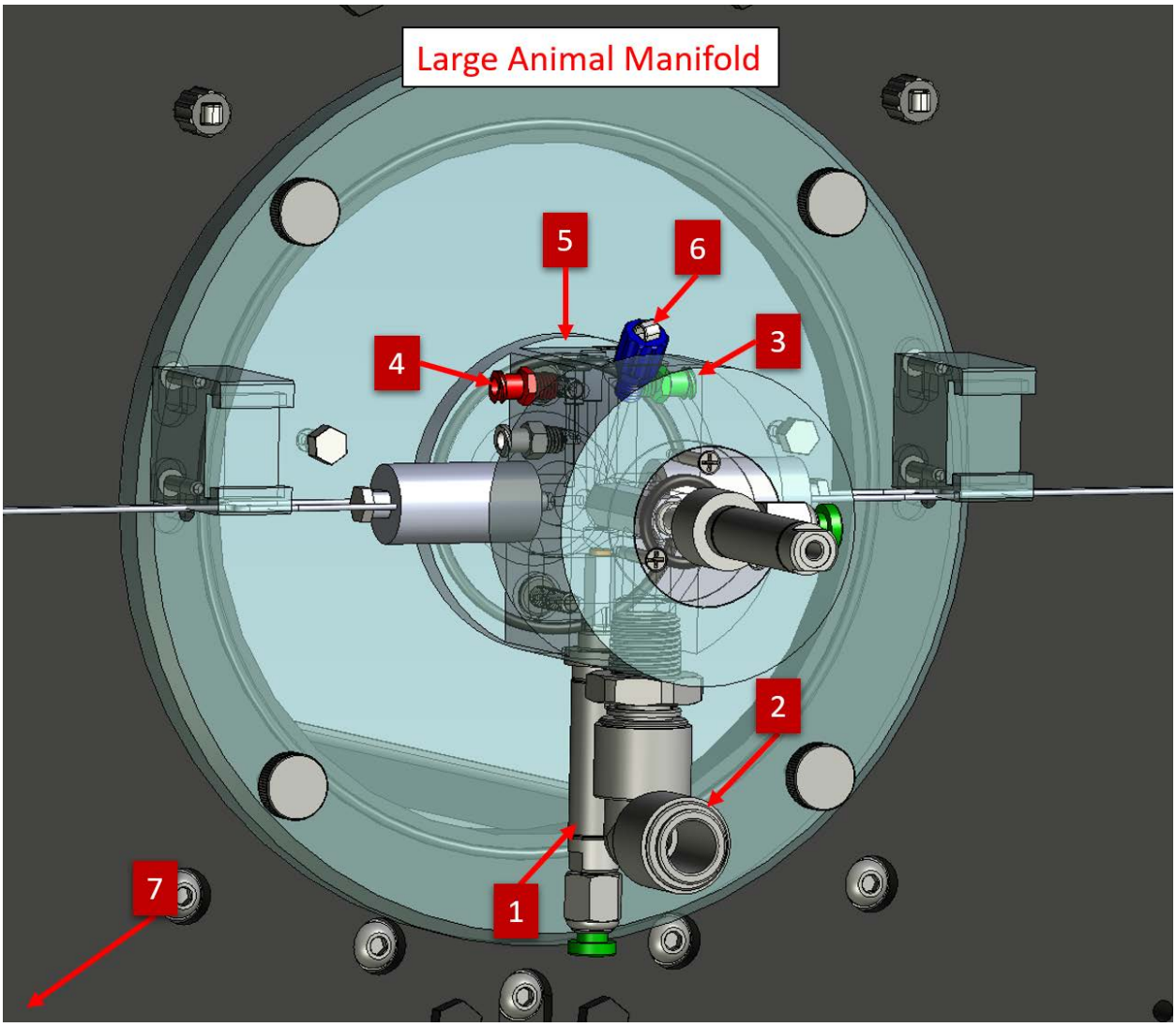
Rat/GP Manifold



The Rat/GP manifold is used for rat and guinea pig chambers

1. FRC valve
2. Fast expiration valve port
3. Slow expiration valve port
4. Inspiration valve port
5. Mouth pressure transducer port
6. Inlet for sampling gases (N, O2, etc.). This port is also used to calibrate the mouth pressure signal.
7. Compressed air source port

Large Animal Manifold



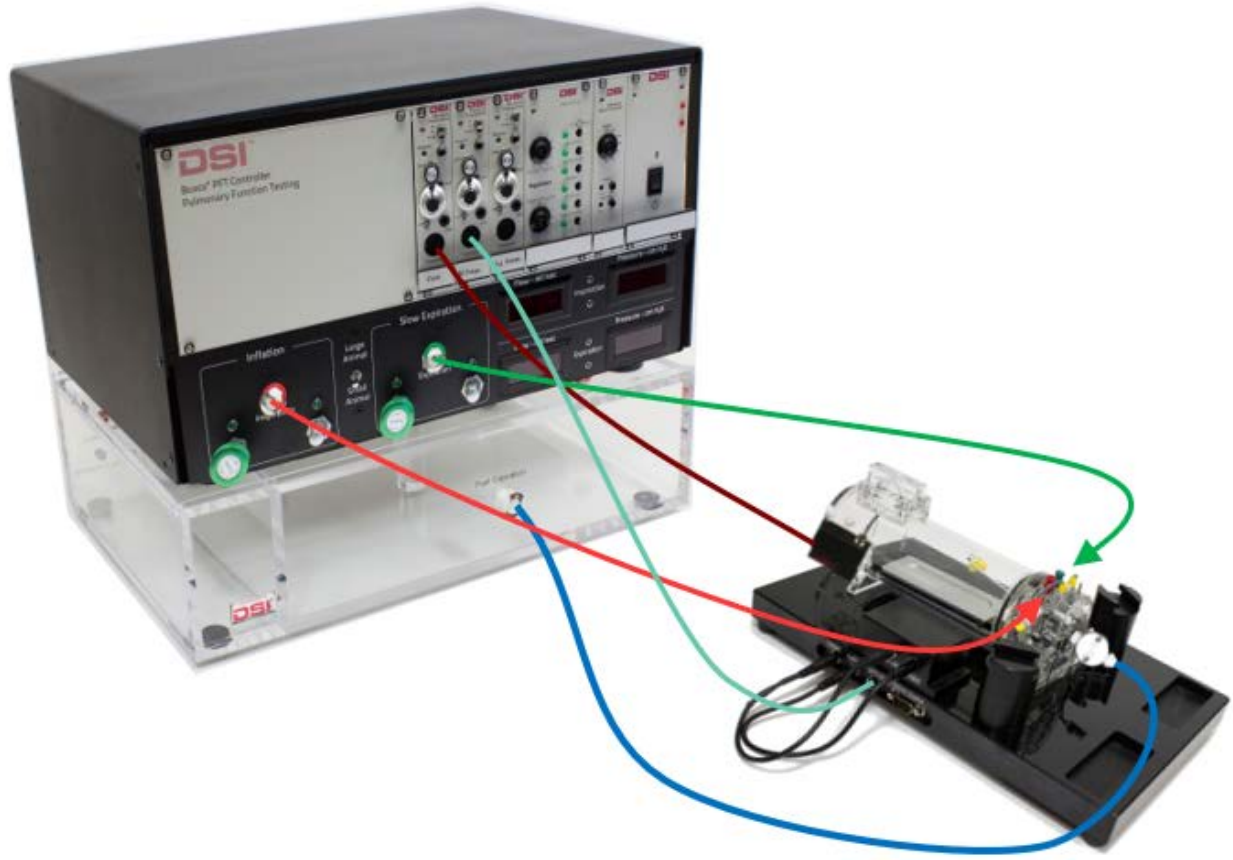
The Large Animal manifold is used for rabbit, ferret, and primate chambers

1. FRC valve
2. Fast expiration valve port
3. Slow expiration valve port
4. Inspiration valve port
5. Mouth pressure transducer port
6. Inlet for sampling gases (N, O2, etc.). This port is also used to calibrate the mouth pressure signal.
7. Compressed air source port (not visible, but is located on the lower left corner of the manifold bulkhead similar to the rat manifold)

Configuring the Hardware

1 - Front Panel Connections

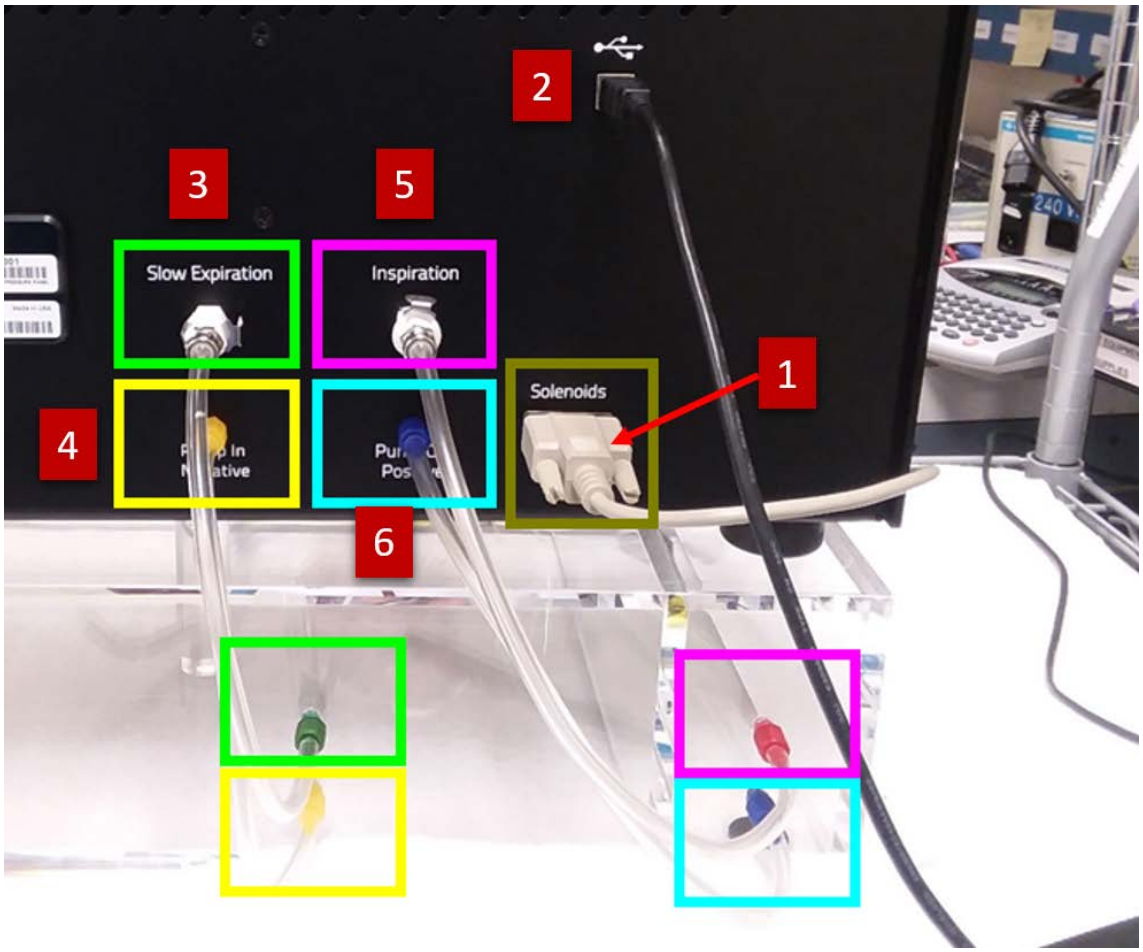
To connect the wiring and tubing, make the following connections to the front of the pressure panel:



1. Use the largest tube to connect the **Fast Expiration** quick disconnect port on the negative reservoir to the **Fast Expiration** valve on the manifold.
2. Connect the narrow tube with green fittings from the **Slow Expiration** quick disconnect port on the front of the Pressure Panel to the **Slow Expiration** valve on the manifold.
3. Connect the narrow tube with red fittings from the **Inflation** quick disconnect port on the front of the Pressure Panel to the **Inspiration** valve on the manifold.
4. Connect the **Pressure transducer** to the connector labelled **Pressure transducer** on the chamber and the other end of the cable to the preamp on the Pressure Panel labeled **M. Pressure**. Ensure that the transducer is oriented correctly. The +/- tips of the transducer must be inserted into the matching holes that are labeled +/-.
5. Connect the **Flow transducer** to the port on the side of the chamber and the cable to the preamp on the Pressure Panel labeled **Flow**.
6. Optionally, connect the **Esophageal Pressure** transducer cable to the preamp on the Pressure Panel labeled **Esophageal**.

2 - Rear Panel Connections

Make the following connections to the back of the pressure panel:

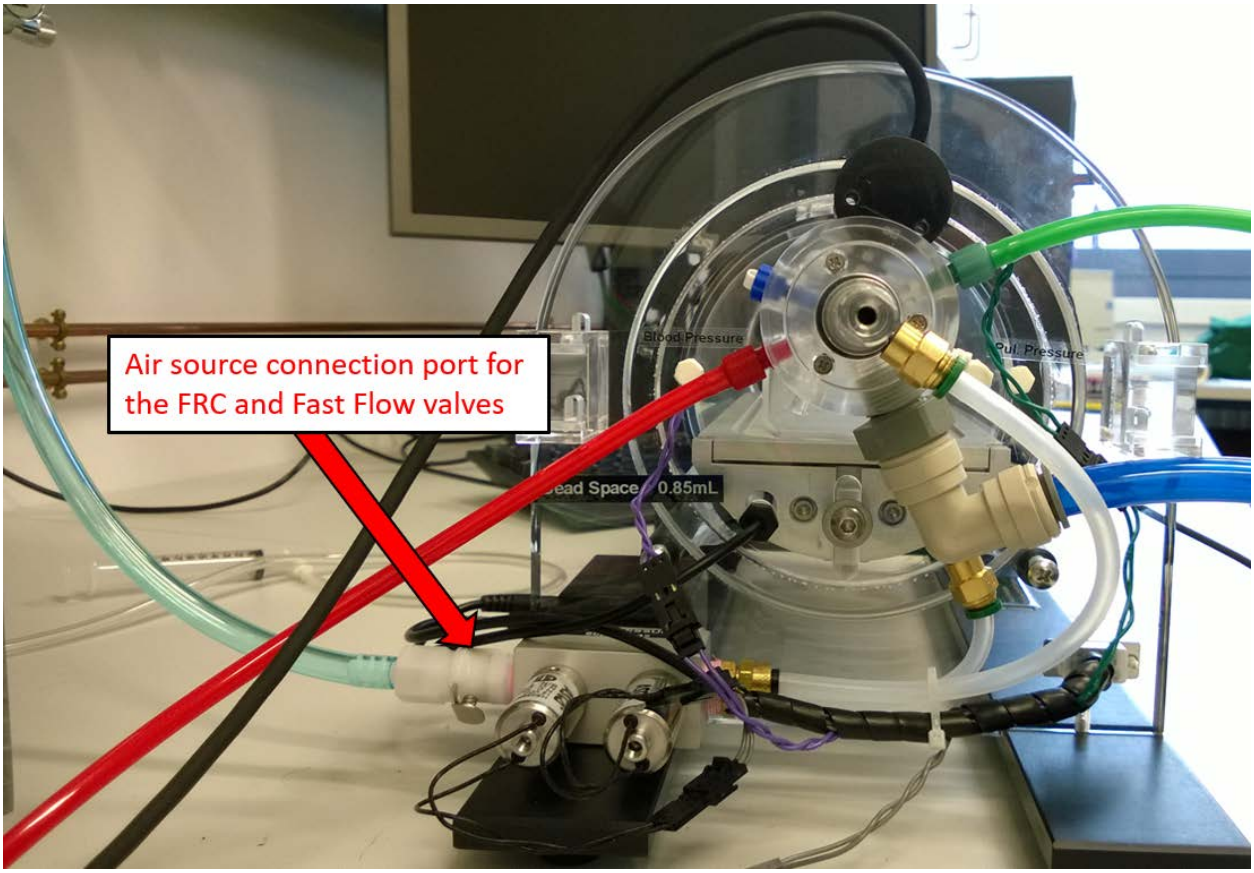


1. Connect the cable from the **Valves Control** connector on the plethysmograph to the **Solenoids** connector on the rear of the Pressure Panel.
2. Connect the **USB** connector to a USB port on the computer. Avoid going through a USB hub (including the hub on the monitor) unless it is approved for use by DSI.
3. Connect tubing from the **Slow Expiration** quick disconnect on the rear of the Pressure Panel to the **green** input on the negative reservoir.
4. Connect tubing from the **yellow Pump in Negative** luer connector on the rear of the Pressure Panel to the **yellow** input on the negative reservoir.
5. Connect tubing from the **Inspiration** quick disconnect on the rear of the Pressure Panel to the **red** input on the positive reservoir.
6. Connect tubing from the **blue Pump Out Positive** luer connector on the rear of the Pressure Panel to the **blue** input on the positive reservoir.
7. Connect the Power Transformer to the power connector. (Not pictured)

3 - Compressed Air Source Connection

If you are using any chamber type other than the mouse chamber you must connect a compressed air source that delivers 40 PSI to 50 PSI of pressure to the pneumatic control valves on the manifold. If the pressure is higher than 60 PSI, the pneumatic valves will be damaged. Possible air sources include low noise air compressors with a pressure regulator, or a laboratory air source with a pressure regulator. Please see the Compressed Air Source section on page 21 of this manual for more details.

Note: The pressure delivered to the air source connection port must not exceed 60 PSI or the pneumatic valves will be damaged!!!



4 - Setting Positive Pressure

Use the 8-position knob labelled **Positive Pressure** on the Valve Control module to set the desired pressure level. For most situations, set this to 50.

Confirm the new pressure by watching the Positive Pressure display. The displayed pressure is approximate so anything within 5% should be acceptable.

Note: If the FRC valve is actuated (the light beside the FRC valve is lit on the Valve Control module), the regulator is inhibited. Make sure the FRC valve is switched off or in the Auto position.

Note: The reservoir regulator works by only pumping air *into* the reservoir. When turning the pressure down, you must vent the reservoir by switching on the Inspiration valve on the Valve Control, otherwise you will not see your pressure reflected on the display

5 - Setting Negative Pressure

Use the 8-position knob labelled **Negative Pressure** on the Valve Control module to set the desired pressure level. For most situations, set this to 50.

Confirm the new pressure by watching the Negative Pressure Display. The pressure is approximate so anything within 5% should be acceptable.

Note: If the FRC valve is actuated (the light beside the FRC valve is lit on the Valve Control module, the regulator is inhibited. Make sure the FRC valve is switched off or in the Auto position.

Note: The reservoir regulator works by only pumping air *out of* the reservoir. When turning the pressure up, you must vent the reservoir by switching on the Slow Expiration valve on the Valve Control, otherwise you will not see your pressure reflected on the display.

6 - Setting Inspiration Flow

Using the toggle switch on the front panel, select Large Animal if you are using a large animal pressure reservoir, or Small Animal if you are using a small animal pressure reservoir. **If you selected large animal, use the large green flow rate adjustment knobs. If you selected small animal, use the small silver flow rate adjustment knobs.**

On the Valve Control module, make sure the **FRC** valve is switched off or in the Auto position. Then flip the **Inspiration** valve switch to ON.

Turn the knob labeled **Inspiration** until the inspiration flow rate display reads the value of your desired flow rate. A general guideline when setting the inspiration flow rate is to use a rate at which it would take 2 or 3 seconds to fill the animal's lung to TLC (Total Lung Capacity).

When you see the correct value displayed on the **Inspiration Flow** digital display, flip the **Inspiration valve** switch back to Auto. Then you have successfully set the Inspiratory flow rate.

Use the table below to estimate inspiration flow rates. Divide the subject's TLC by the number of seconds required for the expiration.

For example, if the subject is a rat and the TLC is 14 ml the flow rate would be 5.6 ml/sec (14 ml divided by 2.5 secs = 5.6 ml/sec). Inspiration flow rates are positive. Please keep in mind that these flow rates are estimates and may need to be adjusted if the subject cannot achieve 30 cm H20 of pressure fast enough during inspiration. You can measure the inspiration time on the flow signal in FinePointe. Measure the time it takes from the pressure to rise from zero to 30 cmH20 during the forced inspiration phase of the breath. These values will vary based the subject's size, especially with larger animal types.

Species	Typical Inspiration Time
Mouse	1 to 2 seconds
Rat	2 to 3 seconds
Guinea Pig	3 to 5 seconds
Rabbit	3 to 6 seconds
Ferret	4 to 7 seconds
Primate (max TLC 350 ml)	4 to 10 seconds

7 - Setting Slow Expiration Flow

Using the toggle switch on the front panel, select Large Animal if you are using a large animal pressure reservoir, or Small Animal if you are using a small animal pressure reservoir. **If you selected large animal, use the large green flow rate adjustment knobs. If you selected small animal, use the small silver flow rate adjustment knobs.**

On the Valve Control module, make sure the **FRC** valve is switched off or in the Auto position. Then flip the **Slow Expiration** valve switch to ON.

Turn the knob labeled **Expiration** until the display reads the value of your desired flow rate. A general guideline when setting the slow expiration flow rate is to use a rate that would expire the vital capacity (VC) of the subject as long as possible for that species.

When you see the correct value displayed on the **Slow Expiration Flow** digital display, flip the **Slow Expiration** switch back to Auto. Then you have successfully set the Slow Expiration flow rate.

Use the table below to estimate slow expiration rates. Divide the subject's VC (Vital Capacity) by the number of seconds required for the expiration.

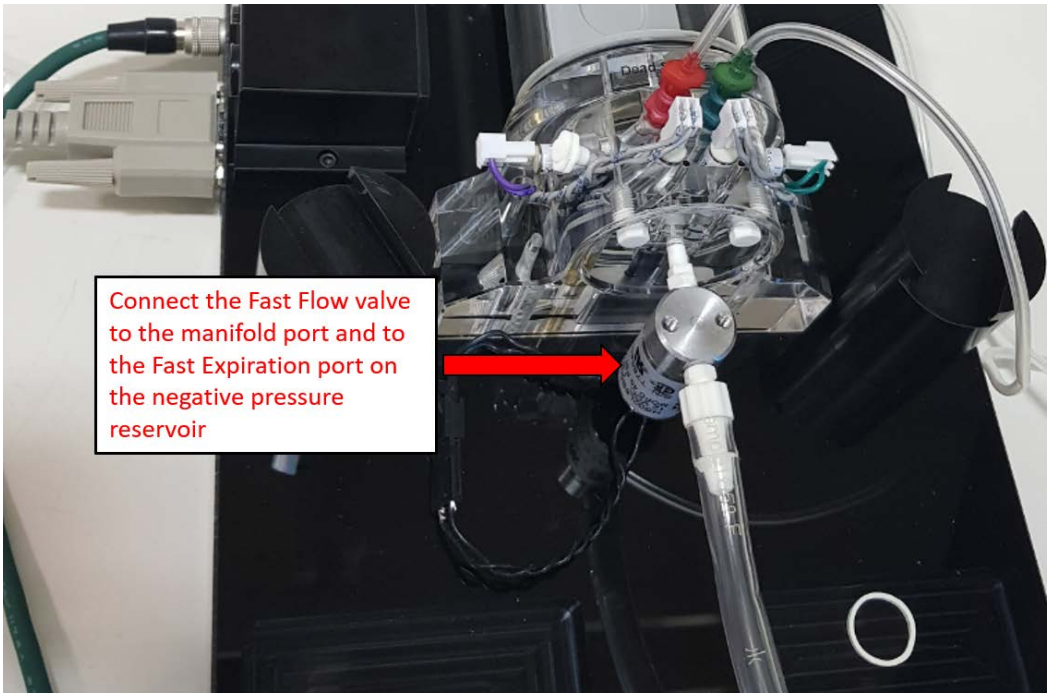
For example, if the subject is a rat and the VC is 10 ml the flow rate would be -2.5 ml/sec (10 ml divided by 4 secs = 2.5 ml/sec). Expiration flow rates are negative. Please keep in mind that these rates are estimates and may need to be adjusted based on the Te (expiratory time) that is measured during the PV (Pressure Volume) test. The Te should fall within the expiration time range of the subject type. The values will vary based the subject's size, especially with larger animal types.

Species	Typical Longest Expiration Time
Mouse	1.5 to 2 seconds
Rat	2.5 to 3.5 seconds
Guinea Pig	3 to 4 seconds
Rabbit	3 to 5 seconds
Ferret	3 to 5 seconds
Primate (max TLC 350 ml)	3- 5 seconds

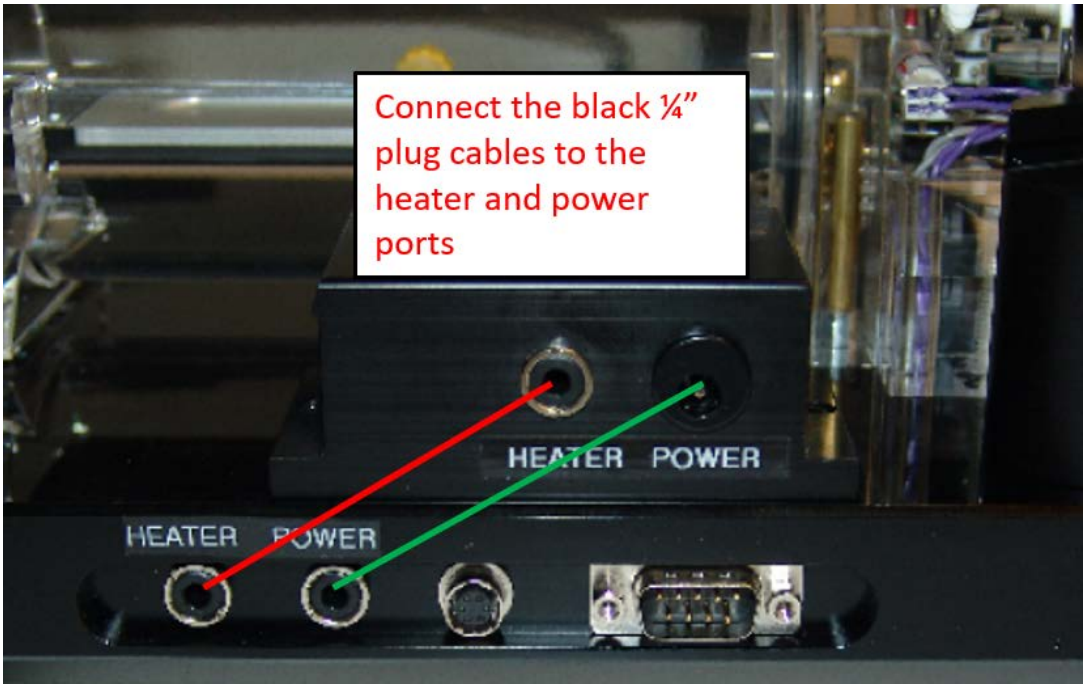
Mouse Chamber Configuration Notes

Several additional steps are required to configure the mouse chamber

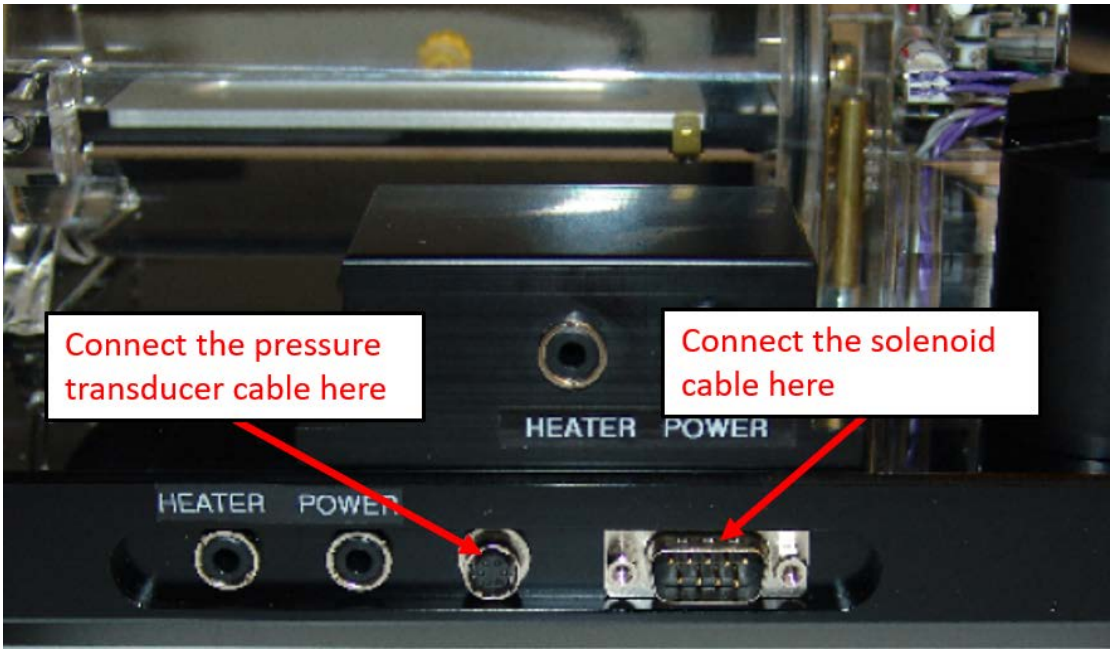
1. Connect the Fast Flow valve to the mouse manifold port and to the Fast Expiration port on the negative pressure reservoir.



2. Connect the black cables with the ¼" jacks to the power and heater ports on the side of the chamber table.



3. Connect the pressure transducer and the solenoid cables to the ports on side of the chamber table.



Switching Between Mouse and Rat

It is common to purchase a Pressure Panel and both a plethysmograph for rat and a plethysmograph for mouse. The two work almost identically, except you are required to adjust a jumper on the Signal Generator module when you switch from using one plethysmograph to using the other. In addition, the first time you calibrate after you switch, you may have to rebalance the transducers and adjust the gains for the system to pass the calibration wizard.

At the time of installation, your installer should have set up two hardware configurations using the same Pressure Panel: one for the rat plethysmograph and one for the mouse plethysmograph.

Use the following procedure as a guide when you switch between rat and mouse plethysmographs:

1. Switch the plethysmographs:
    a. Disconnect the old plethysmograph.
    b. Remove the tubing.
    c. Replace with the new plethysmograph and its proper sized tubing.
    d. Change transducers if necessary. The mouth pressure transducer is built into the mouse plethysmograph, so you will at least have to change this one.
2. Adjust the inspiration flow and slow expiration flow for your new animal.
3. Remove the Signal Generator module from the Pressure Panel and set the jumper labeled **J2** (**Gain Jumper**) to:
    ➔ **x4** for mouse
    ➔ **x8** for rat

4. Re-insert the Signal Generator module back into the Pressure Panel
5. Bring up the FinePointe Software, go to the Laboratory page and re-calibrate the appropriate station.

Measuring Dead Space in the Manifold

In the FRC test, the Pressure and Volume changes caused by the animal's attempts to breathe help us derive the entire volume occluded from atmosphere. This volume is made up of the animal's lung volume at the time of the occlusion plus the dead space. So, if the animal is occluded at FRC, then after we determine the occluded volume, we only need to subtract out the dead space to compute FRC.

The following procedure describes how to measure the dead space. Keep in mind that you should only need to measure this if it is the first time you are using this apparatus or if you change the type of tracheal tube you are using (the dead space of your apparatus should be constant unless you change your apparatus). Once you have measured the dead space, this value must be entered into your hardware configuration in FinePointe Control Panel.

Boyle's Law Method

Before you perform the measurement, make sure the mouth pressure transducer is properly calibrated.

You will need:

- A tracheal catheter with the end sealed (airtight)
- A small syringe, perhaps 100µL or 50µL for a rat chamber. You should plan on injecting 20µL into the valve assembly.
- The current atmospheric pressure P_{atm}, in cm H_2O
- FinePointe open acquiring data so that you can view the mouth pressure signal

Procedure:

1. Attach your sealed tracheal catheter to the tracheal port, on the inside of the chamber. Make a guess at the dead space and set your syringe to roughly 0.02 or 0.03 times that amount. For example, if you guess that there is 0.85 mL of dead space, set the syringe for 20µL.
2. Attach the syringe onto the N_2 sample port.
3. Turn on the FRC valve (to close it).
4. Freeze the waveform in FinePointe Station and use on the cursor to read back the stable pressure. Check it is at zero. If not, make note of the current value. Return the trace to Live.
5. Inject the syringe. The Mouth pressure signal should rise, and if there are no leaks, it will remain steady at that level. If there is a leak, please use the suggestions in the chapter "Test for leaks in the valve assembly".
6. Freeze the waveform in FinePointe and use on the cursor to read back the stable pressure. Find the difference from that stable value to the pressure before you injected the syringe. Use this difference as ***ΔP*** in the following equation.

$$Deadspace = \frac{V_{injected} \times P_{atm}}{\Delta P}$$

P_{atm} and ΔP must be in the same unit (cmH2O).

The computed ***Dead space*** is in the same units that $\boldsymbol{V_{injected}}$ is specified.

Note the following to ensure a good measurement:

- Make sure the pressure signal did not get saturated. If it did, then you should repeat this process with a smaller volume. If you do, make sure your $\boldsymbol{V_{injected}}$ reflects what you injected.
- Your ***ΔP*** should be more than 10cm H_2O. If not, try increasing the volume you inject. If you do, make

sure your $V_{injected}$ reflects what you injected.

- Be sure to specify P_{atm} in cm H_2O units—it should be somewhere near 1030 cm H_2O

Alternate Boyle's Law Method Without P_{atm}

If P_{atm} is not known, you can use this method to measure the dead space. This method requires you to make two injections, and to find two ΔP values. The two injections that you make must differ by a known volume (referred to here as V_b).

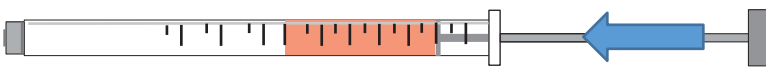
Ideally, you would want to make that known volume about ¼ the volume of the estimated dead space. And the volume you will inject ($V_{injected}$) could be 10 times smaller. It is desirable to use the same syringe to both provide the V_b and to allow you to inject $V_{injected}$.

So, if you estimate the dead space is 0.85ml, then you might want V_b to be around 200µL and you will want to inject about 20µL. This will require you to use a 250µL syringe, and so the accuracy of the 20µL injection is diminished from what it would be if you used a 50µL syringe. In short, you need to make a tradeoff between accuracy of the $V_{injected}$ and the accuracy of V_b.

To improve the situation, instead of injecting only 20µL, inject 50µL. This will improve your ability to have accurate values for both $V_{injected}$ and V_b. However, it requires that you recalibrate the mouth pressure to be less sensitive, to avoid saturation.

Procedure:

1. Calibrate Mouth Pressure so that you get an Effective Range of +/- 90 cmH2O. You will have to reduce the gain on the **M Pressure** preamplifier.
2. Attach your sealed tracheal catheter to the tracheal port, on the inside of the chamber.
3. Turn off the FRC valve (to open it).
4. Attach the syringe onto the N_2 sample port.
5. Pull the plunger of the syringe back 50µL (or $V_{injected}$)
6. Turn on the FRC valve (to close it).
7. Freeze the waveform in FinePointe Station and use on the cursor to read back the stable pressure. Check it is at zero. If not, make note of the current value. Return the trace to Live.
8. Inject the syringe. The Mouth pressure signal should rise, and if there are no leaks, it will remain steady at that level. If there is a leak, please use the suggestions in the chapter "Test for leaks in the valve assembly".
9. Freeze the waveform in FinePointe Station and use on the cursor to read back the stable pressure. Find the difference from that stable value to the pressure before you injected the syringe. Use this difference as ΔP_0 in the equation below.
10. Turn off the FRC valve (to open it).
11. Pull the plunger of the syringe back 250µL (or $V_{injected} + V_b$)
12. Turn on the FRC valve (to close it).
13. Freeze the waveform in FinePointe Station and use on the cursor to read back the stable pressure. Check it is at zero. If not, make note of the current value. Return the trace to Live.
14. Inject the syringe 50µL ($V_{injected}$) to 200µL (V_b) plunger position. The Mouth pressure signal should rise, and if there are no leaks, it will remain steady at that level.



15. Freeze the waveform in FinePointe Station and use on the cursor to read back the stable pressure.

Find the difference from that stable value to the pressure before you injected the syringe. Use this difference as ***ΔP₁*** in the equation below.

$$Deadspace = \frac{\Delta P_1 \times V_b}{\Delta P_0 - \Delta P_1}$$

Pressure Conversions and Useful Information

Conversion	Multiply by this value
Inches Mercury to cm H2O	34.53
Torr to cm H2O	1.3595
mm of Mercury to cm H2O	1.3595
Pascal to cm H2O	0.0102
Kilopascal to cm H2O	10.197
mb to cm H2O	1.0197

Sizes	
ID of 14 Gauge Sheath (Rat)	1.524 mm
ID of PE260 Tubing (Rat)	1.778 mm
ID of 19 Gauge Stainless Tubing	0.889 mm
ID of 18 Gauge Stainless Tubing	1.0668 mm
ID of 17 Gauge Stainless Tubing	1.27 mm

Hardware Configuration in FinePointe Control Panel

To setup the software, you need to create a hardware configuration in the FinePointe Control Panel. The hardware configuration will appear as a station from within FinePointe. This only needs to be done once. The hardware configuration tells the system how the hardware is setup and what species you intend to run. To create the configuration the software will step you though a 3-page wizard.

If you intend to use the same Pressure Panel for both Rat and Mouse chambers, then you will setup a separate hardware configuration for each.

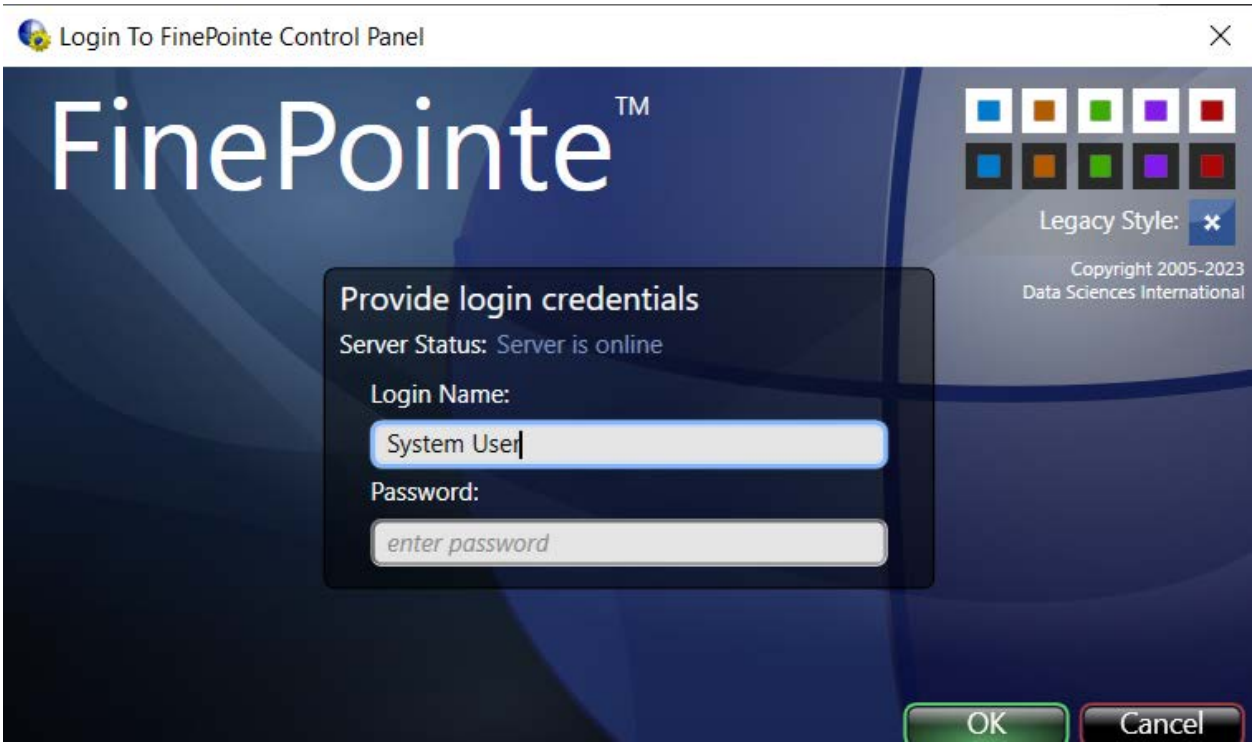
Once this is complete you will be able to create PFT studies which store the data within FinePointe Review and use the Station you defined in the Control panel to acquire data into them.

If you are installing the software for the first time, refer to the FinePointe Installation Guide for details. In this description, we assume the software has been installed and configured on the network appropriately.

Begin by running the FinePointe Control Panel. FinePointe Control panel requires administrative access to the computer, so if UAC (User Access Control) is enabled on your computer, you may be asked if you want to allow FinePointe Control Panel to make changes to your computer. You must respond Yes. Keep in mind that it is possible that UAC can prevent FinePointe Control Panel from running at all if your administrator has locked the system down. So, if FinePointe Control Panel does not appear to respond, contact a network administrator for assistance.

Note: The shortcut for FinePointe Control Panel must have the Run As Administrator option checked on the Compatibility Tab of the shortcut Properties form.

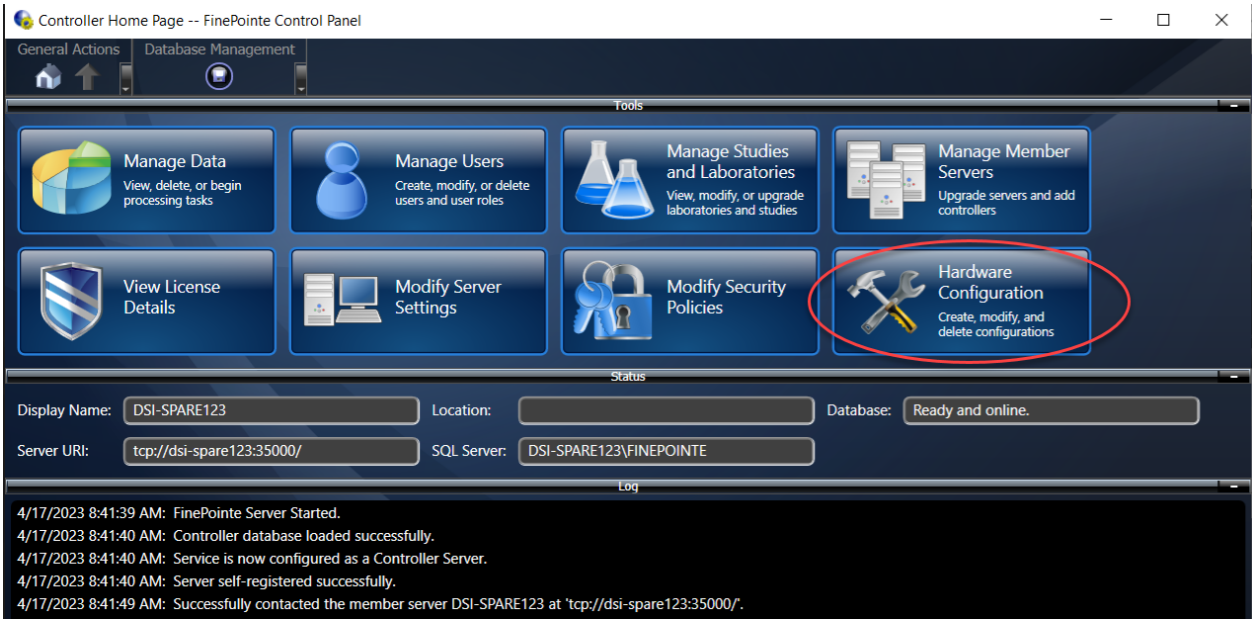
Assuming FinePointe Control Panel is allowed to run, you will first be presented with a login form as follows:



The Login Name and Password must be an administrator of this computer (not necessarily the user who logged in at the start of this Windows session). If you are on a network, you may have to explicitly specify the network domain where the Login Name can be found. It can be specified using one of the following formats:

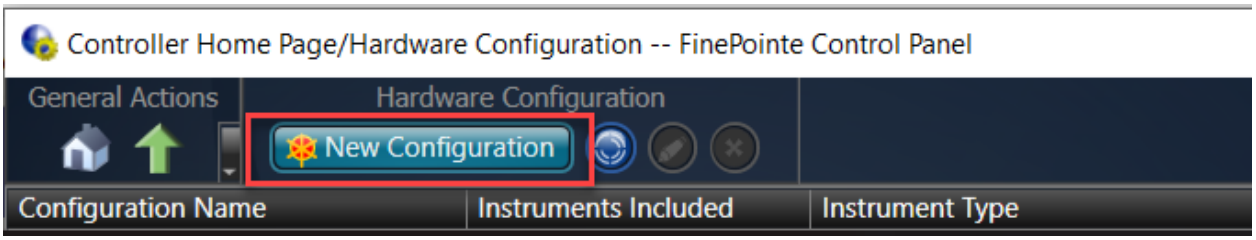
Login Name Format	Example
<Username> → Joe	Where the username is "Joe". The network domain is decided by the system.
<Domain>\<Username> → Buxco\Joe	Where the network domain is "Buxco", and the username is "Joe". A network domain can also be the local computer name. If you specify the local computer name, it will look for a local administrator account named "Joe".
<Username>@<Domain > → Joe@Buxco.com	Where the network domain is "Buxco.com", and the username is "Joe". A network domain can also be the local computer name. If you specify the local computer name, it will look for a local administrator account named "Joe".

Once you login successfully, you are presented with the main summary page of the server. This page may differ depending on the type of server it was configured as: Controller Server or Member Server. The most common is the Controller Server. In this case the following shows what you can expect to see.



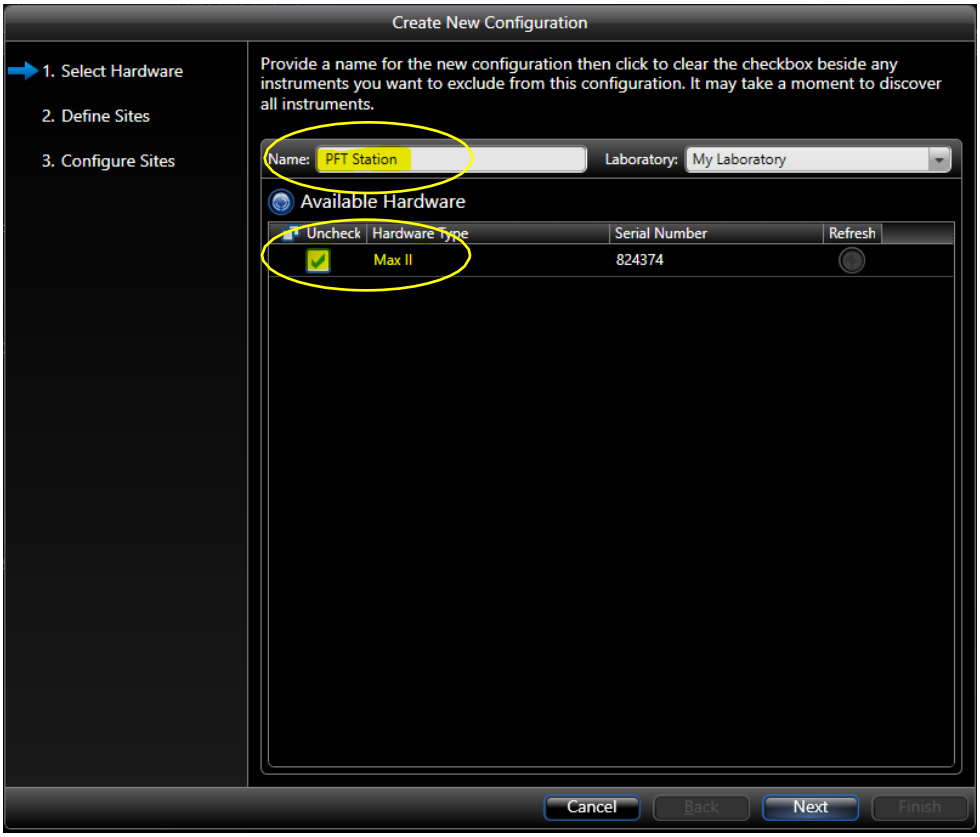
This picture shows FinePointe Control Panel Home Page for a Controller Server. A member server Home Page is similar but only has Data Upload, Modify Server Settings, and Hardware Configuration Buttons.

Click the Hardware Configuration button to add the hardware configuration.



The upper portion of the Hardware Configuration Page. Shown here, no Hardware Configurations have been created yet (so the list is empty).

Click the New Configuration button to start the Create New Configuration wizard.

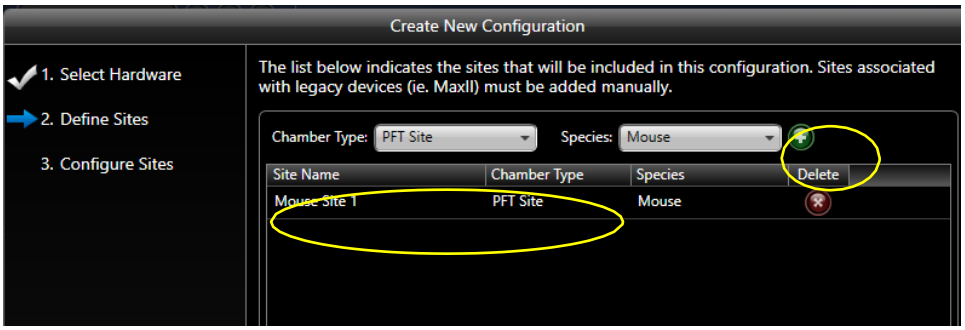


The first page of the Create New Configuration wizard.

The first page of the Create New Configuration looks similar to the one pictured above. Typically, you will only see the instruments you have connected to the PC listed here. Since the PFT Pressure Panel has a Max II amplifier built in, it appears as a Max II.

Make sure only the Pressure Panel is checked here and give the configuration a descriptive name. If you intend to share your Pressure Panel with a mouse and a rat chamber, then be sure to indicate the species in the name. For example: "Mouse PFT Station", "Rat PFT Station".

When you are satisfied click **Next**. Keep in mind also that you can change all these settings later if you feel you did not do it correctly.

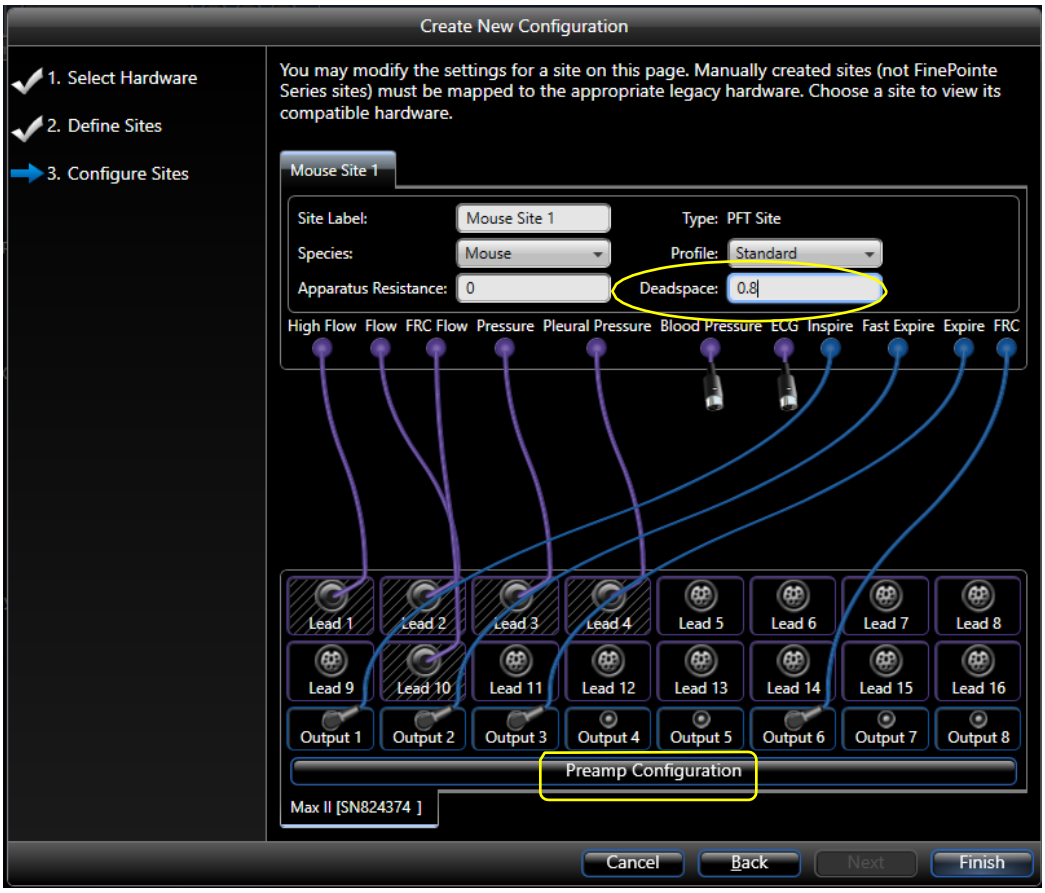


On this page you define the Site for your new PFT station.

Site is a term used throughout the software which refers to all the collection apparatus associated with the data collection of a single subject.

A Pressure Panel can be associated with no more than one PFT site. So here you will only put down as many sites as you have Pressure Panels (typically 1).

In the selection by Chamber Type, select "PFT Site" as shown. Next select the correct Species by the species selection. Finally, click the Green **+** button to add the site to the list. The Green **+** is circled. Your new site is listed in the list below those selection controls. Click **Next**.

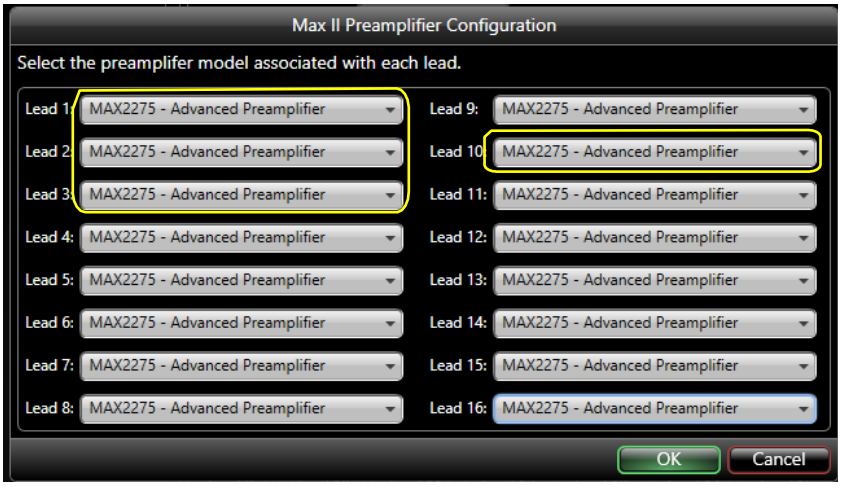


This page is where you need to indicate how each signal on the built-in Max II is configured. You do this by dragging connectors from the Site above to the jacks on the Max II instrument below. Make the same connections as you see here. Every Pressure Panel is built the same way, so it should be connected the same way here.

Pictured above a connection is made from Pleural Pressure to Lead 4. Most systems are not sold with this option. If your system was NOT sold with it, do not connect this lead, and simply leave the Pleural Pressure Lead on the Site disconnected.

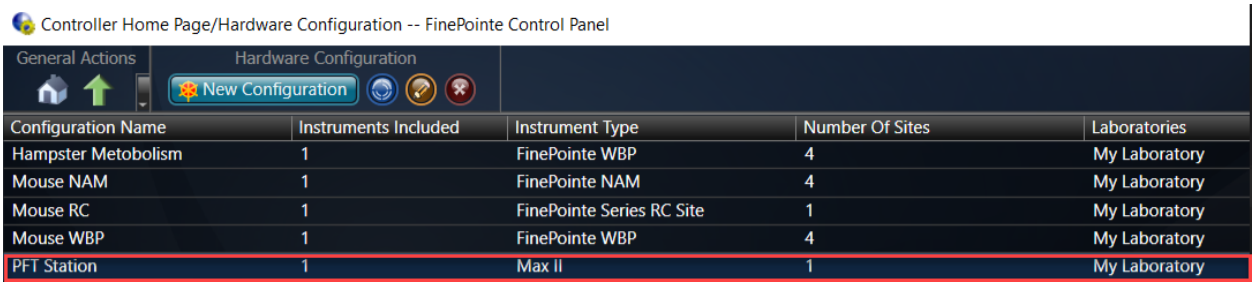
Once you have made those connections, enter the dead space value in the edit box beside Dead space as circled above.

Finally click the Preamp Configuration button. This button presents a dialog box which you use to indicate the hardware module which is currently creating each signal. This information is used by the system to customize the calibration wizard precisely to your system. It will not affect data collection beyond that.



In this form you need to tell the system what type of preamplifier you have connected to Lead 1, Lead 2, Lead 3, (optionally) Lead 4, and Lead 10. Lead 1, Lead 2, and Lead 10 are all from the Flow Preamplifier (at different gain settings). If you look at the preamplifier, it is either labeled Advanced Preamplifier or simply Strain Gauge Preamplifier. Shown here, all the relevant leads are connected to the Advanced Preamplifier. You should set yours to the type of preamplifiers you have in your system.

Click **OK**. Then click **Finish** on the Create New Configuration wizard.



Configuration Name	Instruments Included	Instrument Type	Number Of Sites	Laboratories
Hampster Metobolism	1	FinePointe WBP	4	My Laboratory
Mouse NAM	1	FinePointe NAM	4	My Laboratory
Mouse RC	1	FinePointe Series RC Site	1	My Laboratory
Mouse WBP	1	FinePointe WBP	4	My Laboratory
PFT Station	1	Max II	1	My Laboratory

Once you have created your hardware configuration, it appears in the list as shown in the image above. You can select it and click the Pencil button to edit it, or you can create another one of a different species if you intend to share your Pressure Panel with different chambers.

System Calibration

To calibrate the PFT signals, you need to calibrate 2 or 3 transducers. These include flow, mouth pressure, and (optionally) pleural pressure. The flow transducer requires 3 separate calibrations since it appears on 3 leads, each lead at a different sensitivity: High Flow, Flow, and FRC Flow.

You should calibrate once at the start of each day you intend to use the system. Calibration is a quick check that key parts of your system are functioning properly. For example, when you calibrate Mouth pressure, you also test the manifold for leaks. When you calibrate flow, you test the plethysmograph for linearity and gross changes in sensitivity.

Definitions of Signals

The following is a list of leads that you might use during your maneuvers.

Lead Name	Definition
FRC Flow	A more sensitive flow signal which is used to measure flow while the animal is occluded. Flow x 5.
Flow	Flow is amplified an extra 4 or 8 times depending on the species, (jumper selectable on the Signal Generator Module) for normal flow rates. Flow is negative on inspiration.
High Flow	High Flow is the low gain flow signal for measuring high flow rates during the FV tests. High Flow is negative on inspiration.
Mouth/ Lung Pressure	Mouth Pressure is the pressure at the mouth of the subject. Its purpose is to monitor the lung inflation and to protect the subject's lungs from dangerous pressure levels. Mouth pressure is positive when higher pressure is applied to the airway.
Pleural/ Pulmonary Pressure	Pulmonary Pressure is the lung pressure. This can be esophageal pressure measured with an esophageal catheter.

Valves

The four solenoid valves in this system can set to be controlled by the computer (Auto mode) but may also be thrown manually (On or Off). The combined operation of these valves distinguishes the three different maneuvers.

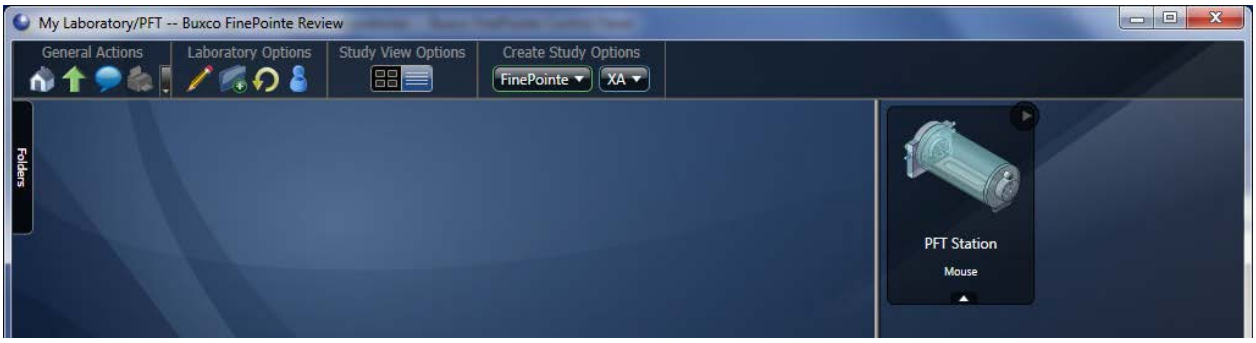
Digital Out	Valve	Description
1	**Inspiration**	When open for inspiration, air is forced into the animal's lungs at the set flow rate.
2	**Fast Expiration**	This valve performs a fast expiration. It is connected directly to the Negative Reservoir. When open, there is little to no resistance as the air is quickly sucked out of the animal's lungs.
3	**Slow Expiration**	This valve is connected to the negative reservoir through the Slow Expiration Flow control valve on the front panel. When open, this valve forces the animal to expire at the set flow rate.

6	**FRC**	This is a low resistance valve. When activated (closed), it blocks the animal's air path. When no test is running, it is open, allowing the animal to breath voluntarily.

Calibration Procedure

1 - Open the Calibration Window

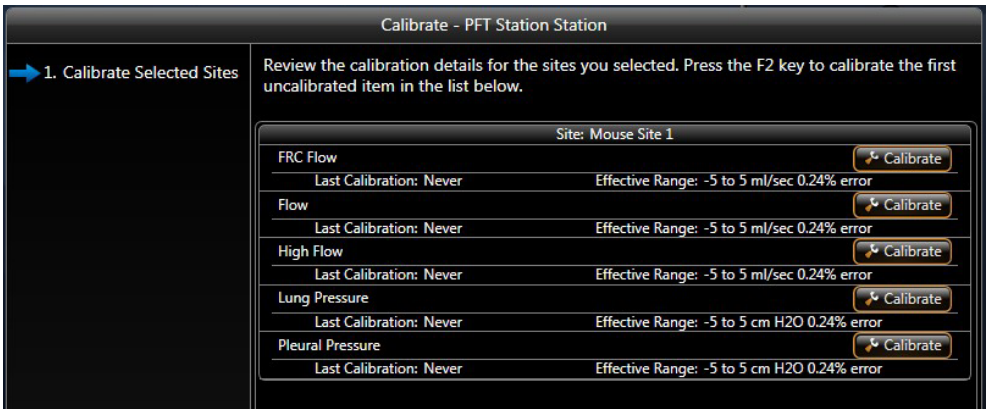
To calibrate, bring up the Laboratory page in FinePointe Review.



The area on the right is where you see icons for the stations. As shown here, there is one station icon named PFT Station.

When you move the mouse over the station icon, a little toolbar becomes exposed. This toolbar presents buttons for actions you can perform on the station. One of the actions is **Calibrate** the station. The following image illustrates this toolbar.

Hover the mouse over the station icon you want to calibrate and click on the wrench icon.



This picture shows the main calibration page for a PFT Station before it has been calibrated. Once calibrated, the "Last Calibration" will indicate when the signal was last calibrated, and the "Effective Range" will indicate the last effective range determined during calibration.

When you calibrate, it is important that the flow calibrations be performed in the order presented on the calibration page. You must calibrate FRC Flow first, followed by Flow, and then High Flow last.

If preamplifier adjustments to the Flow preamplifier are necessary, you should do so only during the calibration of the FRC Flow. If you need to adjust the preamplifier gain during the Flow or High Flow calibration, you must repeat calibration of the other flow leads.

You can calibrate the **Lung Pressure** at any time, and same is true of the **Pleural Pressure** (if you have that optional signal).

Typically, the first time you calibrate, you will need to adjust the preamplifier which will not be necessary for subsequent calibrations. Because of this, your first calibration will take more time. But afterwards, you will be able to run through this very quickly, usually making no preamplifier adjustments and only applying the calibration standards.

2 - Calibrate Flows

Before you begin calibrating the flows, place a luer plug in the tracheal port to ensure the manifold is isolated from the chamber.

Begin by calibrating **FRC Flow**. Click the Calibrate button for **FRC Flow**.

The calibration wizard begins at step 2 “Balance the Transducer”. As with all wizards in FinePointe software, the list at the left shows you the steps you will need to complete in order to finish the wizard, indicating which step you are on by the blue arrow. The scrolling chart on the right shows you the current live

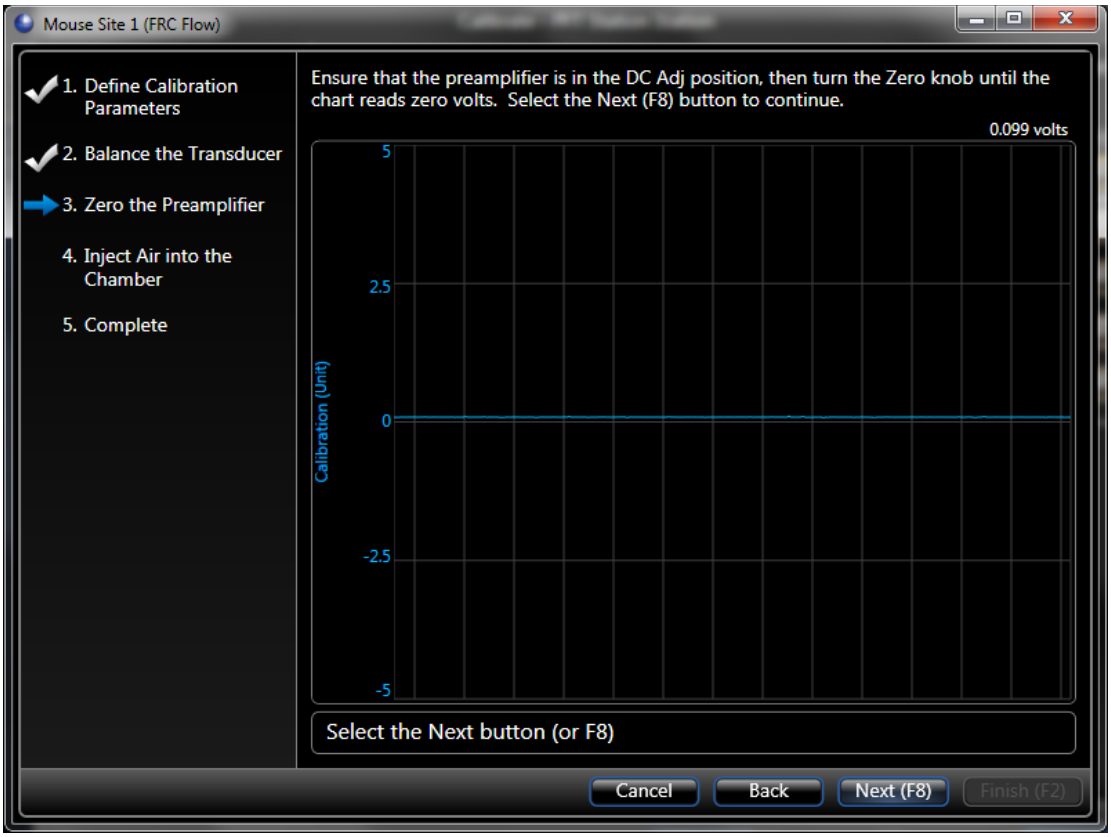
waveform. The text above the chart describes what you need to do during this step.

Your calibration wizard may be slightly different if you are not using the Advanced Preamplifiers. If you see differences, please follow the instructions as they are stated on your calibration wizard.

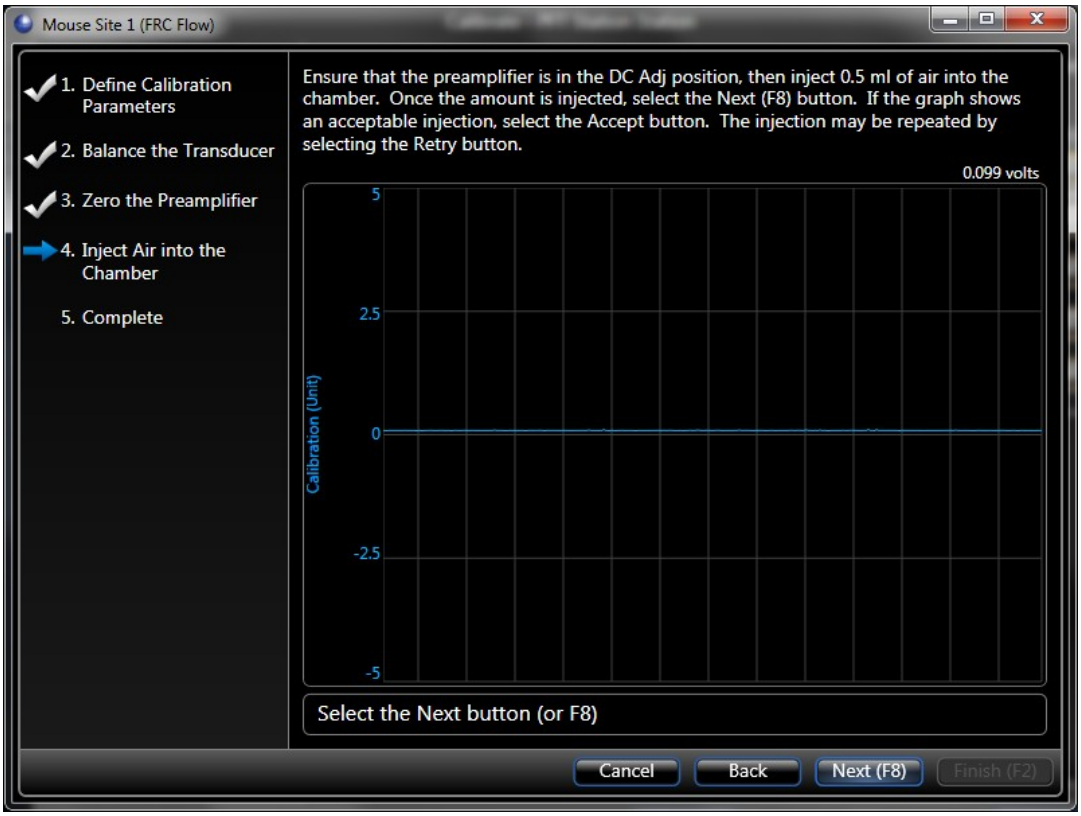
As indicated above the chart in the step, you need to turn the Balance screw on the preamplifier module until the chart reads 0 volts. You can see the voltage reading in the upper right-hand corner of the chart (circled in yellow). It does not have to be exactly 0 volts. The system will check to determine if you are close enough.

When you hit **Next**, the FinePointe software checks the waveform to determine if you have followed the instructions.

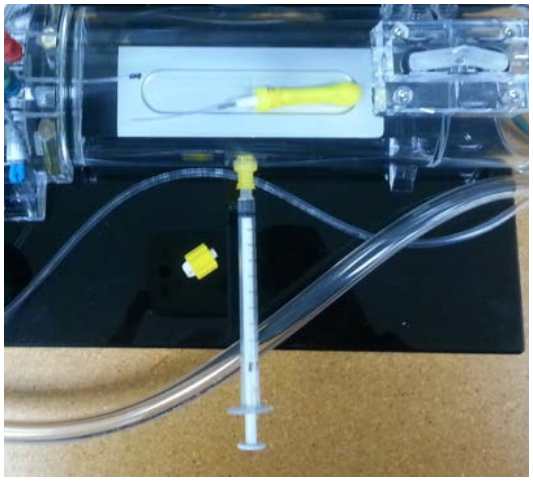
Any warnings are indicated below the chart as shown in the following screenshot. In this example, the instructions say you must turn the Balance screw until the voltage is at zero. FinePointe checked and the signal is too high (it is 0.693V). Click **Next**.



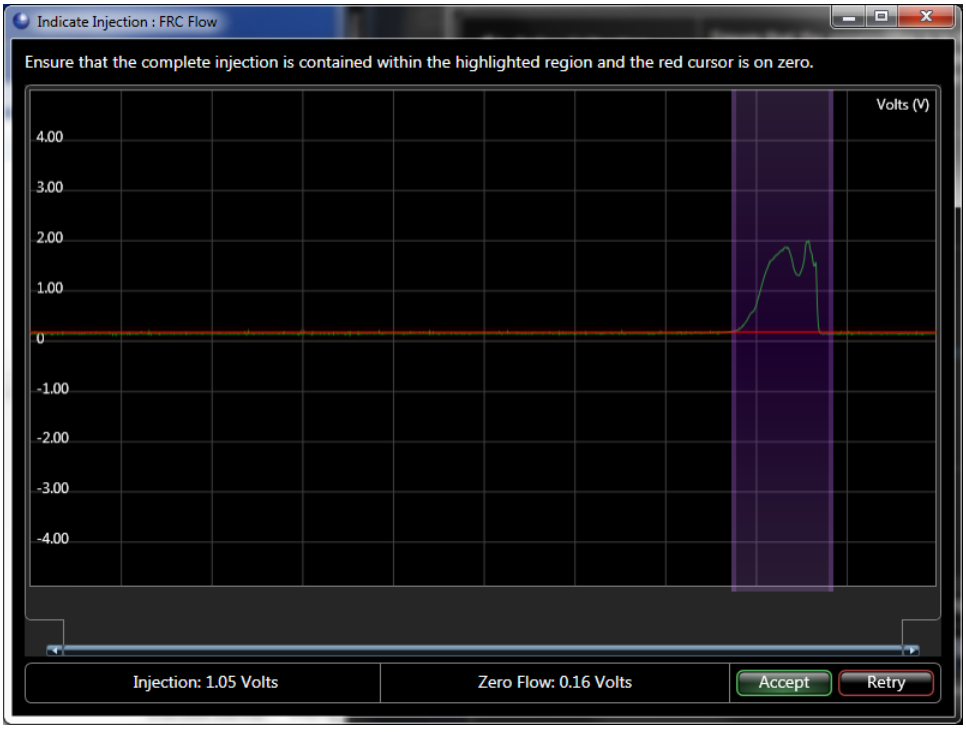
This time, FinePointe wants you to turn the zero knob until the signal is 0 volts. Do so and click **Next**.



In this step, you must inject 0.5mL into the chamber.



Use the 1mL syringe and inject the 0.5mL volume fast enough, but without exceeding ±5volts on the chart. This may take some practice and you can repeat it as many times as you want. Before you deliver the volume, make sure the entire chart is at zero. When you feel you have a good injection, click **Next** while the entire injection is still on the chart. If part of the injection runs off the chart, then you must repeat it. After you click **Next**, and FinePointe has checked your delivery is good, it will freeze the injection for you to accept.

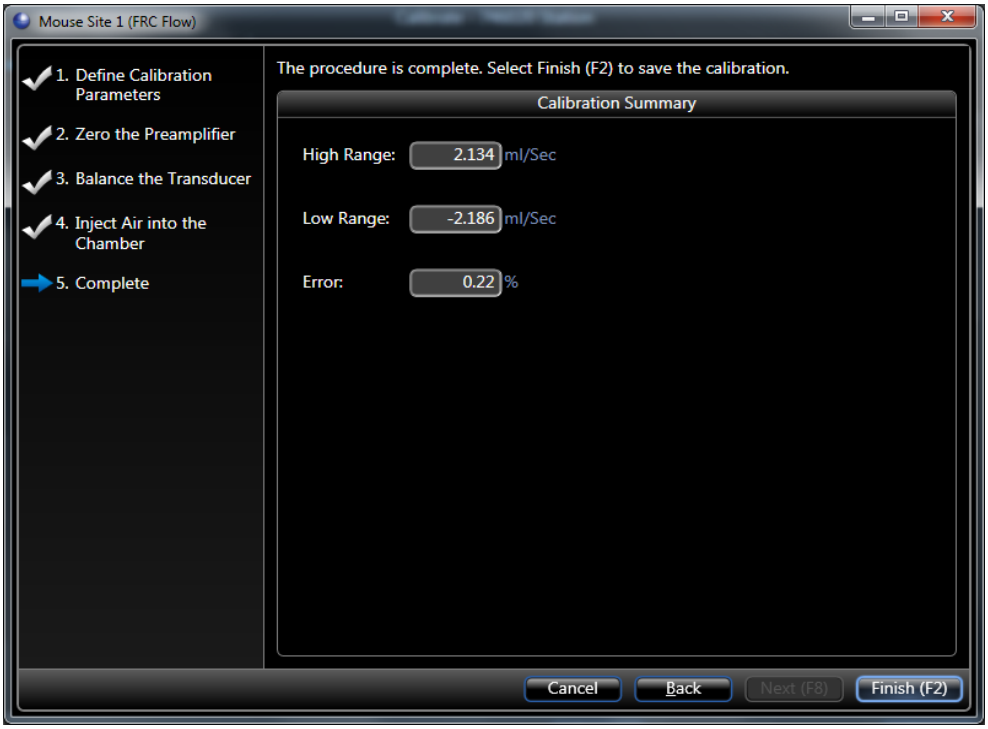


Note: The polarity of the voltage signal may be inverted from what you see here. That is due to the orientation of the transducer. If you choose, you can leave it inverted. The calibration process will correct it. Or you can reverse the way the transducer is plugged in and retry this injection.

When you look at the frozen screen like this, you should go through the following check list:

Is the entire injection captured?
As shown above it clearly is. You can see this because the signal starts at zero, rises, and then returns to zero. If not, you must **Retry** the injection.
Is the red line firmly on zero?
If not, you can move it down to zero or **Retry** the injection.
Is the entire injection within the shaded blue area?
If not, you can adjust the shaded area using your mouse, or **Retry** the injection.

If you are satisfied that your injection, **Accept** the injection to continue to the last page of the wizard.



The final page of each calibration presents you with the effective range of that signal as determined by that calibration.

The calibration wizards for the other two flows are essentially identical except the volume you inject is progressively larger, and you should not touch the preamplifier at all. If you do, you need to repeat the **FRC Flow** calibration.

Since all the flows are derived from the same preamplifier and are created by applying fixed gains, you should observe that same relationship between the effective ranges of each of the flows. So, since the **Flow** gain is 4x (for mouse) or 8x (for all other species) the gain used for **High Flow**, and **FRC Flow** gain is 5x **Flow** gain, then the **High Flow** effective range is 4x or 8x the effective range of **Flow**, and the effective range for **Flow** is 5x the effective range of **FRC Flow**.

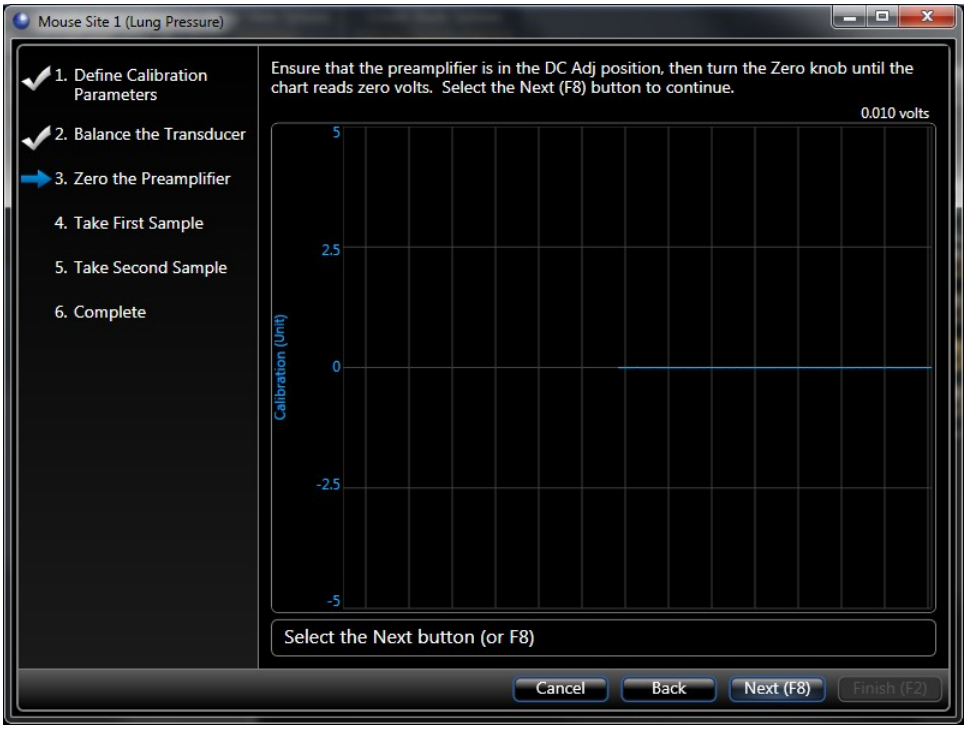
3 - Calibrate Pressure

Before you begin, put a luer plug in the tracheal port. Attach the calibrator tubing and a 10 mL syringe to a luer T, then attach the luer T to the N_2 Sample port.

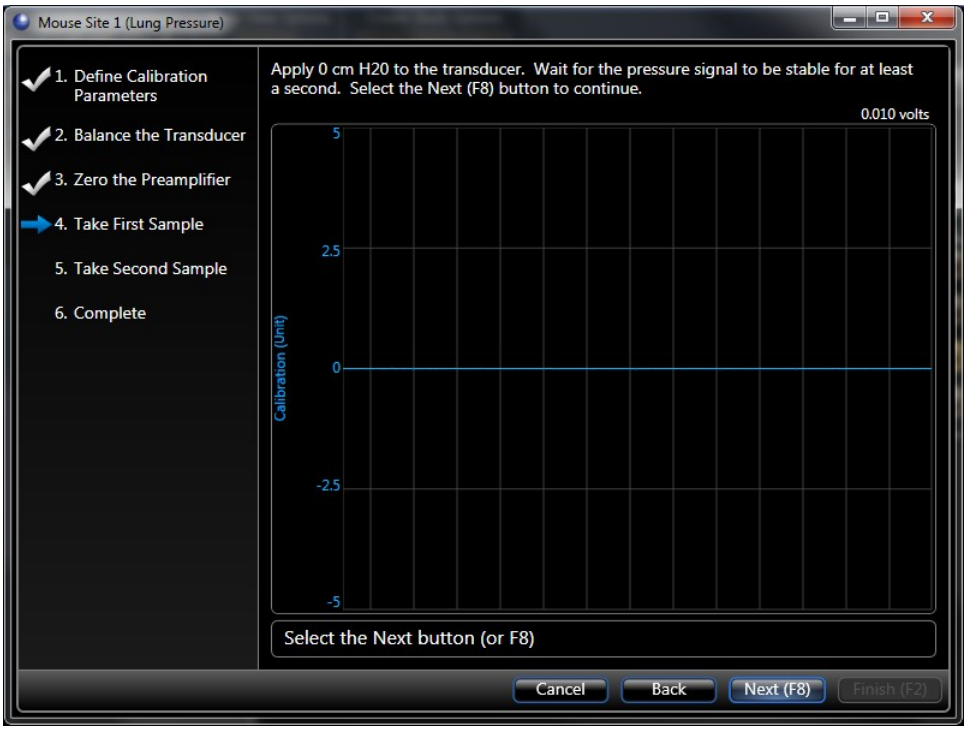
You will need this so that you can push air into the manifold and the calibrator simultaneously.

Click the Calibrate button for **Lung Pressure**.

The calibration wizard begins at step 2 “Balance the Transducer”. Turn the Balance screw until the voltage reads zero. If it is close to zero, just click **Next**. If FinePointe accepts it and goes to the next step, then you are close enough, otherwise it will warn you are described earlier in the Flow calibration.



Next switch to the DC Adj switch position on the preamplifier and turn the zero knob to zero volts. If it is close to zero, just click **Next**. If FinePointe accepts it and goes to the next step, then you are close enough, otherwise it will warn you are described above in the Flow calibration.

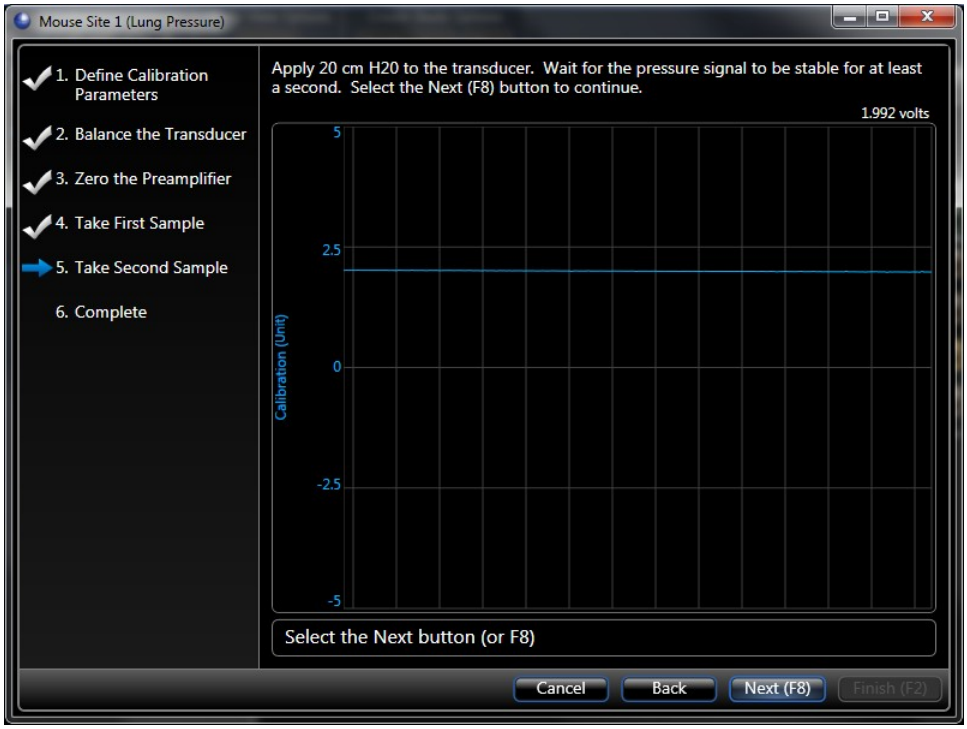


Make sure the FRC valve is off (open). By turning the FRC valve off, you ensure the pressure is at zero.

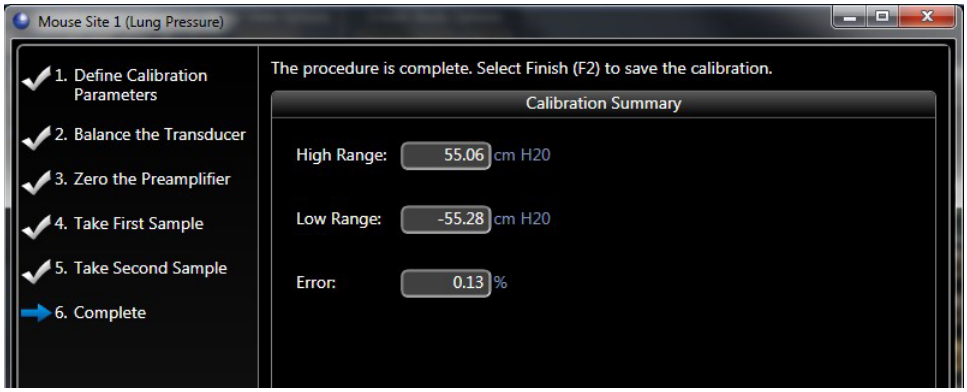
Click **Next**.

With the FRC Valve off, slowly draw the syringe back so that the syringe is full of air.

Turn on the FRC Valve to close off the manifold, and then force the plunger of the syringe down until the calibrator is at 20cm H_2O.



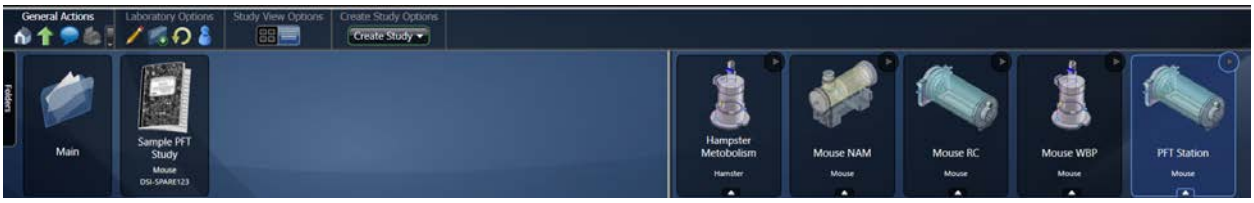
At this point, the voltage should not drift down. If it drifts down, then you have a leak, and you should identify and fix the leak in the manifold. If it is stable (like pictured above), click **Next**.



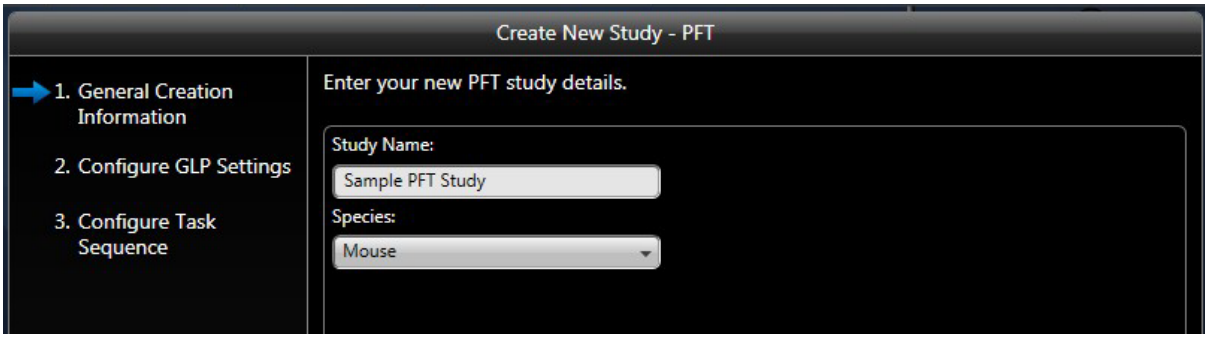
The final page of the **Lung Pressure** calibration presents the effective range of the pressure. This calibration should be the same for all species and should be between ±45cm H_2O and ±60 cm H_2O.

Creating a PFT Study

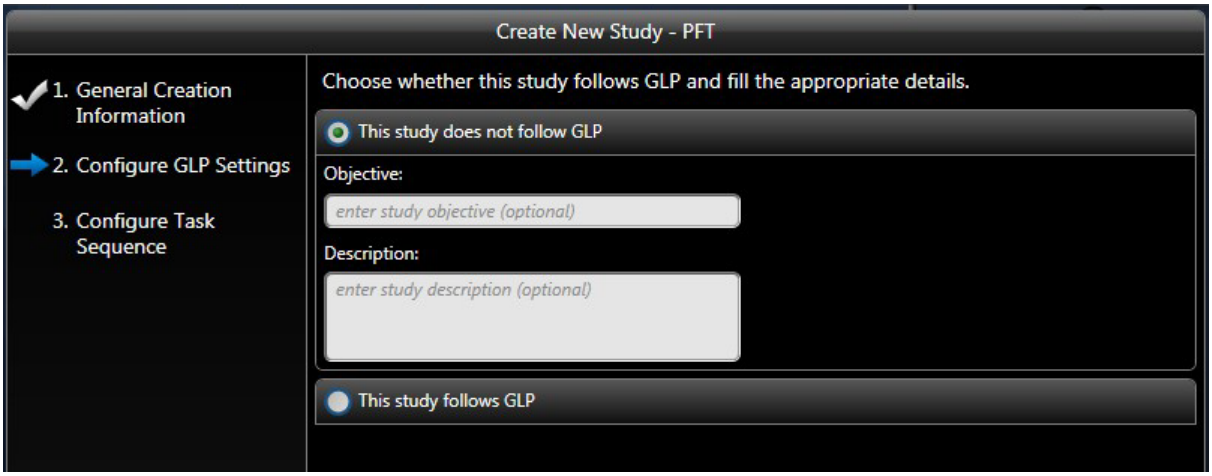
To create a PFT Study, start at the Home page in FinePointe Review.



Click the **FinePointe** → **PFT Study** button under **Create study Options.**

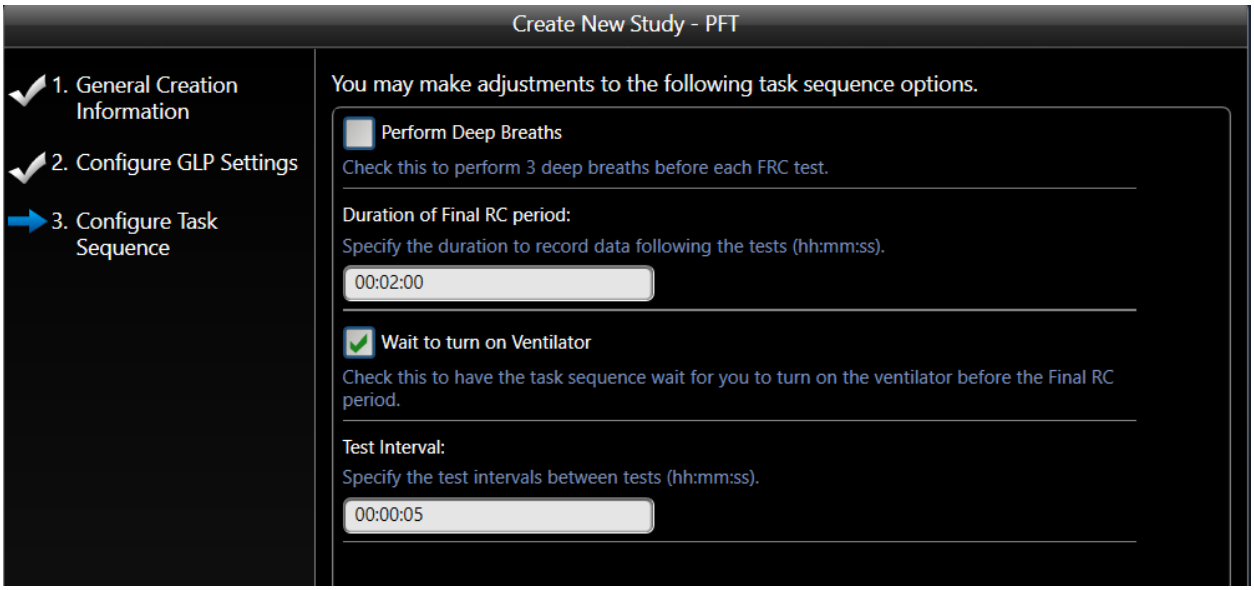


Give your study a descriptive name and select the species you intend to acquire. Click **Next**.



This page allows you to provide some descriptive information about the study. All this content is optional.

Click **Next**.



On this page you can fill in some specifics about how you want to collect data. Generally speaking, we recommend you save the tidal breathing for the end (after you perform the tests). This way the subject is fresh for the FRC tests. So, fill in the following:

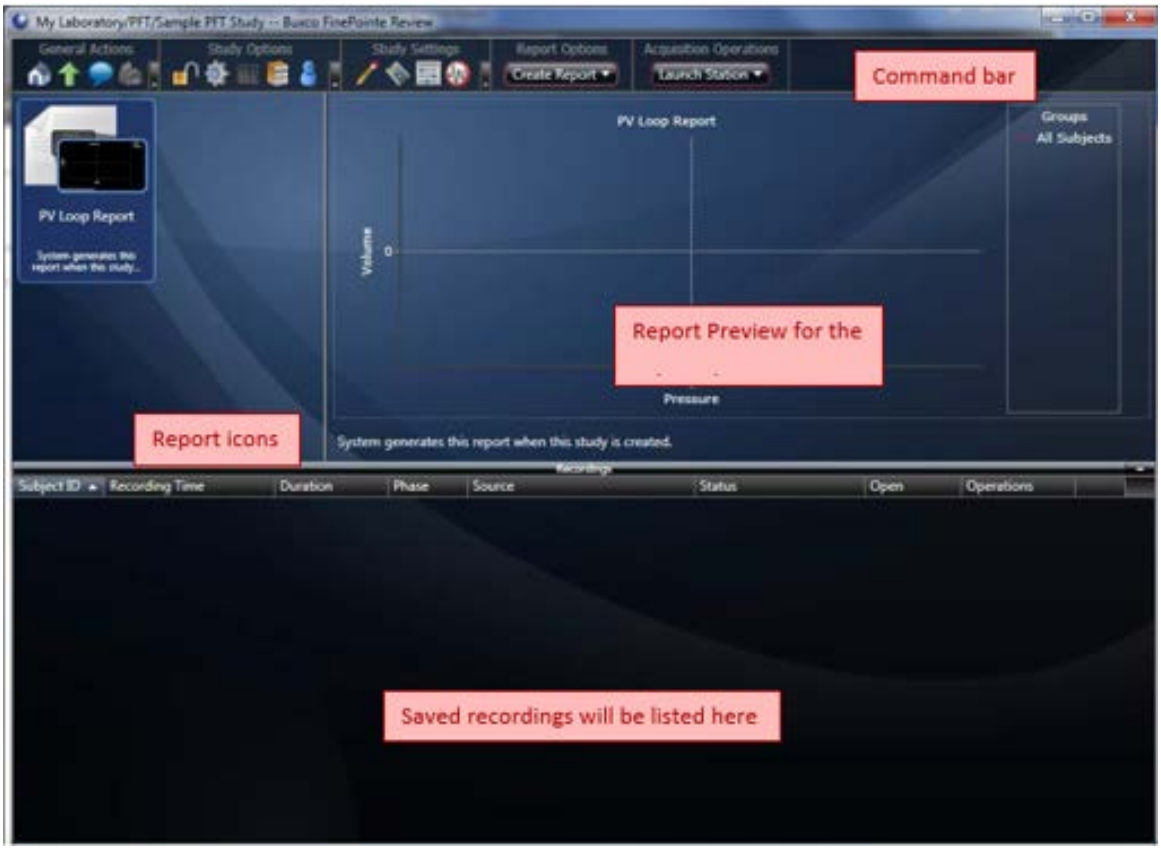
Duration of Acclimation period: **00:00:00**

Duration of Final RC period: **00:02:00**

Test Interval: **00:00:05** (You may want to increase this for larger animals).

Click **Finish**.

Once you click finish, FinePointe creates the study database and opens the study. The image below shows what an empty study will look like.



The study page has a command bar across the top. This bar has buttons which allow you to access forms and other pages within the study. If you hover the mouse over a button, a tool tip will provide you with a text description of the operation performed, page navigated to, or form opened.

The report icons show a single icon for each report. You can have as many reports as you want within a study. If you double click on a report icon, you can open the report and get complete details about the report. Also, you can modify characteristics of the report.

The report preview gives you a visual preview of the report icon selected in the report icons area. You can quickly review data in the reports by single clicking each report icon and looking at the report preview.

The list below the reports shows you the recordings you made. Each time you acquire data for a subject, you will have a row added to this list once the data has been committed to the database. If you double click on a row, you can open the recording and view the data just as it was recorded.

Acquiring Data

When you acquire data, you will find that the actual acquisition of the data takes very little time. The key to consistency and validity of the data is how you manage the subject within the apparatus. One factor which determines much of what you do is the dead space in the system. In the smaller animals, the dead space in the system is at least large compared to the tidal volume of the subject, so you will need to be vigilant about clearing CO_2 from the manifold.

When you acquire data from a mouse, the dead space is much greater than the tidal volume of the mouse. In the case of a rat, the dead space makes up a significant percentage of the rat's tidal volume. In all tests, it is important that the test begins at FRC, and if the animal is rebreathing air from the manifold, the FRC will fall rapidly as the animal breathes deeper to get more oxygen.

Moreover, in previous systems, we used to recommend that the user run first by ventilating the subject for a couple minutes while acquiring baseline Resistance & Compliance data. We found that mice would become hyperventilated and as a result, would not try to breathe when the FRC tests were run. The users would have to turn off the ventilator for about a minute before the mouse would begin to breathe on its own again. It may still be possible to run this way, but the preferred approach is to begin running the FRC test almost immediately and save the ventilated Resistance & Compliance baseline collection for after the execution of the tests.

So here are some things you should do:

- Be sure to cut the tracheal tube to a length as short as possible
- Whenever the subject's tracheal tube is attached to the apparatus, and not currently running a test, turn on the **Inspiration** valve on the Valve Control module so that the subject can get fresh air, and make sure the FRC valve is set to Auto (open).
- Be quick an efficient when you run the tests. Taking extra time opens the opportunity for you to deprive the subject of oxygen, and this will adversely affect your result.
- After you complete the tests, turn on the ventilator and get tidal breathing data (RC).

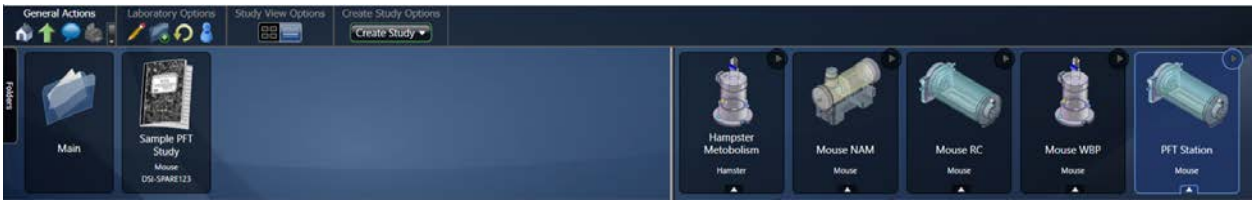
Launching Acquisition

Before you can begin data collection, you will need to create a PFT Study. If you've already created one before, and even collected data, you can use that study to collect additional data. Keep in mind that the reporting capabilities of FinePointe exist within a single study, so you will only want to collect data into a study which you intend to report together.

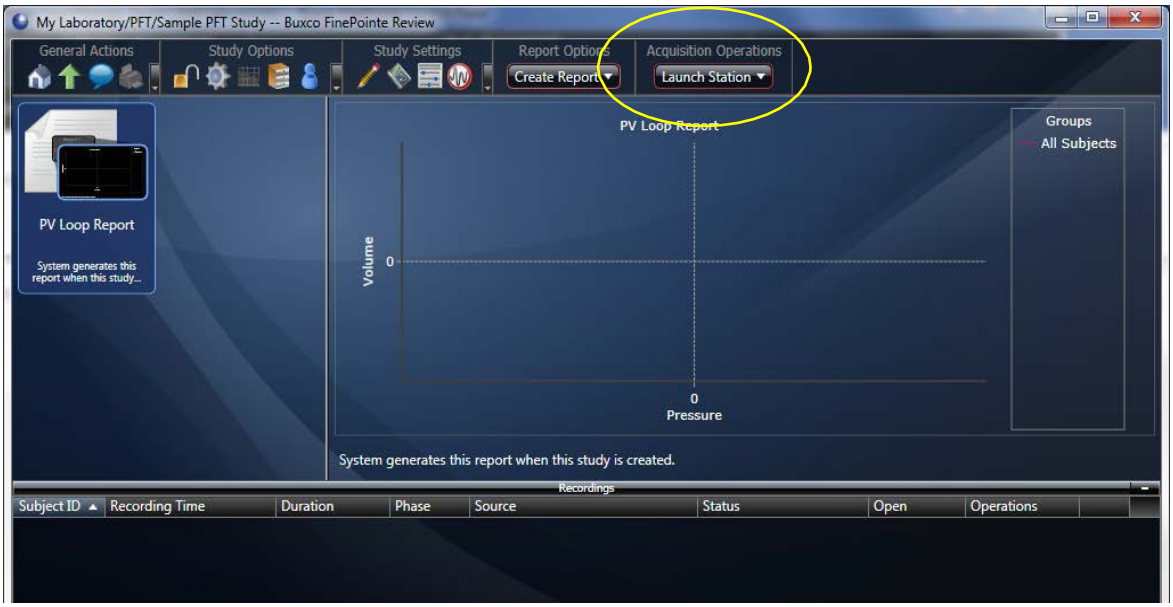
In addition to having a study already created, you will also need to calibrate beforehand. Refer to the section on Calibrating for instructions in doing this.

There are two ways to launch acquisition. You can launch acquisition from the FinePointe Home page, or you can launch it from the Study page.

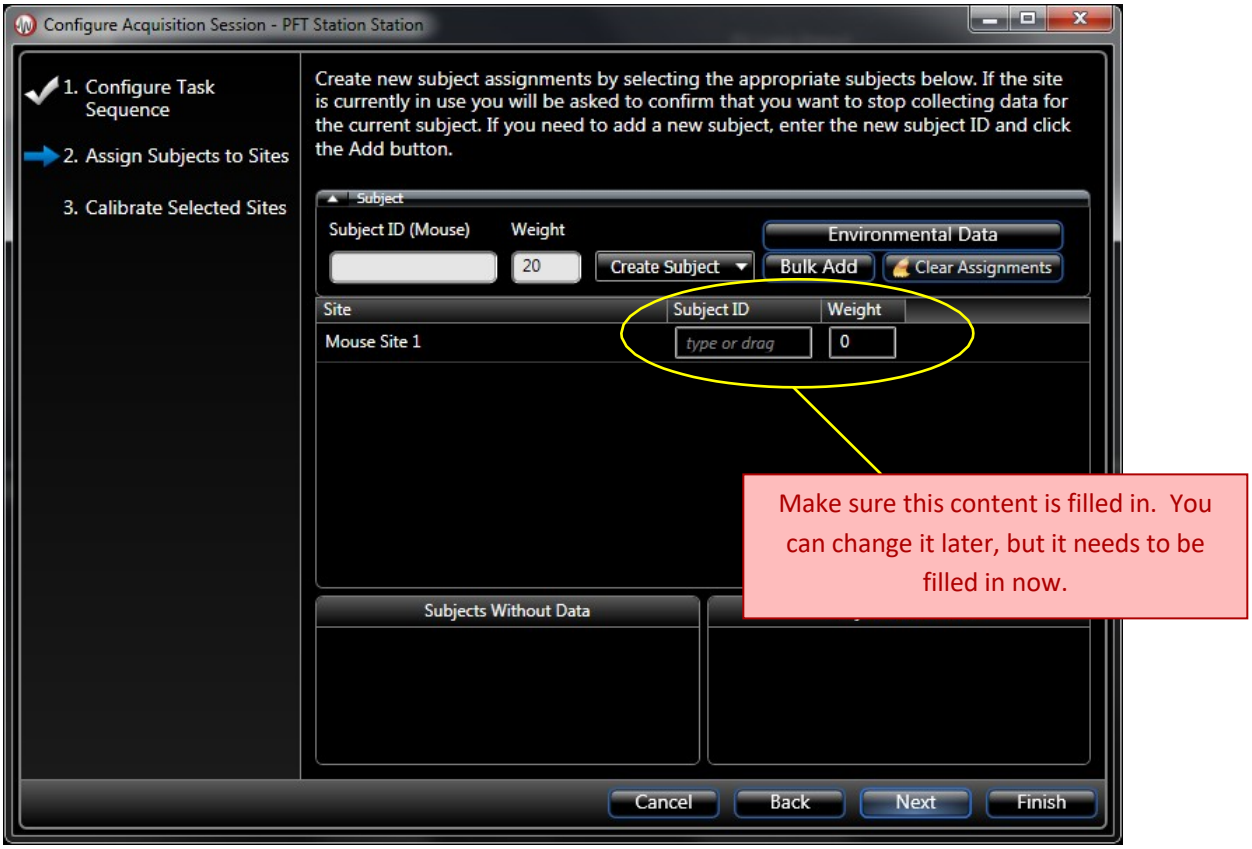
To launch from the Home page, you can select the study you want to acquire to, and drag the study and drop it on the station icon you want to acquire with.



To launch acquisition from the Study page, open the study you want to acquire data to, and select Launch Station button on the command bar of the Study page.



After you launch acquisition, FinePointe Station opens to the Assign Subject IDs page. In this page you identify the subject in the PFT chamber. The subject ID can be almost any nonblank text.



The Assign Subjects page provides a flexible way to associate a subject ID with this recording. In this page, you can create a new subject ID or use an ID which has already been created in the study. The page has a Subject ID and

Weight edit box followed by a Create Subject and Bulk add buttons. These controls allow you to create new subject IDs. The **Clear Assignments** button restores the form back to its initial state (like you see above).

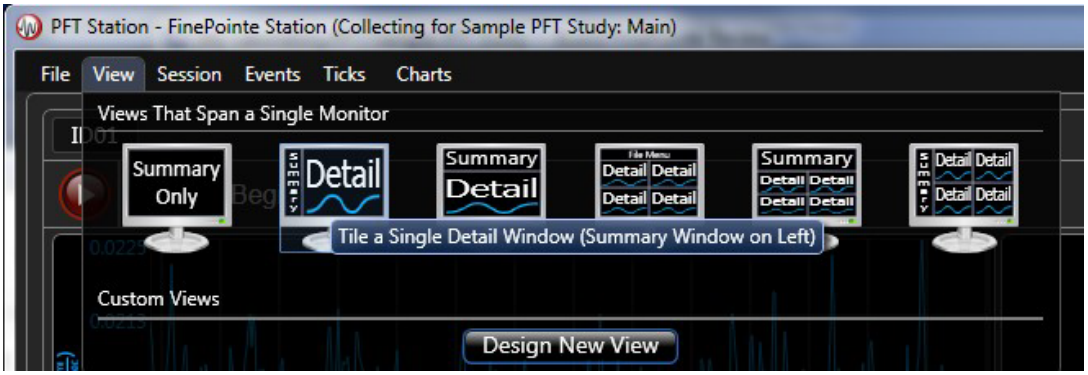
When you click Create Subject, the subject ID and weight that you entered in the edit boxes beside the button is added to the next unassigned Site. In this case there is only one site.

Subject ID's, which are already in the study, are presented in the two lists at the bottom of the page: **Subjects Without Data**, and **Subject With Data**. Just as the description suggests, the subject IDs in the **Subjects With Data** list already have data acquired in the study. **Subjects Without Data** contains subject IDs which have been created in the Study, but there has not yet been any data acquired for them. You can drag an item from these lists and drop them on the Site to assign an existing subject ID to that site.

You should also be sure to fill in the environmental data too. This data is used by the FRC analysis and will affect the results. Should you forget, it can also be modified later from within the study and by clicking the **Edit Environmental Data** in the Operations column of the recording.

After you have specified the subject ID and clicked the **Finish** button, FinePointe station begins acquiring live data. If you are running for the first time, it starts up with the Summary view.

You can easily change the view by selecting a view you feel is suitable under the **View** menu. For this description, the view second from the left is chosen.



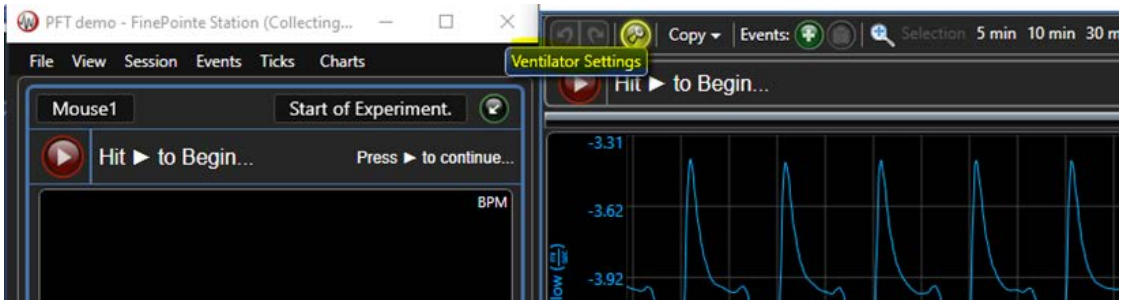
After you select the view, FinePointe Station reconfigures the display as follows:

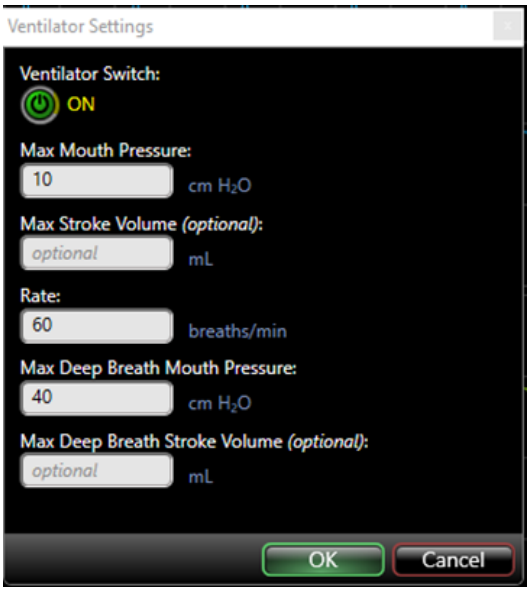
FinePointe Stations remembers the last view you selected so next time you run, you will not need to do this.

Loading the subject

At this point, you are ready to load the subject. Here is a suggested procedure:

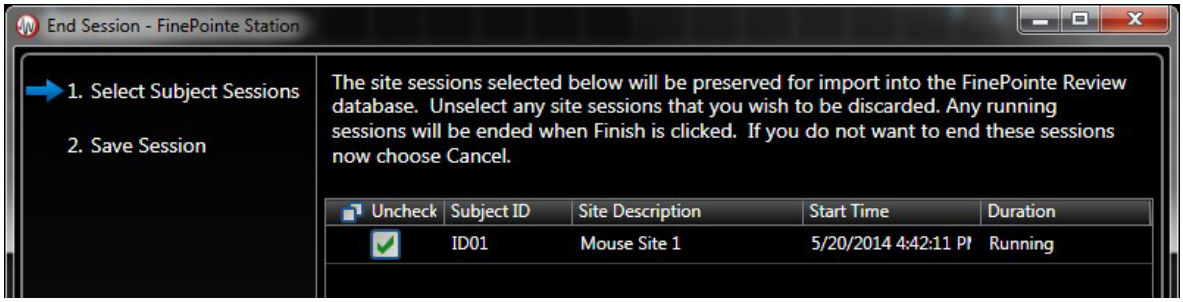
1) Make sure the **FRC** valve is **off** - in Auto Position - to open the manifold to the atmosphere.
2) Make sure the **Inspiration** valve is **on** to bring fresh air in the manifold and clear CO2 build up
3) Other switches on the Pressure Panel should be in the Auto position
4) Connect the subject's tracheal tube to the manifold inside the chamber
5) Make sure that there is no leak around the trachea. If there is a leak, it won't be possible to inflate the lungs to TLC during the PV and FV tests.
6) Inspect the height of the subject to ensure the end of the tracheal tube is not obstructed by the trachea itself due to the subject's position. Add tissue paper beneath the subject or lower the bed as necessary.
7) Inspect the tracheal tube to ensure it is aligned straight. This is critical for the FV test.
8) Close up the chamber.
9) Watch the flow and pressure signals on the chart to ensure the subject is breathing with a regular and even rhythm.
10) Flip the **Inspiration** valve to the Auto position.
11) Immediately press the Play (▶) button on the screen to start the task sequence.
12) The tests will run automatically (3x FRC tests, 3x PV tests and 3x FV tests).
13) After the tests have completed, turn on the ventilator for the RC measurements at the end.





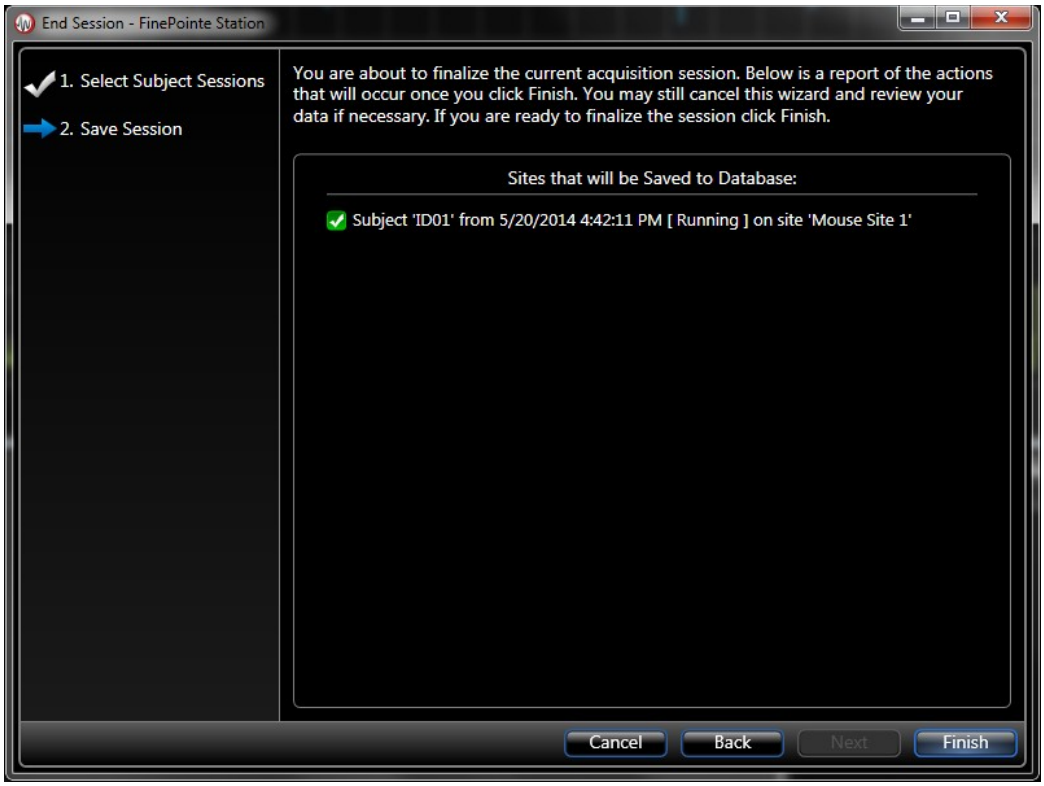
Saving Your Data

After you complete the task sequence, exit FinePointe Station (**File->End Session**) and be sure to leave the box checked to save the data to the study.



Note that any *unchecked* recordings are *deleted and not saved* to the study.

Once you have made your selection, click **Next**.



The final wizard page presents you with a summary of which recordings FinePointe will save to the study. Click **Back** or **Cancel** if you want to make changes, otherwise click **Finish**.

If you click Finish, FinePointe will begin the process of importing the data into the SQL study. This can take a few minutes, but you can launch FinePointe Station again (even before the data is fully imported) and begin data collection of the next subject.

Reports

The reporting capabilities built into FinePointe are designed to provide a convenient means to quickly combine subjects into treatment groups and summarize your data. Since the report processing is automated, you can see your results almost immediately. To fully understand how the reporting works within FinePointe, you need to understand some simple concepts.

Reports in FinePointe produce basic ANOVA group statistics and group summaries. A **group** is a group of subjects identified with a unique ID. These groups can be set up at any time and modified at any time. When a group is modified, the reports that refer to that group are automatically recalculated.

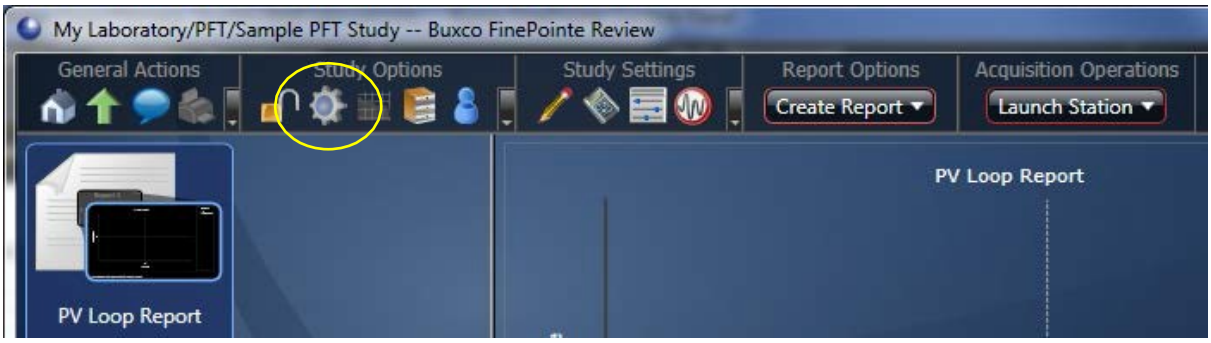
To create your reports, you first need to identify **Measurements** within each recording. A **measurement** represents a region of time which will be summarized in one or more reports. Typically, the measurements are placed automatically when you acquire data. However, if you choose, you can modify the placement of those measurements in the "Place Measurement" View. Anytime you modify a measurement, the reports which summarize that measurement are automatically updated.

FinePointe allows you to mark ranges of data, recordings, or individual tests as rejected. When data is rejected, it remains stored in the study, but is excluded from reports.

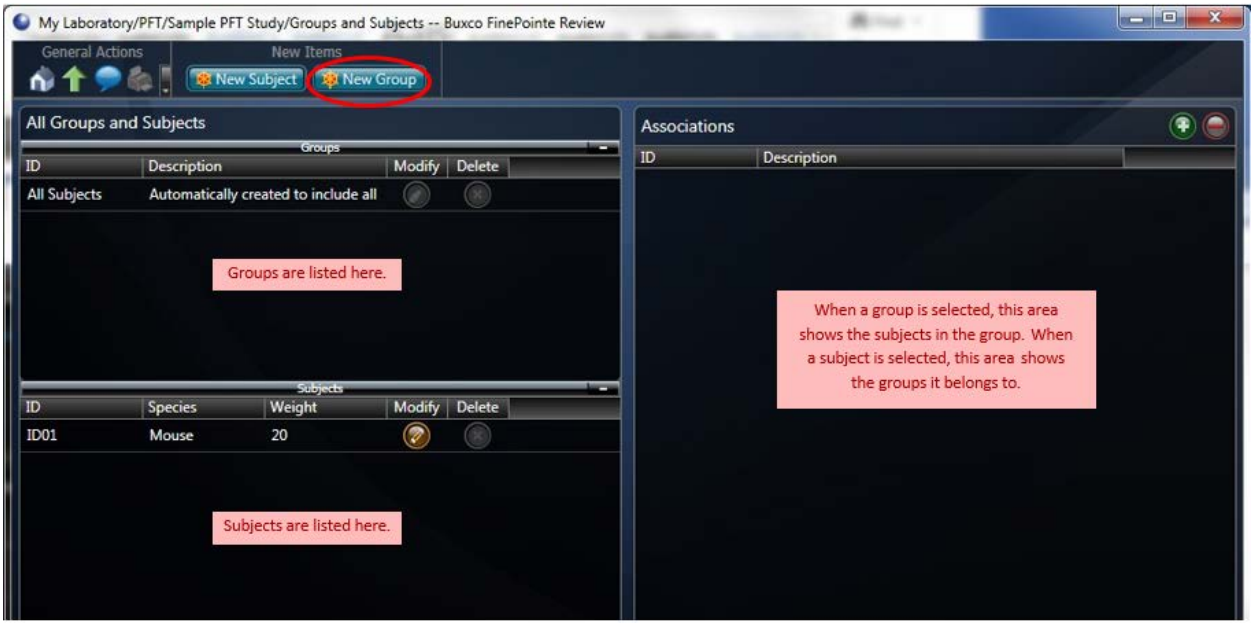
When you create a report, you will need to indicate which measurements and groups are to be summarized. In addition, you need to specify the formula used to summarize a measurement. Common formulas include Average, Maximum, or Minimum of a parameter, but formulas can be any algebraic combination of multiple parameters.

Creating and Modifying Groups

To create or modify your subject groups, click on the “Manage Groups and Subjects” button from the study page.

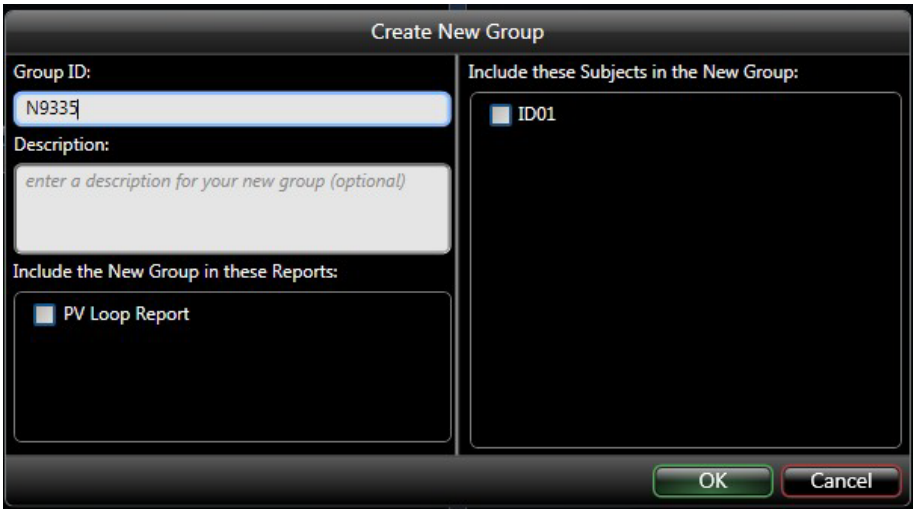


When you click on that button, the Groups and Subjects page is presented. From this page, you can create groups and subject IDs. If you choose, you can create them all at once, and as you acquire data from the subjects, the reports will automatically be updated. Alternatively, you can create the subjects as you acquire data from them in the Assign Subjects form of FinePointe Station.



The page shown above shows how the subject and group content is arranged. The left side of the display is divided into 2 lists: groups and subjects. The groups list shows you the groups which are currently created. You can modify or delete each of them by clicking the appropriate button under Modify or Delete header. The content on the right of the page changes based on what is selected on the left. If a group is selected on the left, then the right shows the subjects that belong to that group. You can modify the constituents of the group by selecting the group and clicking the + or - button over the right list of subjects.

To create a Group, click the **New Group** button on the command bar.



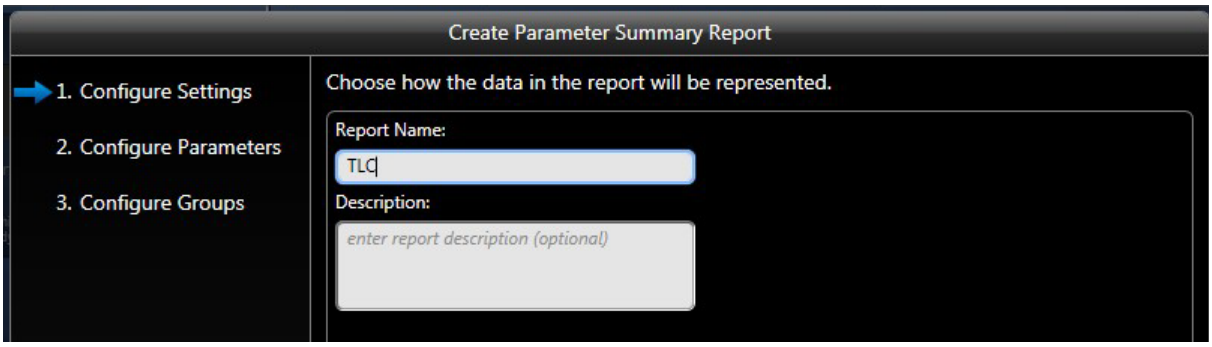
The group ID is the identifier which you will see in the reports describing this group of subjects. At this time, you can add any relevant subjects you already have created to this group. You can also add them later too. Also, if you have already created reports and you want to include this group in any of those reports, you can check the reports in the list below the description.

Click **OK** when done.

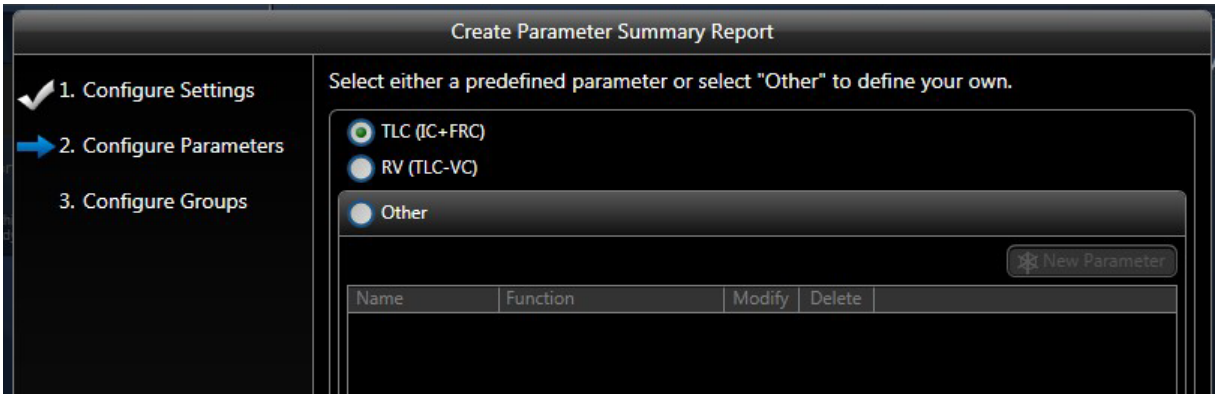
Creating Reports

There are several kinds of report you can produce within FinePointe. From the study page, you pull down the button named **Create Report** and select the type of report you want to create. The Parameter Summary report summarizes a parameter (or formula). In addition to that report, you can also produce group average Flow-Volume or Pressure-Volume curves. And if you are interested in modeling the Pressure-Volume curve to a sigmoid there is a special report named PV Sigmoid Report.

If you select **Create Report->Parameter Summary Report**, the wizard is presented.



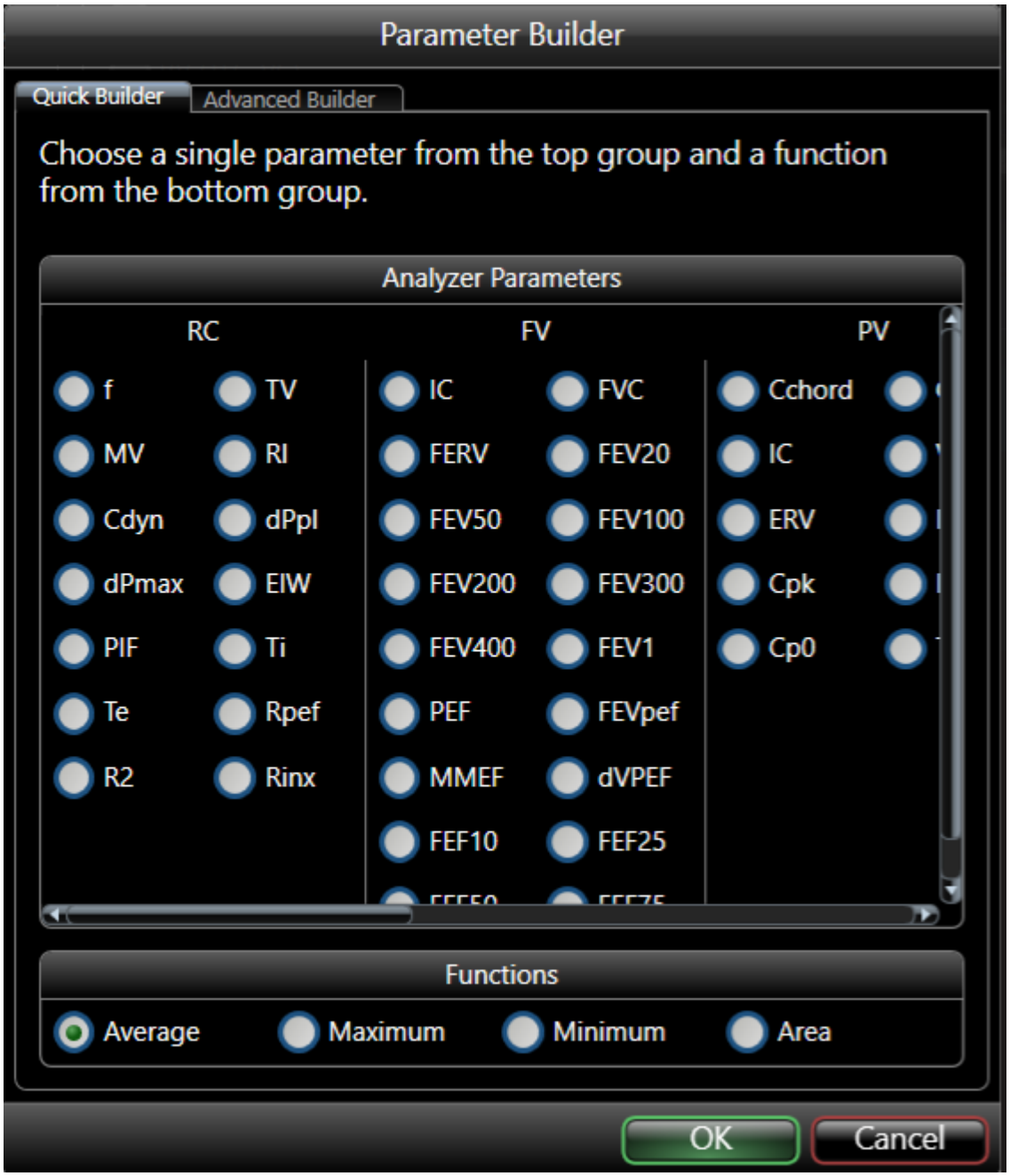
On the first page, specify a descriptive report name. Click **Next**.



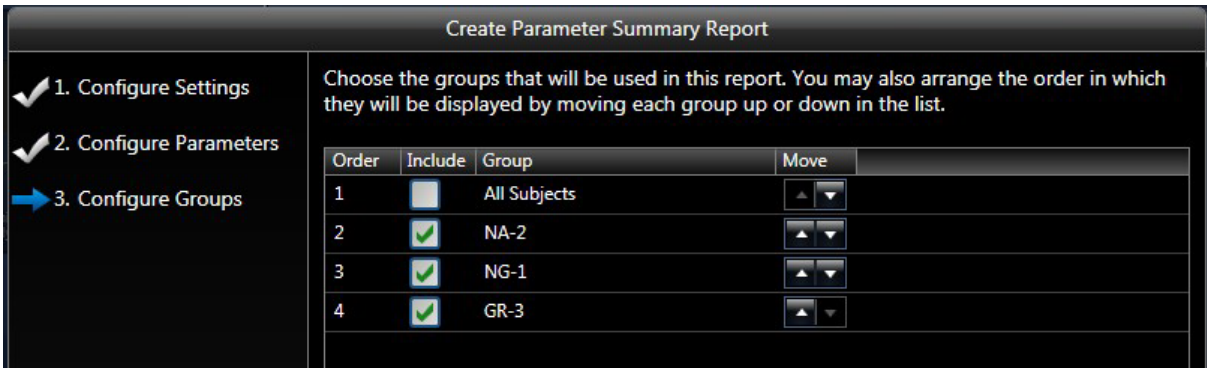
Select the parameter to summarize. The Parameter Summary provides two special formulas: **TLC** and **RV**.

These are common values which are computed from more than one parameter.

Alternatively, you can select **Other** and then select one of the other parameters or an algebraic combination of them.

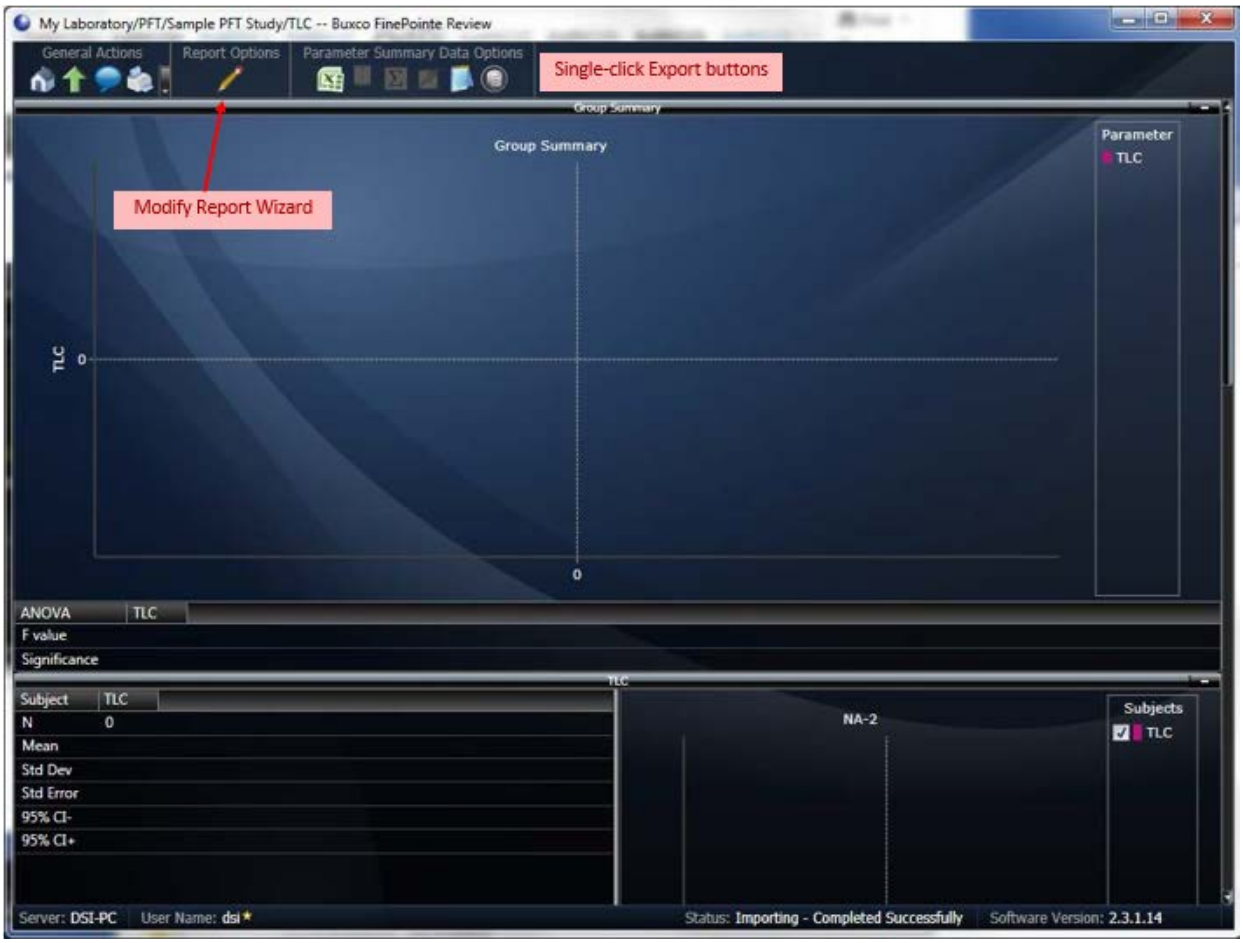


Click **Next**.



And lastly, select which subject groups to summarize in this report. You can also set the order that the groups are summarized by clicking the ▲ and ▼ buttons.

Click **Finish**. The report will open.

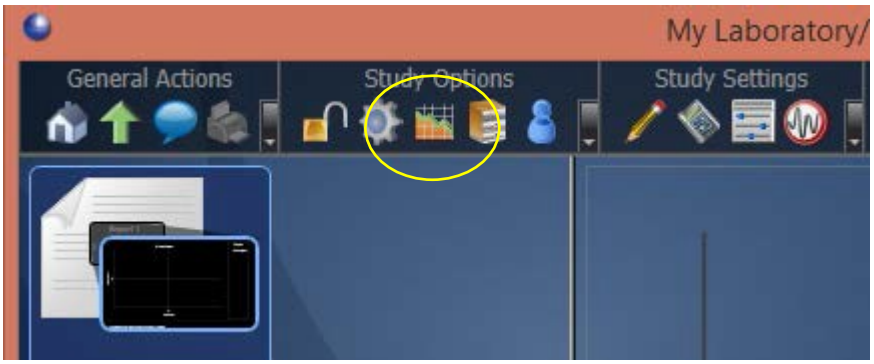


Reports can be modified via the Modify Report Wizard.

Reports can be exported to several different formats.

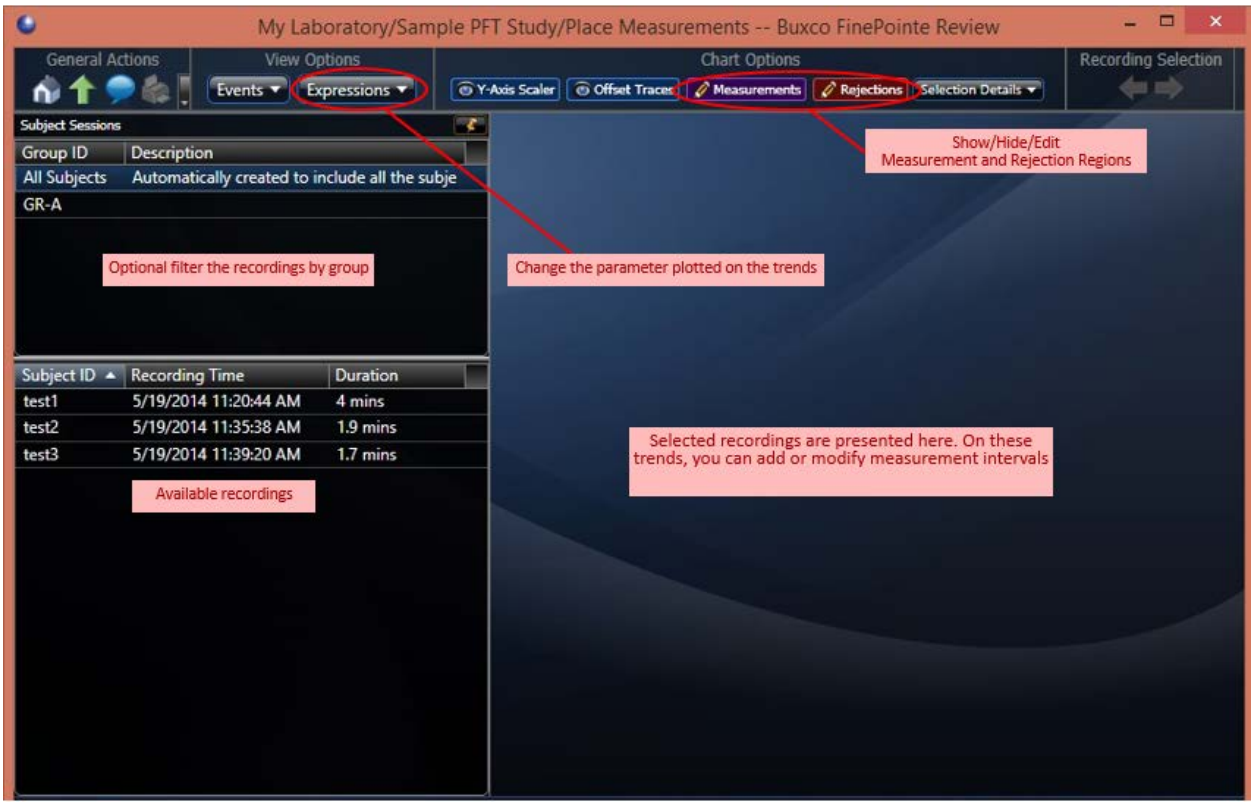
Modifying Measurements

If you choose not to use the automation of the tests, and run them manually, you will need to place the measurements in the recordings yourself. To do this, go to the Place Measurements view of the study.



Click the button pictured above.

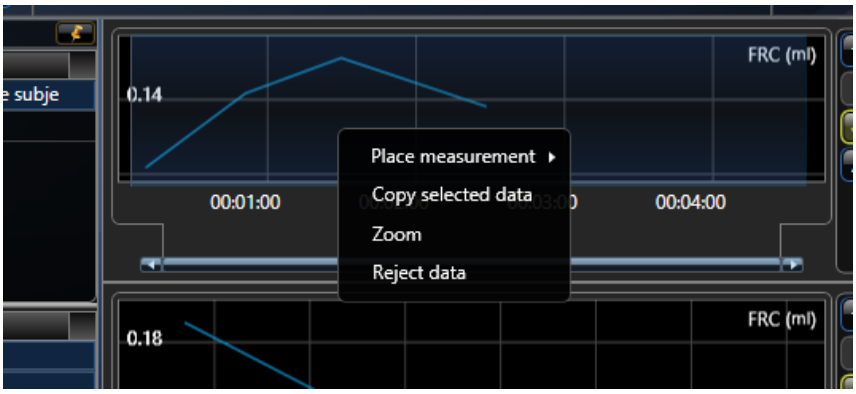
The Place Measurement view opens as shown below.



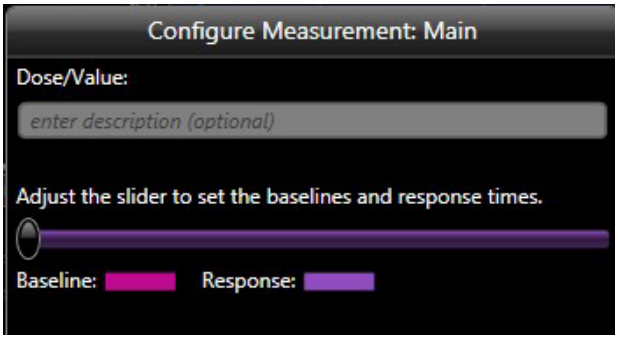
Select the recordings you want to adjust or create measurements on. When you do, trends are plotted in the area on the right for each recording you select. It will not be practical to select more than 4 at a time since the trend will probably get too small.

When you select a recording, any measurements that have already been placed will appear as shaded blue regions. If there is not any (as shown in the previous image), you will see no such region. In this case, you can place a measurement by clicking and dragging a region, then right click on that region to Place Measurement.

The following image shows a region drawn the full width of the recording and the context menu which is presented by right clicking on the region. With a PFT study, your measurement will usually span the entire recording. With other study types, this is generally not the case.



To place a measurement, you will select **Place measurement->Main** (or you may replace **Main** with one of the available measurements presented by the submenu). The following form is presented to give you an opportunity to give the measurement a baseline region. In PFT data, there should not be any baseline data.



Once you have placed all the measurements, you can still modify them by dragging the edges.

Rejecting Data

You can reject data in much the same way that you place a measurement. You select the region you want excluded from reporting. Then you right click on that region and select the **Reject Data** content menu option.

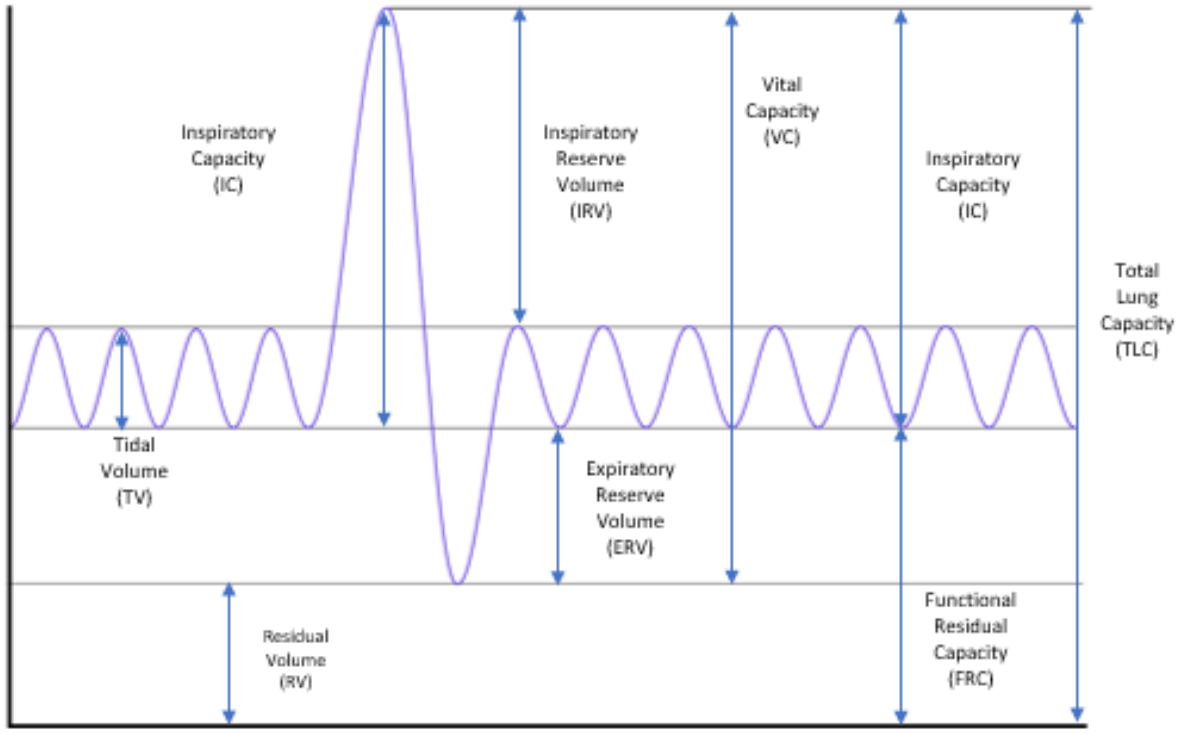
This way to reject data is convenient for continuously monitored data but may not be so good for a PFT test.

The best way to reject a test is to open the recording up from the study page directly, and right clicking on the row you want to reject, in the table. Once you have done that, select **Reject**. That will reject that individual test.

If you reject data, modify the constituents of your treatment groups, change your report expressions, or measurements, the reports are automatically recalculated ensuring that you are looking at the most up-to-date information. In addition, should your needs go beyond the basic ANOVA statistics which are provided within FinePointe, you can, with a single click, export your data to Microsoft Excel, GraphPad Prism, or to a variety of other statistics packages.

Test Results

The picture below illustrates all the lung volumes.



FRC

The FRC tests measures the FRC lung volume by using Boyle's Law. FRC lung volume is the Functional Residual Capacity. This is the volume of air that remains in the lung at the end of a normal expiration. This lung volume is important because by knowing this lung volume, it is easy to know all the other lung volumes.

The FRC test produces the following parameters:

Parameter	Description	Units
FRC	Functional Residual Capacity.	mL
R2	Goodness of Fit. A value between 0 and 1 indicating how well the data fits the assumed model.	
DeadSpace	The amount of volume that is in the hardware. Subtracted from the volume result of Boyles Law to arrive at the FRC value.	mL
DPmax	The maximum pressure change of all the efforts.	cmH_2O
DVmax	The maximum volume change of all the efforts.	mL
Tocclude	The time the subject was occluded	s

Quasistatic Pressure Volume

The PV test provides information about the static lung properties. Static lung properties are described by the pressure-volume relationship of the lung. The slope of the Volume-Pressure curve is the instantaneous compliance at a given pressure.

The PV test produces the following parameters:

Parame	Description	Units
Cchord	Chord Compliance, between 0-10 cm H_20	mL/cmH_2O
Cfvc50	Compliance at 50% VC	mL/cmH_2O
IC	Inspiratory Capacity. Volume measured from the start of the test to TLC.	mL
VC	Vital Capacity. Volume measured from TLC to the end of the test.	mL
ERV	Expiratory Reserve Volume. The difference between VC and IC.	mL
Pfrc	Pressure at Return to FRC during expiration	cmH_2O
Cpk	Peak Compliance	mL/cmH_2O
Ppk	Pressure at Max Compliance	cmH_2O
Vpk	Volume at Max Compliance	mL
Cp0	Compliance at Zero Pressure	mL/cmH_2O
Te	Duration of expiration	S

Fast Flow Volume

The FV test provides information about the dynamic lung properties. Dynamic lung properties are described by the flow-volume relationship of the lung. The Flow-Volume curve shows the flow at a given expired volume. Since the pressure across the lung is essentially constant during the acquisition of the flow-volume data, the flow value is proportional to the conductance of the limiting airways at a given expired volume.

The FV Test produces the following parameters:

Parameter	Description	Units
IC	Inspiratory Capacity. Volume measured from the start of the test to TLC	mL
FVC	Forced Vital Capacity. Volume measured from TLC to the end of the test.	mL
FERV	Forced Expiratory Reserve Volume. The difference between FVC and IC.	mL
FEV20, FEV50, FEV100, FEV200, FEV300, FEV400 and FEV1(000)	Forced Expiratory Volume nn. Volume expired during the first 20 milliseconds of expiration. Same for the first 50, 100, 200, etc.	mL
PEF	The peak expiratory flow value during the expiration	mL/s
FEVpef	Expired volume up to the instant that PEF is achieved	mL
MMEF	Mean Mid Expiratory Flow. The average flow between 25% and 75% of expired volume	mL/s
dVPEF	This number refers to the percent of FVC that remains in the lung when the Peak Expiratory Flow is reached.	%
FEF75, FEF50, FEF25 and FEF10	Forced expiratory flow at 75% (25% expired), 50, 25, and 10% of FVC	mL/s

Resistance & Compliance

The resistance and compliance analysis produce the following:

Parameter	Description	Units
f	respiratory Rate	BPM
TV	Tidal volume (volume inhaled)	mL
MV	Minute volume (the rate of ventilation)	mL/min
Rl	lung resistance	$cmH_2O/mL/s$
Cdyn	Dynamic compliance	mL/cmH_2O
Cstatic	Static compliance (Optional with special ventilation)	mL/cmH_2O
dPpl	Compliant pressure, pressure change from the start to the end of inspiration	cmH_2O
dPmax	The maximum pressure change over the breath	cmH_2O
EEW	End expiratory work, work of breathing	cmH2O ■ mL
PIF	Peak inspiratory flow, peak flow during inspiration	mL/s
PEF	Peak expiratory flow, peak flow during expiration	mL/s
Ti	Inspiratory time	S
Te	Expiratory time	S
Ve	Expired volume	mL
R2	Coefficient of determination	
Rinx	Rejection index, percentage of breaths rejected	%

Appendix A - Troubleshooting

This section provides general information that may help you resolve common user questions or problems.

If you require additional assistance, please contact DSI Technical Support or email support@datasci.com.

Common Reasons PFT Tests Might Fail

This section lists the most common reasons the FRC, PV, and FV tests fail

FRC Test

- Occlusion is not triggered
    - This can happen if the end of the breaths cannot be determined during the breath history phase. Occlusion is triggered at zero pressure when the previous breath ends.
    - Make sure the calibration zero is good for all leads.
    - Check the system for leaks (**See the Leaks section below)**.
    - If breaths are erratic during the breath history phase, check the anaesthesia.
- No breathing efforts during occlusion
    - This can happen if the subject has been ventilated and is hyperventilated and is not trying to breath. See the **Appendix C** section below for more details

PV Test

- The inspiration to 30 cm H20 phase of the PV test is not triggered
    - This can happen if the end of the breaths cannot be determined during the breath history phase. The inspiration phase is triggered at zero pressure when the previous breath ends.
    - Make sure the calibration is good for all leads.
    - Check the system for leaks (**See the Leaks section below).**
    - If breaths are erratic during the breath history phase, check the anesthesia.
- 30 cm H20 of pressure cannot be achieved during the inspiration phase
    - There could be a leak in the tracheal tube surgery. Check the surgery site and the tubing connection to the manifold.
    - The inspiration flow rate is too low. Increase in inspiration flow slightly and repeat the test (**See the Setting Inspiration Flow section on page 25)**.
    - There could be a leak in the valves (**See the Leaks section below**)
    - If you have a test lung for your species type, connect it and attempt the PV test. If the test passes, the issue was probably with the tracheal surgery or the connection to the manifold. If it still fails, try the other items in this list.
    - 30 cm H20 must be reached within a unique specified time for each species, or the inspiration will time out. Keep in mind that the rate you need to achieve for a “good” test is usually less than the time out value (**See the Setting Inspiration Flow section on page 25**). For example, with mice the inspiration time out is 5 seconds. However, the best inspiration rate for the average sized mouse is to reach 30 cm H20 in 1-2 seconds.
- The slow expiration phase was too slow or too fast
    - After the PV test is complete, check the Te value and compare to the values listed for your species type (**See the Setting Slow Expiration Flow section on page 26).** If the Te is too high, lower the slow expiration flow rate slightly. If the Te is too low, increase the slow expiration flow rate slightly.

FV Test

- The inspiration to 30 cm H20 phase of the FV test is not triggered
    - This can happen if the end of the breaths cannot be determined during the breath history phase. The slow expiration phase is triggered when the breath hold phase ends.
    - Make sure the calibration is good for all leads.
    - Check the system for leaks (**See the Leaks section below).**
- 30 cm H20 of pressure cannot be achieved during the inspiration phase
    - There could be a leak in the tracheal tube surgery. Check the surgery site and the tubing connection to the manifold.
    - The inspiration flow rate is too low. Increase in inspiration flow slightly and repeat the test (**See the Setting Inspiration Flow section on page 25)**.
    - There could be a leak in the valves **(See the Leaks section below**)
    - If you have a test lung for your species type, connect it and attempt the PV test. If the test passes, the issue was probably with the tracheal surgery or the connection to the manifold. If it still fails, try the other items in this list.
    - 30 cm H20 must be reached within a unique specified time for each species, or the inspiration will time out. Keep in mind that the rate you need to achieve for a "good" test is usually less than the time out value (**See the Setting Inspiration Flow section on page 25**). For example, with mice the inspiration time out is 5 seconds. However, the best inspiration rate for the average sized mouse is to reach 30 cm H20 in 1-2 seconds.
- The fast expiration phase failed
    - Check the connection from the fast flow valve to the negative pressure reservoir.
    - Ensure the negative pressure dial is set to -40 on the PFT controller. It can be set as high as -50 cmH20 if needed.
    - Check the system for leaks (**See the Leaks section below).**

Leaks

The principal cause of problems you will find are due to leaks.

Leaks may occur at any point where there is a detachable junction between two air passages.

While the chamber and valve assembly are shipped with no leaks, the equipment is very fragile and even the jostling during shipping may cause some leakage. However, we have found that the most common place for leaks is the tracheal connection, where the cannula enters the animal. Other common causes for leaks include - equipment being moved from one place to another, and air tubes being connected and disconnected.

Common Symptoms

If any of the following are occurring in your experiment, consider that there may be a leak in your system, and use the "Guide to Fixing Leaks".

- The system has trouble delivering inspirations ("Unable to inflate to 30 cmH2O").
- The system has trouble performing forced expirations.
- Hold period does not appear flat, but drifts upwards (in the PV maneuver).
- Expiration does not cross zero volume (also in the PV maneuver).
- The software has trouble analyzing data.

General Suggestions

- In general, make sure that all connections are snug and airtight.
- Check the connection of the tracheal tube to the animal. Make sure the tube is tied in tightly,

without any air leaking through. This is a very likely place to find a leak.

- To improve the quality of your seal, try using a greasy substance (like vacuum grease or silicone grease) around the outside of the air tube. Make sure that no grease gets inside the air tube.
- Thin Teflon tape may be applied or wrapped around the area in suspicion, providing a semi-malleable and snug connection. Be careful not to add too much Teflon tape, however, because too much will only prevent you from making the connection completely.

Never glue air tubes, valves, or any other connections together. This may interfere with the air flow. It will also prevent you from ever moving or adjusting previously detachable parts.

Test for Leaks in the Main Chamber

If you suspect a leak in the PFT chamber, then you can repeat the FRC flow calibration.

If the calibration is successful, then the chamber is likely not leaking.

If you want to verify more thoroughly that the chamber does not leak:

- Plug the tracheal tube port and close the chamber,
- Seal the pneumotach opening with painter's tape or electrical tape,
- Pull the plunger of a 10 cc syringe back 5 mL,
- Connect the syringe to the syringe port of the chamber and to the calibrator at the same time using a Luer "T" fitting,
- Inject the air, keeping pressure on the plunger to make sure it does not get pushed back,
- Make sure that the water level in the calibrator does not rise and stays steady.

Test for Leaks in the Valve Assembly

If you suspect a leak in the valve assembly (the Lucite manifold that houses all the valve connections, at the end of the chamber). Simply follow the instructions listed in the Pressure Calibration chapter. After pushing the syringe plunger until the calibrator is at 20 cm H_2O, the water level in the manifold should not raise, and the voltage should not drift down. If the voltage drifts down, then you have a leak, and you should identify and fix the leak in the manifold.

Common places to look for a leak in the Valve Assembly:

- N_2 Sample port. Make sure it is plugged.
- Mouth Pressure Transducer.

If the leak is not in either of these places, you will need to narrow it down by removing one valve at a time and plugging the hold with tape or a bung. Start with the **Slow Expiration**, then **Inflation**, etc.

If you cannot solve your leak problem, then you may have a faulty valve or transducer. Please call your DSI representative in that situation.

Animal Breathes During Tests

If your animal is breathing throughout the tests, consider the following possibilities:

- Inspiratory Flow Rate is too low.
- Expiratory Flow Rate is too low.
- Anesthesia dose should be increased.

Appendix B – Anesthesia

The following is a list of suggestions for anesthetizing an animal.

First Choice

For **Guinea Pig, Rat, and Mouse**, choose the following:

Ketamine (50 mg/kg) + Medetomidine (0.33 mg/kg) combo

Delivery : i.p. 1 mL/kg

For example:

10 mL of 1 mg/mL Medetomidine

15 mL of 100 mg/mL Ketamine

5 mL water FI

This cocktail keeps them spontaneously breathing but allows plenty of time for the Quasi-static maneuver to complete.

For **Monkey**, choose the following:

Diprivan (Propofol) 3-15 $\frac{mL}{h \times kg}$

The dose varies, start with 3 $\frac{mL}{h \times kg}$ but you might have to increase it up to as much as 15 $\frac{mL}{h \times kg}$

The monkey metabolizes this substance rapidly, so recovery is also relatively quick.

Second Choice

Thiopentone sodium 100 mg/kg.

Delivery: i.p. 1 mL/kg

Third Choice

Nembutal (50 mg/mL solution of Pentobarbital)

Delivery: i.p. 0.8 mL/kg

Notes

- Pentobarbital is ok but you will get some of them breathing as you perform the test; you just have to be patient. Thiopentone tends to be better.
- It is also recommended that the i.p. injection be made to two sites, not just one. Be patient.
- Concerning the Ketamine cocktail:
  Xylazine hydrochloride (Rompun) may be used in place of the Medetomidine, at the same dose.

Appendix C - Interpretations PFT Results

This appendix describes things you should look for when you review results from the Pulmonary Maneuvers. While some things you look for only apply to a given test, many of them apply to all the tests.

Overall, the passing of time will affect all tests. If you run a test 15 times, it is likely that some of the results you get early on will be very different from the results you get later. A very likely cause of this change in results is the effect of anesthesia. Over time, the anesthesia becomes lighter, causing the test results to degrade.

FRC (Functional Residual Capacity) Test

For the FRC test to successfully return good data, FinePointe must occlude the subject at the end of expiration (at FRC). In addition, the subject must attempt to breathe while its airway is occluded. Finally, there can be no leaks in the manifold. Leaks in the manifold with violate a basic assumption which is made in this test that the volume is constant.

Occlude at FRC

One of the most challenging objectives to meet with this test is to ensure that when the occlusion takes place, the animal is at FRC.

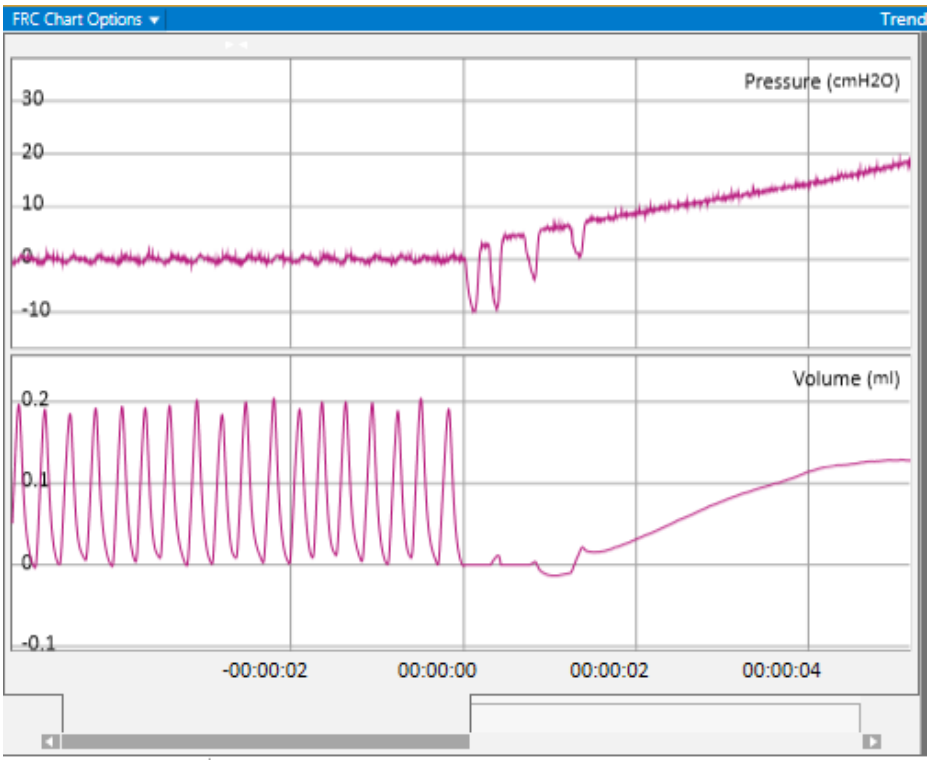
In the figure above, you can decide if FinePointe occluded the subject appropriately at FRC.

If it does not look correct, right click on the line corresponding to the test and select **Reject Data**.

Rejected data will be excluded from reports.

No Efforts During FRC Occlusion

If you ventilate the subject prior to running the FRC test, it is possible that when FinePointe occludes the airway, the subject is hyperventilated and does not try to breathe. The data returned may look something like the following:



The volume appears to drift away. FinePointe actually uses the Pressure to determine if an effort is present. The data above does technically have an effort or two, but this data should be rejected since the subject did not try to breathe past those couple of effort. To correct this, turn off the ventilator until the subject begins to breathe again on its own. Once the subject is breathing regularly again, you can start your test.

Data Assumptions Met

You can also get an indication of how well the pressure and volume data agrees with the model by looking at the R2 parameter. FinePointe attempts to compute FRC on as many samples as it can. Not every pressure and volume pair yields the same FRC result. This parameter is a number between 0 and 1 representing the how well each pair agrees. If all the pairs agree precisely, the result will be 1. If something has gone wrong, making them all differ, then the result can start to fall. Experience will tell you what is a good R2, and certainly if you intend to reject the worst one, criterion which can help you decide is the R2 result. Achieving R2 better than 0.95 should not be difficult.

If you see R2 results below 0.9, you should look for a leak around the tracheal tube or possibly in the manifold.

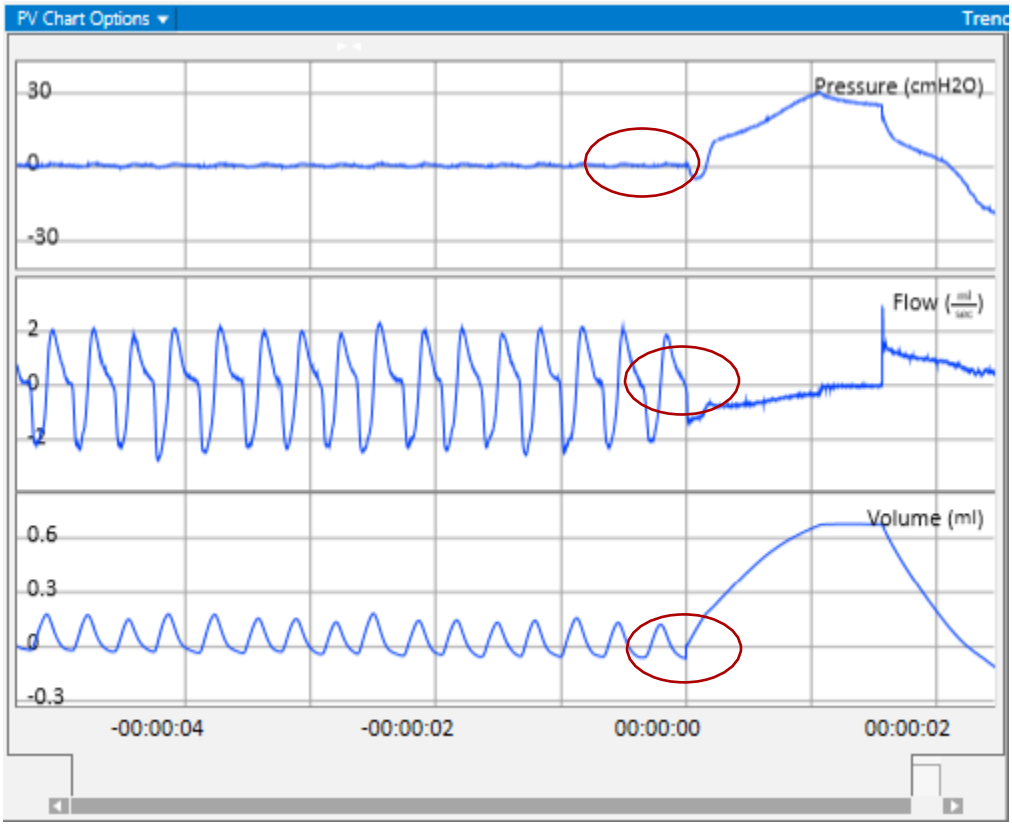
PV (Pressure Volume) Test

In this test, you need to watch out for a few things:

- The flow was properly zeroed during calibration.
- The expiratory duration is long enough and without attempts from the subject to breathe on its own.
- There should be no leaks in the tracheal line.
- Dead space in the expiratory line affecting the upper side of the curve.

Test Starts When Flow Is Zero

Just like the FRC test, the PV test must begin at FRC so that it can accurately determine IC. If IC is not measured properly, your TLC measurement will be off as well. Check that the test starts when the flow is at zero, and the volume is at a minimum. The volume curve is reset at the start of the test, so you may see it step up, but you should look to ensure that the volume appears to be at a minimum.



At TLC, Volume Rises as Pressure Falls

If pressure drop is accompanied with a slight rise in the volume, then some air escaped from the trachea into the chamber. Check that you tied the tracheal cannula tightly.

Sources of leak to consider:

- where the tracheal cannula attaches to the manifold,
- where the cannula is tied to the trachea,
- in the cannula itself.

At TLC, Volume Drops as Pressure Falls

If the pressure drop is accompanied with a slight decrease in the volume, then the air has escaped from the trachea directly to atmosphere. This can only be due to a leak in the manifold, and most probably one of the following:

- The **N2 Sample** port,
- Around mouth pressure transducer,
- The **FRC** valve.

Almost Vertical Volume Drop Immediately after the Hold Period

With certain apparatus, it is possible for the dead space in the expiratory line to be excessive. This is particularly true if you are using a pressure panel that is meant for a rat, with a mouse. The result is that you will lose the higher-pressure portion of the PV curve. To correct the problem, try the following in the following order:

- Shorten the tubing to the pressure panel, if possible,
- Place a flow limiter or a crimping attachment near the slow expiration valve on the manifold,
- Reduce the negative pressure reservoir.

Expiratory Duration Should be Long Enough

The system requires that the expiratory duration must be at least 1 second, otherwise it will not attempt to compute results. What the duration should be depends upon the species.

- Mouse should be around 1.5 seconds
- Rat should be about 2.5 - 3.5 seconds
- Dog and monkey should be 3 - 5 seconds

As far as the test results are concerned, this duration cannot be too long. If it is too long, then the animal tries to breathe and ruins your data. To lengthen the expiratory duration, you must reduce the expiratory flow on the pressure panel.

Volume Should Rise Steadily During Inspire to TLC

If the rise of the volume during this period is diminishing near the top pressure, then it is likely that the positive pressure in the Pressure Panel is set too low. The reason is that as the mouth pressure increases, the pressure difference between what is in the lung and what is in the pressure reservoir diminishes. This results in a steadily decreasing flow. If the pressure difference between the mouth pressure and the reservoir pressure is very small at TLC, your flow will be too.

You can increase the positive pressure in the reservoir. The positive pressure in the reservoir should always be at least 40 cm H2O, but 50 cm H2O is certainly safe too.

Volume-Pressure Curve is Smooth

Particularly in the negative pressures, it is possible to observe little oscillations on the Volume-Pressure curve. These oscillations are generally due to the animal trying to breathe before the test is complete.

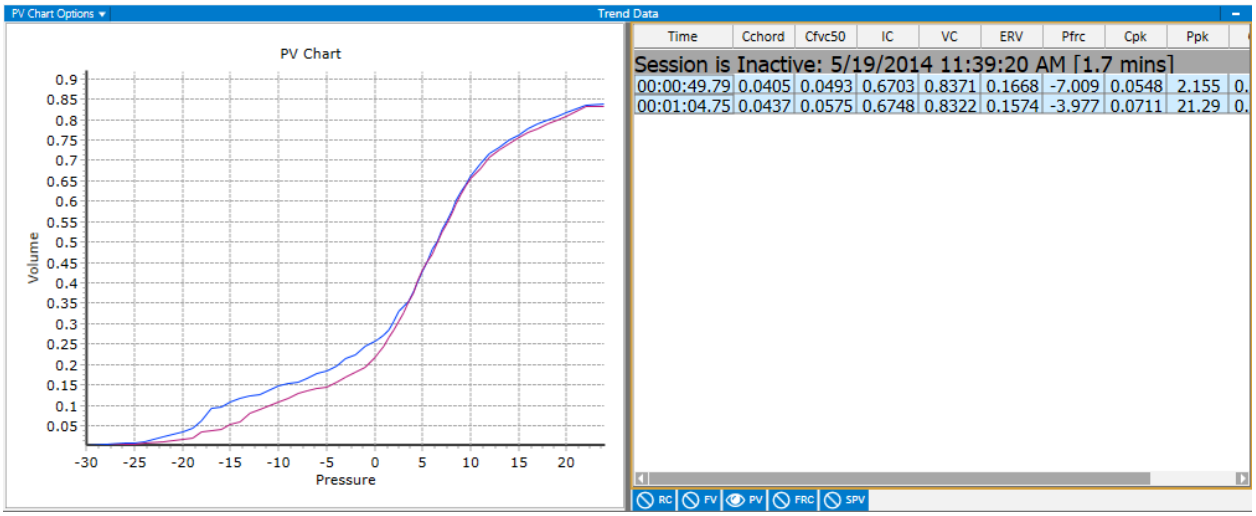
To verify that, simply select “PV Chart Options > Show Time Domain” and look at the Pressure curve to see if the animal attempted to breathe at the end of the slow expiration (in the negative pressures).

If that is the case, it may simply be that your expiratory duration is too long for the animal to tolerate. If it is, increase the expiratory flow on the pressure panel. However, if the duration is reasonable, try the following:

- Consider increasing the inspiratory flow to shorten the inspiration.
- Consider ventilating the subject prior to running this test.
- Consider adjusting (increasing) the anesthesia.

Overlays of Consecutive PV Tests Differ

When you are looking at PFT test results, you can select multiple rows in the PV table to overlay their results as illustrated below:



One test for repeatability of data is to overlay 2 consecutive tests. With the PV test you should get very consistent results, so an overlaid test should trace almost identically. If one particular curve is an outlier, right click and select “Reject Data”.

FV (Flow Volume) Test

With this test, more than any other, you should be concerned about the position and comfort of the animal.

Ripples or Squiggles Near the End of the Flow-Volume Curve

If you observe squiggles near the end of the Flow Volume curve, it is probably because there is mucus, goo, or condensation in the lung or trach tube. For larger animals, we recommend you suction it out. For mice, just change either the trach tube or the mouse.

A Flat Top of the Flow Volume Curve

This does not occur often, but if you look at a Flow-Volume curve, and it is perfectly flat at the top, this is most likely a saturated flow transducer. What to do:

- Make sure the calibration procedure was performed properly.
- Make sure that the High Flow input is connected to Lead 1.
- Reduce the gain on the flow preamp and recalibrate the High Flow, Flow, and FRC flow.
- Make sure that there are no obstructions in the tracheal line of the manifold.

A Dip in the Center of the Flow-Volume Curve

A dip in the flow near mid volume of the Flow-Volume curve could be a real result of a physiological condition, however, when it is observed you should check a couple things to make sure it is not something else. This shape is caused by a temporary obstruction in the expiration line. One example may be a collapsed tube either in or outside the lung. A balloon without cotton inside will almost always do this as the flabby piece of balloon covers the hole in the trach tube temporarily. You might just try repositioning the animal to make sure the tracheal line is straight and not putting the trachea at any angles.

Generally Rounded Flow-Volume Curve

The FVC parameter (forced vital capacity) should not be too far from what you expect. If the tracheal tube is inserted too deeply in the lung, the end of the tracheal tube may pass the first bifurcation of the lung. The result is that you are performing your test on only one half of the lung.

FVC Value is Wildly Different Than IC

Though rare, we have seen data where the expired volume is very small when compared to the inspired volume. While this can be caused by a physical obstruction in the airway or tubing, for example, a tube crimps during the expiratory phase, you may want to confirm the calibration for the Flow and High Flow signals. In general, the effective range of the High Flow should be 8 times larger than the effective range of the Flow signal. A jumper on the Signal Generator card can change this to 4 times.

4 times has been recommended for mouse systems. The High Flow signal is only used during expiration of the FV test, so problems with the High Flow calibration should not impact other tests.

Appendix D - Suggested Reading

This list of selected studies is recommended reading in the field of Pulmonary Maneuvers and may provide information beneficial to your own research.

DuBois, Arthur B., Stell Y. Botelho, George N. Bedell, Robert Marshall, Julius H. Comroe, Jr. **A rapid plethysmographic method for measuring thoracic gas volume: A comparison with nitrogen washout method for measuring functional residual capacity in normal subjects**. Journal of Clinical Investigation, 35: 322-326, 1956.

Green, M., J. Mead, F. Hoppin and M. E. Wohl. **Analysis of the forced expiratory maneuver.** Chest 63: 335-365, 1973.

Mauderly, Joe L**. Assessment of Pulmonary Function and the Effects of Inhaled Toxicants.** Concepts in Inhalation Toxicology – 2nd Ed. Edited by Roger McClellan and Rogene Henderson, 1995.

Paiva, M., J.C. Yernault, P. Van Errdeweghe and M. Englert. **A Sigmoid Model of the Static Volume-Pressure Curve of Human Lung**. Respiration Physiology 23,317-323, 1975: North Holland Publishing Company, Amsterdam.

Sabo, John P., Edgar C. Kimmel, and Louis Diamond. **Effects of Clara Cell Toxin,4-ipomeanol, on pulmonary function in rats**. J Appl Physiology: 54(2) 337-344,1983.

Takezawa, Jun, Fred J. Miller, John J. O'Neil. **Single-breath diffusing and lung volumes in small laboratory animals**. J Appl Physiology: 48(6), 1052-1059,1980.

Contact Information

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the DSI Support Center at https://support.datasci.com to find articles and helpful information in our knowledge base, Chat with an agent, or setup time to receive one-on-one consultation. We are happy to help!

DSI Technical Support—North America

Email: Support@datasci.com
Support Center Website: https://support.datasci.com

DSI Technical Support—Europe

Email: Europe-support@datasci.com
Support Center Website: https://support.datasci.com