Compliance Measurement

Chelsea Stowell - Oct 17, 2019, 4:55 PM EDT

Note: it is often easiest and most economical to use samples tested for compliance again for burst pressure.

Setup

DAQ

Obtain an AD Instruments PowerLab DAQ with at least two input channels, capable of recording voltages up to +/-10 V (e.g. PowerLab 8/30). Connect it via USB to a PC with the associated LabChart software installed (e.g. LabChart 7).

Open the settings file 20180627_CETS_Compliance. If this file is not available, recreate it as such. Most of these settings can be accessed under Setup > Channel Settings.

- Turn off and hide display of input channels 3-8.
- Under "Channel Settings" for channel 1, apply the following:
  - Title: Pressure
  - Sampling Rate: 10/s
  - Range: 2 V
  - Input Settings:
    - Range: 2 V
    - Lowpass: Off
    - Single-ended
    - AC coupled: not checked
    - Mains filter: not checked
    - Invert: not checked
  - Units:
    - Point 1: 0 V, 0 mmHg
    - Point 2: 2 V, 200 mmHg
    - Units: mmHg
    - Decimal places: 0
    - Unit conversion: On
  - Computed input:
    - Function: raw data
    - Raw data input: 1
    - Range: 2 V
  - Calculation:
    - Smoothing
      - Source channel: Ch1
      - Triangular (Bartlett) window
      - Samples: 9
- Under "Channel Settings" for channel 2, apply the following:
  - Title: Diameter
  - Sampling Rate: 10/s
  - Range: 10 V
  - Input Settings:
    - Range: 10 V
    - Lowpass: Off
    - Single-ended
    - AC coupled: not checked
    - Mains filter: not checked
    - Invert: not checked
  - Units:
    - Point 1: 0 V, 0 mm
    - Point 2: 10 V, 10 mm
    - Units: mm
    - Decimal places: 3
    - Unit conversion: On
- Computed input:
  - Function: raw data
  - Raw data input: 2
  - Range: 10 V

- Calculation:
  - Smoothing
    - Source channel: Ch2
    - Triangular (Bartlett) window
    - Samples: 9

The smoothing and sampling rate of either channel may be adjusted if preferred.

Set up the display as desired.

Pressure Monitor

Obtain a Living Systems Pressure Monitor 4. Connect the associated in-line pressure gauge to either the P1 or P2 inputs using the telephone jack connectors in the back. (On our lab's unit, the P1 input is currently broken, so the P2 must be used.) Turn the dial on the front of the monitor to that input channel so that the LED display will show that channel's readings.

The monitor should be calibrated for each new pressure gauge connected.

- With the gauge open to atmosphere, turn the screw denoted "zero" until the display oscillates about 0.0 mmHg.
- Connect the monitor's inline pressure gauge to a closed system including a pump and a trusted pressure gauge. The inline gauge has male LuerLock connections. The configuration of the system is not important, but it should be as compact and rigid as possible to minimize inter-sensor lag. We have traditionally used a sphygmometer with a hand bellows as the trusted gauge and pump.
- Increase the pressure in the system to just below 200 mmHg, which is the inline gauge's upper detection threshold. Hold the pressure constant and note the reading on the monitor. Adjust the "scale" screw on the monitor to increase or decrease the input scaling factor, and repeat until the monitor reading matches the trusted gauge. With our sphygmometer, we have typically achieved +/- 2 mmHg precision.
- Release the pressure and disconnect the gauge from the closed system. Disassemble the closed system.

Connect the monitor output channel chosen (P1 or P2) to PowerLab input channel 1 via a coaxial cable. Calibration of the monitor-DAQ interface was already accounted for in the channel unit conversion.

Laser Micrometer

Obtain an LS-7601 laser micrometer. Two coaxial cables exit the back of the monitor. Furthermore, there are 23 screw terminals just above the cable exits. Use a multimeter to validate that one coaxial cable's shield is tied to the screw of input 21 (0 V) and that its pin is tied to the screw of input 22 (OUT1). If the connections are bad, uninstall the monitor from its base and check the wiring on the monitor underside. Be especially careful that the inputs are not shorted. When finished, connect this coaxial cable to input channel 2 on the PowerLab.

Power on the laser micrometer. Ensure that the LCD display is measuring the vertical diameter of a black object onscreen, that it is in "Run" mode, that "OUT1" is displayed, and that the "Standard" value displayed is 0.00000. If it is not, consult the owner's manual for how to correct these settings. Whether the display shows "GO", "LO", "HI", or any other message in that field is irrelevant to the measurement accuracy. The micrometer function may be tested using an object of known width.

2173 mg + 1243 mg
25%

If the micrometer LCD display is showing a black field, check the connections on the cables running to the measuring heads. Usually this is a poor signal transmission resulting from cable displacement.

System Assembly

If needed, install tubing and connectors into the axial distension stage as shown. Use a stiff tubing to minimize pressure lag from compliance effects. Termini internal to the stage should be male LuerLock.

2173 / 250 = 8.692 mg / 1 mL

39.3%.

500 mg 1 mL

393 mg 1 mL

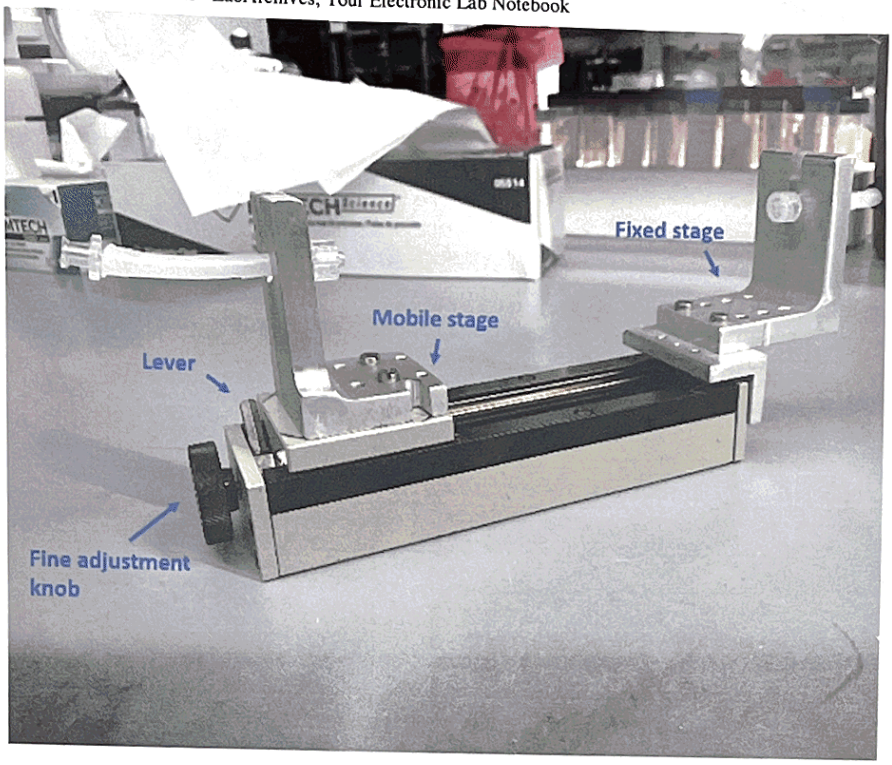


Fig. 1. Axial distension stage with installed tubing. The knob allows fine position adjustment. The push lever allows manual adjustment of the stage or locks it into knob control only. This stage was fabricated by Glenn Swan in the Chemical Engineering machine shop.

Place the laser micrometer track on top of the axial distension stage between the two vertical tubing supports. Support the laser micrometer track as needed. If desired, the micrometer and/or stage may be placed within a shallow basin to contain any splashing or leaks.

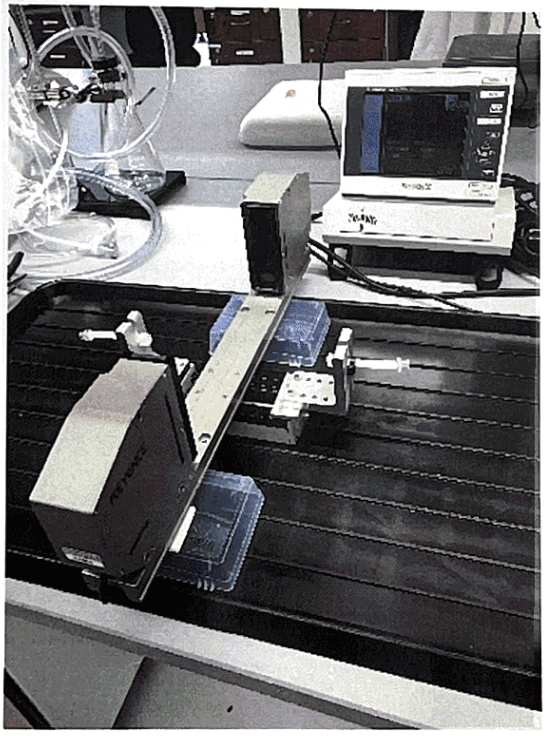


Fig. 2. Positioning of laser micrometer track and axial stage.

Install a syringe in a syringe pump (e.g. New Era NE-1000) and program the pump with the syringe diameter. Use more tubing in any required configuration to connect the syringe to the tubing on one side of the axial stage.

HSW 10 mL 15-90 mm

Connect the in-line pressure gauge to the tubing on the other side of the axial stage. Connect a cap or stopcock to the distal side of the connector.

Select a compliance test chamber with a port just larger than the inner diameter of the conduit to be tested.

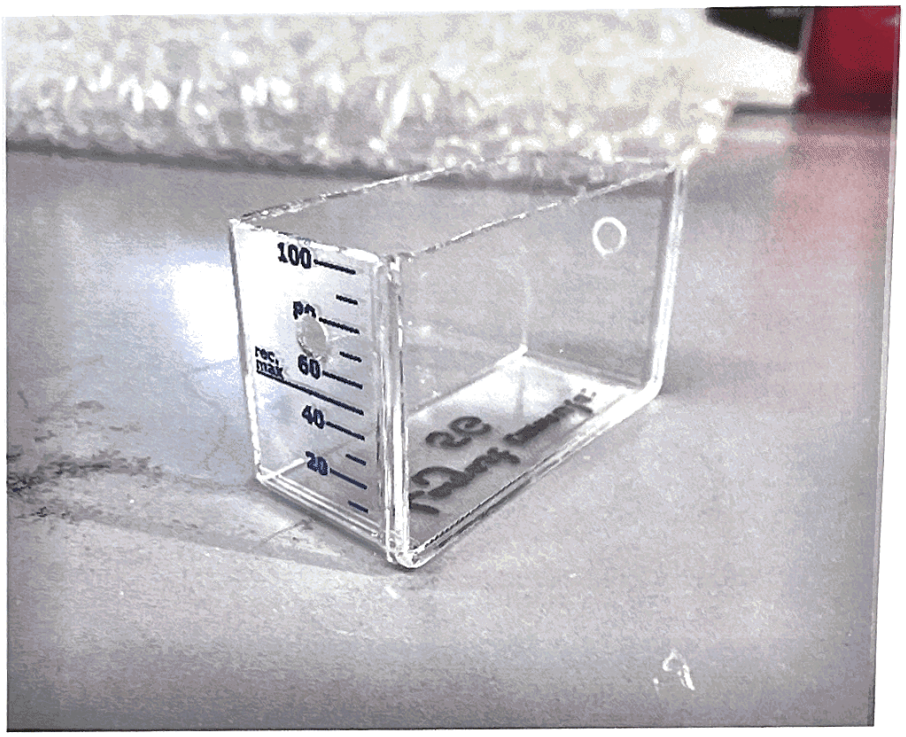


Fig. 3. Compliance test chamber. The chamber should be constructed of optically clear and uniformly thick material with no markings in the field of view (e.g. tissue culture flask polystyrene). Ports should be drilled into two opposite sides. When the chamber is placed on the axial distention stage, the ports should align with the internal Luer termini. The chamber should be tall enough so that water can cover the conduit by several mm. Wider and deeper chambers facilitate later graft mounting.

Select a blunt, female Luer, steel cannula (e.g. McMaster 6710A15 or a blunt-ended needle) that is only slightly smaller than the inner diameter of the conduit to be tested, such that it can be easily inserted into the conduit lumen. Cut a ~2 mm segment of Parafilm and wrap it around the tip of the cannula to a few layers' thickness. Then, cut a <1 mm segment and wrap it around the extreme tip of the cannula to a few layers' thickness. This should create a thicker Parafilm barb on the end of the cannula, with a thinner cushioning layer extending further inwards. Adjust layer thickness until the cannula can be just barely inserted into the conduit.

Prepare a second cannula in the same way. The inner diameter of the conduit may not be quite the same on each end, so ensure each cannula is a good fit to its designated end.

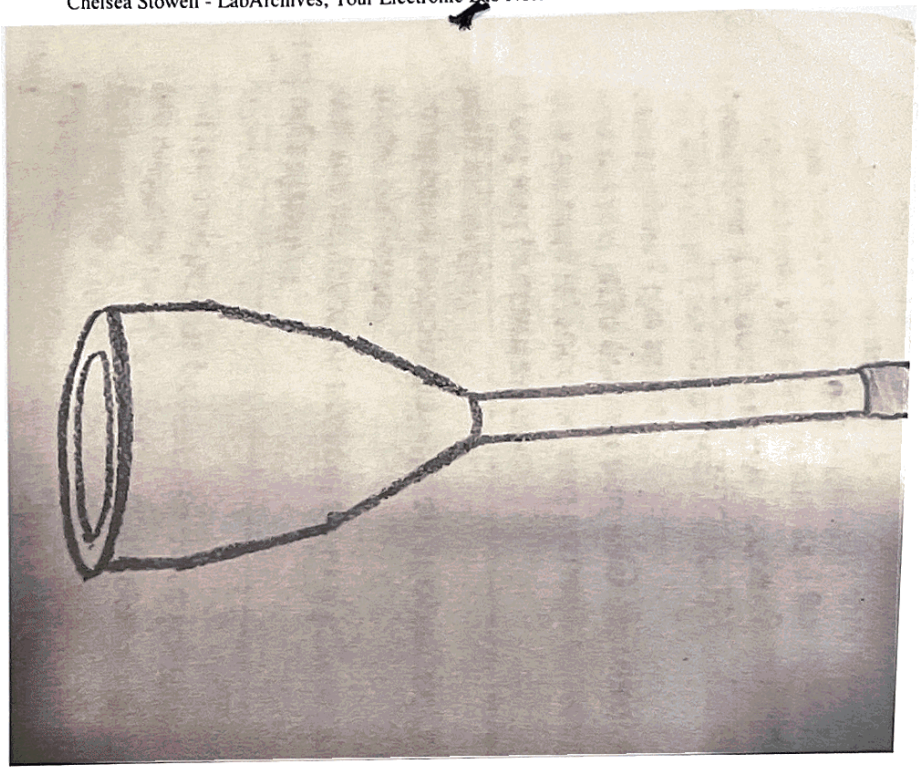


Fig. 4. Sketch of Parafilm barb on needle (not to scale)

Install the cannulae onto the Luer termini within the test chamber. Ensure the cannulae are coaxial.

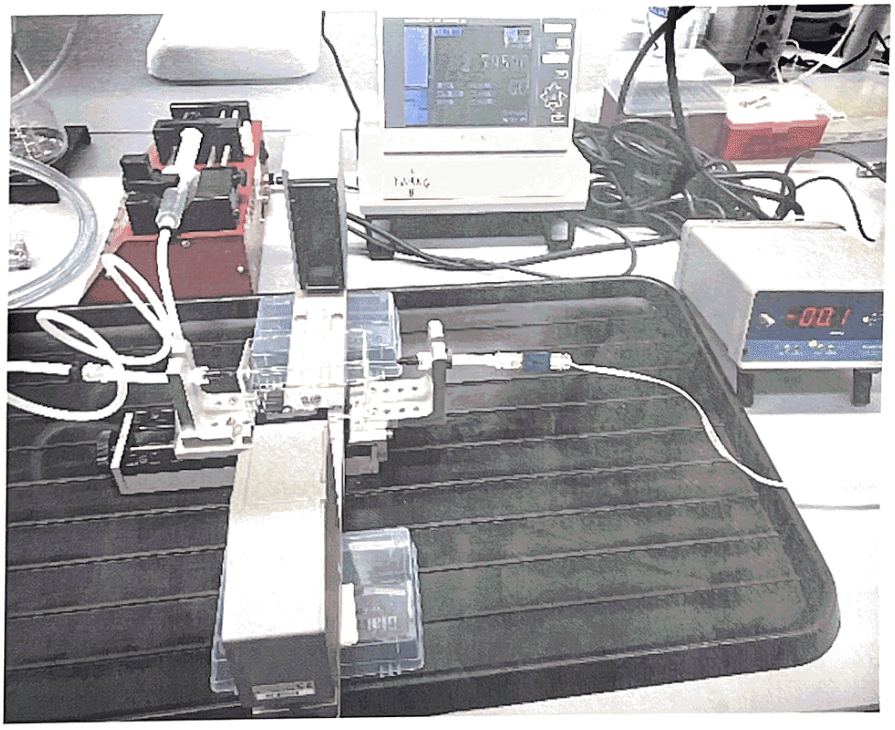


Fig. 5. Assembled system.

Graft Mounting

Cut a segment of undamaged graft or artery ("conduit"). Any branches should be tightly ligated. The segment length should be at least 5 * (approximate inner diameter in mm) + 5 mm. Soak the segment in RT RODI (graft) or DPBS (artery) to hydrate. Throughout the test, keep the conduit wet.

Using calipers, mandrels of various sizes, or a dissecting microscope, measure the exact inner and outer diameters of the conduit. Remember that live arteries may go into spasm. Use topical papaverine or

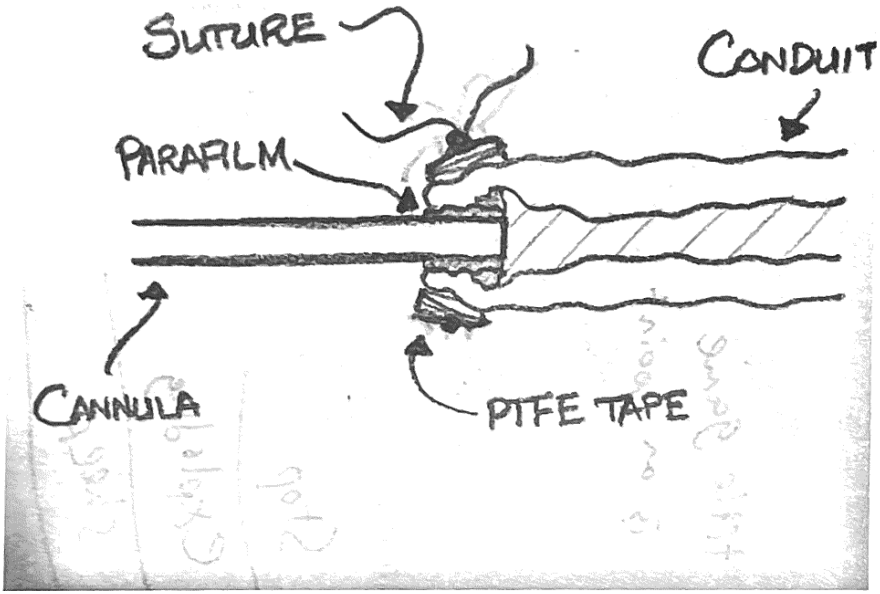


Fig. 8. Cutaway schematic of conduit mounted on cannula.

Final Setup

Extend the axial distention stage until the conduit is just barely taut. Record the distance between the ties as the unpressurized length. If tests are to be performed at different axial stretches, adjust the length to the desired ratio of this reference length.

Ensure that the center of the conduit is centered in x, y, and z within the micrometer field of view and that the conduit axis is perpendicular to the laser axis. Plug up any open space in the cannulae ports.

Fill the compliance chamber to several mm above the conduit with water (graft) or DPBS (artery). If the water level drops into the micrometer field of view, the micrometer will interpret the water surface as an object, disrupting measurement. To prevent this, blind the relevant portion of the micrometer's field of view by taping over portions of one glass panel. Confirm only the conduit is being measured before proceeding.

Fill the syringe with the desired test fluid and flush the system, reinstalling the end cap when finished. If necessary, give the conduit time to hydrate before beginning testing.

Testing

Start sampling on LabChart. Record at least 5 s at zero pressure. Then start the syringe pump at a low flowrate. Increase the flowrate until the pressure reaches the test range within an acceptable period of time. (Since many of our grafts leak, the rate of pressurization has been hard to standardize.)

Using this flowrate, run the graft through the desired test regimen. If the line has small leaks, halting the pump may result in a natural pressure decrease. Alternatively, the pump may be run in "withdraw" mode to decrease the pressure.

When the test is finished, reduce the pressure to zero and vent the line. Stop sampling.

Sample Reuse

Consider inflated samples damaged. They may be reused for burst pressure testing while still mounted on the cannulae, but should not be used in any other mechanical or structural test.

Data Analysis

Data may be exported from LabChart 7 for further processing.

Most analyses require the graft inner diameter. Assuming incompressibility, this can be found from the unloaded dimensions, the axial stretch, and the measured outer diameter:

$$D_i = \sqrt{D_o^2 - \frac{1}{\lambda_z}\left(D_{o,\varnothing}^2 - D_{i,\varnothing}^2\right)}$$

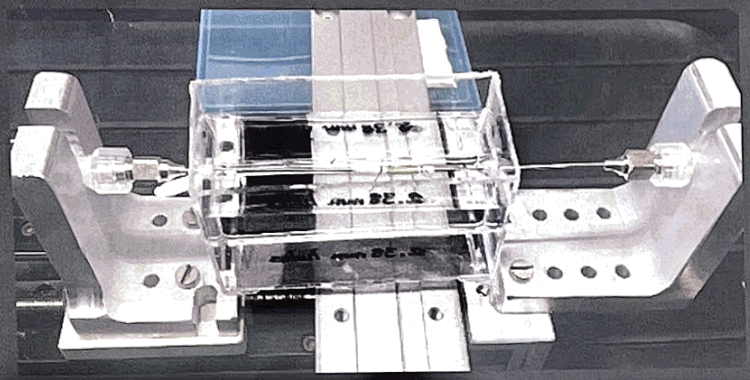
According to the supplementary methods of Wu, W. et al. (2012). *Nature Medicine 18*:1148-53, compliance (%/100 mmHg) is defined as:

$$C = \frac{D_{high} - D_{low}}{D_{low}(P_{high} - P_{low})} * 10,000$$

D, the inner diameter, may be in any units, but *P* should be in mmHg. The 10,000 converts to the %/100 mmHg units.

Compliance & burst pressure

Compliance at 120 mmHg:
3.40 ± 1.56 mmHg



Burst pressure:
1333 ± 299.03 mmHg