

# PRESSURE MYOGRAPH SYSTEM

## MODEL 110P





# PRESSURE MYOGRAPH SYSTEM MODEL 110P

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This document was, as far as possible, accurate at the time of printing.  
Changes may have been made to the software and hardware it describes since then.  
New information may be supplied separately.

This documentation is provided with the DMT Pressure Myograph System – Model 110P

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## SAFETY

The Pressure Myograph System has been designed for use only in teaching and research applications. It is not intended for clinical or critical life-care use and should never be used for these purposes: nor for the prevention, diagnosis, curing, treatment, or alleviation of disease, injury, or handicap.

- Do not open the unit: the internal electronics pose a risk of electric shock.
- Do not use this apparatus near water.
- To reduce the risk of fire or electric shock, do not expose this apparatus to rain or moisture. Objects filled with liquids should not be placed on the apparatus.
- Do not block any ventilation openings. Install in accordance with the manufacturer's instructions.
- Do not install near any heat sources such as radiators, heat registers, stoves, or other apparatus that produce heat.
- Only use attachments and accessories specified by the manufacturer.
- Unplug this apparatus during lightning storms or when unused for long periods of time.
- This apparatus must be grounded.
- Use a three-wire grounding-type cord similar to the one supplied with the product.
- Do not defeat the safety purpose of the polarized or grounding-type plug. A polarized plug has two flat blades, one being wider than the other. A grounding type plug has two blades and a third (round) grounding pin. The wide blade or the third prong is provided for your safety. If the provided plug does not fit into your outlet, consult an electrician for replacement of the obsolete outlet.
- Be advised that different operating voltages require the use of different types of line cord and attachment plugs. Check the voltage in your area and use the correct type. See the table below:

<b>Voltage</b>	<b>Line plug according to standard</b>
110–125 V	UL817 and CSA C22.2 No. 42
220–230 V	CEE 7 page VII, SR section 107-2-D1/IEC 83, page C4.
240 V	BS 1363 of 1984. Specification for 13A fused plugs and switched and unswitched socket outlets.

Protect the power cord from being walked on or pinched: particularly at power plugs and the point where they connect to the apparatus.

Refer all servicing to qualified service personnel. Servicing is required when the apparatus has been damaged in any way; such as, the power-supply cord or plug is damaged, liquid has spilled onto or objects have fallen into the apparatus, the apparatus has been exposed to rain or moisture, does not operate normally, or has been dropped.

## EMC / EMI

This equipment has been tested and found to comply with the limits for a Class B Digital device, pursuant to part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference in residential installations. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception (which can be determined by monitoring the interference while turning the equipment off and on), the user is encouraged to correct the interference by one or more of the following measures:

- Reorient or relocate the receiving antenna.
- Increase the separation between the equipment and receiver.
- Connect the equipment into an outlet on a circuit different to that which the receiver is connected to.
- Consult the dealer or an experienced radio/TV technician for help.

## APPROVALS

Complies with the EMC standards: EMC 89/336/EEC: EN 50 081-1 and EN 50 082-1  
FCC part 15, Class B  
CISPR 22, Class B

Certified with the safety standards: EN 60 065 (IEC 60065)

Complies with the safety standards: UL6500  
CSA E65

# CERTIFICATE OF CONFORMITY

---

DMT A/S, Skejbyparken 152, 8200 Aarhus N., Denmark,  
hereby declares its responsibility that the following product:

*Pressure Myograph System - Model 110P*

is covered by this certificate and marked with CE-label conforms with the following standards:

EN 60 065 (IEC 65)	Safety requirements for mains operated Electronic and related apparatus for household and similar general use.
EN 50 081-1	Electromagnetic compatibility – Generic emission standard Part 1: Residential, commercial and light industry.
EN 50 082-1	Electromagnetic compatibility – Generic immunity standard Part 1: Residential, commercial and light industry.

With reference to regulations in the following directives: 73/23/EEC, 89/336/EEC

## UNPACKING THE PRESSURE MYOGRAPH SYSTEM

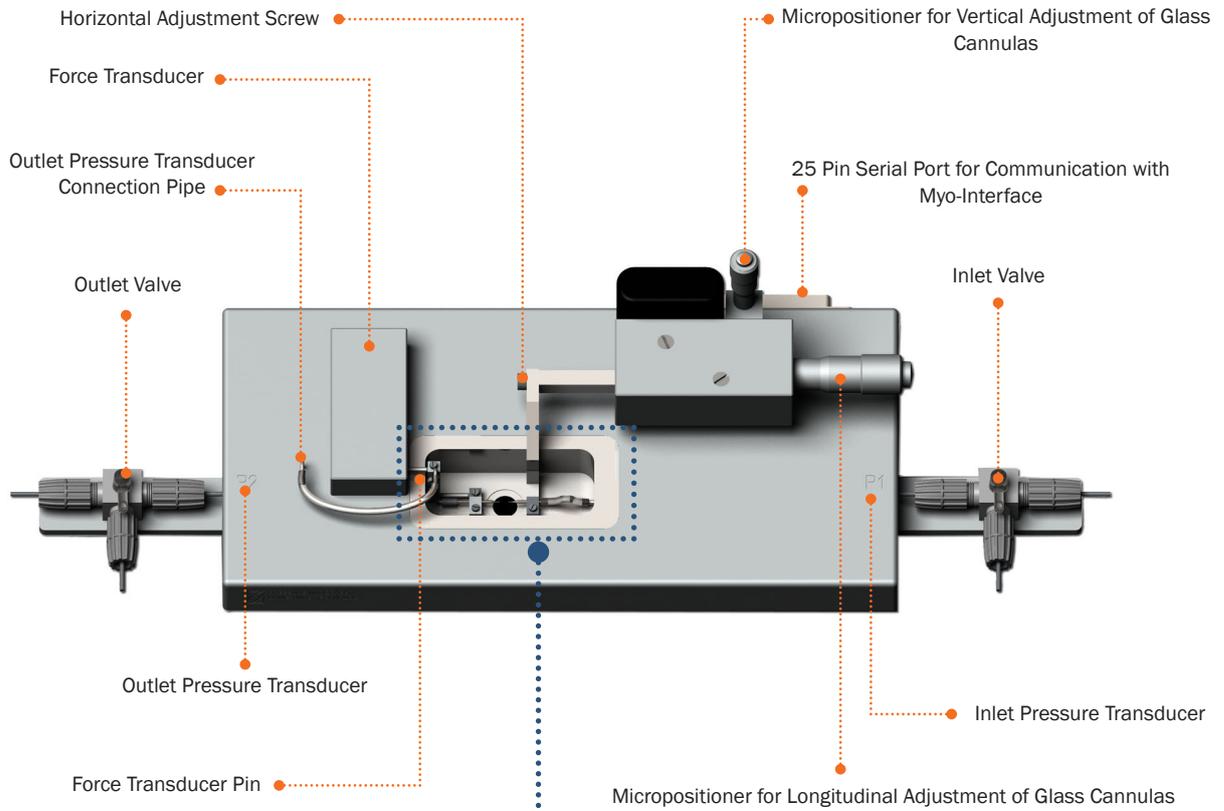
Please take a few minutes to carefully inspect your new Pressure Myograph System for damage, which may have occurred during handling and shipping. If you suspect any kind of damage, please contact us immediately and the matter will be pursued as soon as possible. If the packing material appears damaged, please retain it until a possible claim has been settled.

We recommend that you store the packing material for any possible future transport of the Pressure Myograph System. In case of transport and the original packing material is unavailable, please contact DMT Sales Department for advice and packing instructions. After unpacking your new Pressure Myograph System, please use the following list to check that the system is complete:

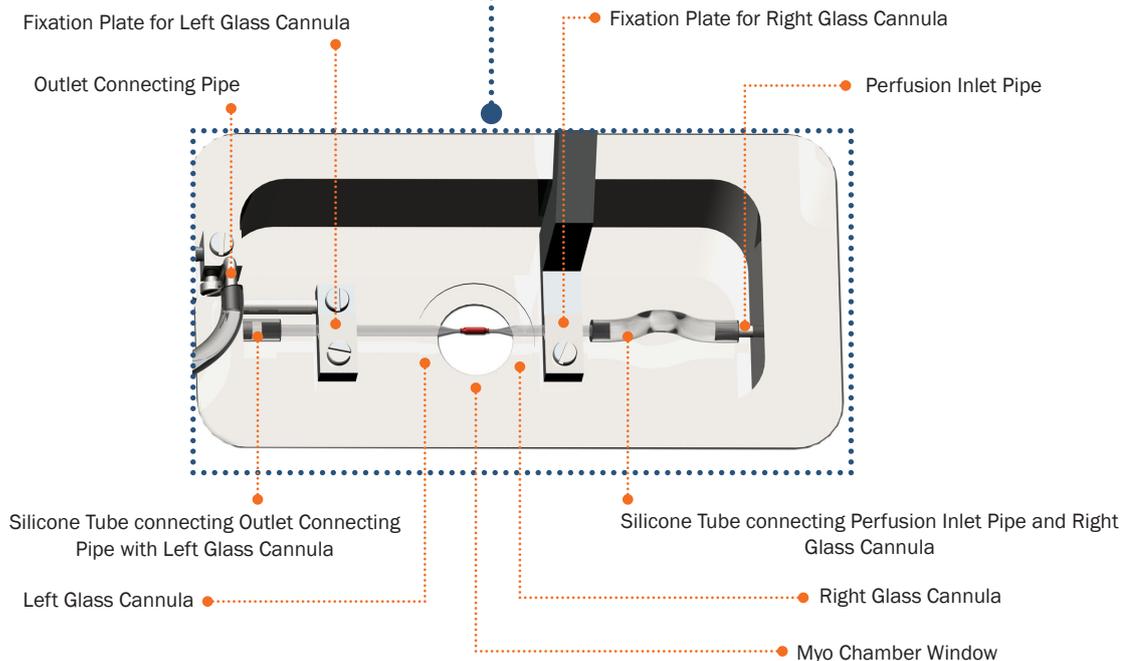
- **Pressure Myograph Unit:**
  - Force calibration kit (Including “bridge”, “balance” and 2 gram weight)
  - Resistance tube shunt (Calibration tube)
  - Two glass cannulas
  - Chamber cover
- **Pressure Myo-Interface:**
  - Pressure Myograph Unit connection cable with a temperature probe
  - Power cord (the shape of the AC plug varies by country; be sure that the plug has the right shape for your location)
  - Serial Cables for PC connection or serial connection to other DMT Pressure Myograph Systems
- **Pressure Regulator:**
  - Power cord (the shape of the AC plug varies by country; be sure that the plug has the right shape for your location)
  - Atlas Copco Cablair hose for main pressure supply
  - Two 500ml Schott Duran Bottles incl. special designed screw caps
- **Riester “Big Ben” Pressure Manometer**
- **Accessories:**
  - Allen key
  - Spare screws
  - Small screwdriver
  - Nylon suture
  - Tube of high vacuum grease
  - Tube of grease for linear slides
  - Silicone tubing, internal diameter 1mm
- **Hardware, Software & Manuals:**
  - USB digital Camera
  - CD with user manual “Pressure Myograph System – Model 110P”
  - CD with DMT Vessel Acquisition Suite Software (DMT VAS) (optional)
- **FlowMeter – Model 161FM (Optional):**
  - User manual “FlowMeter – Model 161FM”
  - MyoFlow™ FlowMeter software including drivers (CD-ROM)
- **Peristaltic Pump for Superfusion Flow (Optional):**
  - Alita Watson Marlow400, VS2-10R-Midi, 2.5-50rpm
- **Computer (Optional)**

# CHAPTER 1 - SYSTEM OVERVIEW

## 1.1 Pressure Myograph Unit



**Figure 1.1** The Pressure Myograph Unit

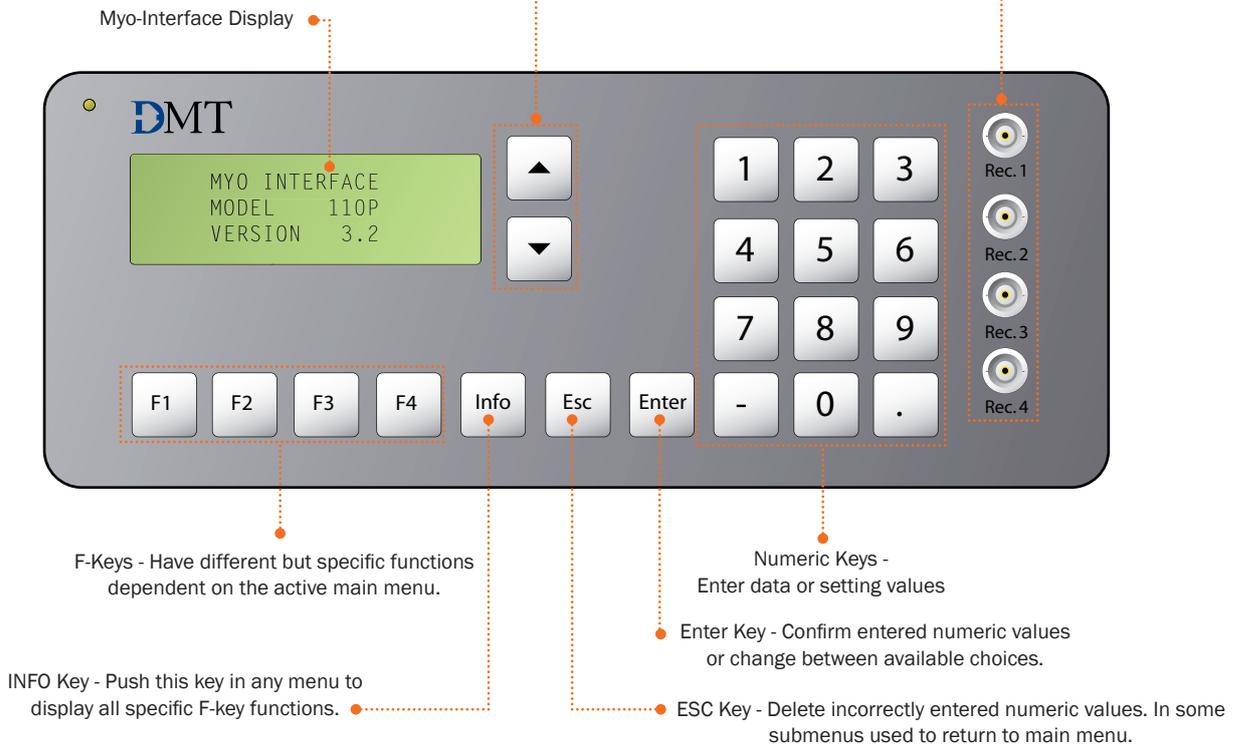


**Figure 1.2** The Pressure Myograph chamber - close up

## 1.2 Pressure Myo-Interface

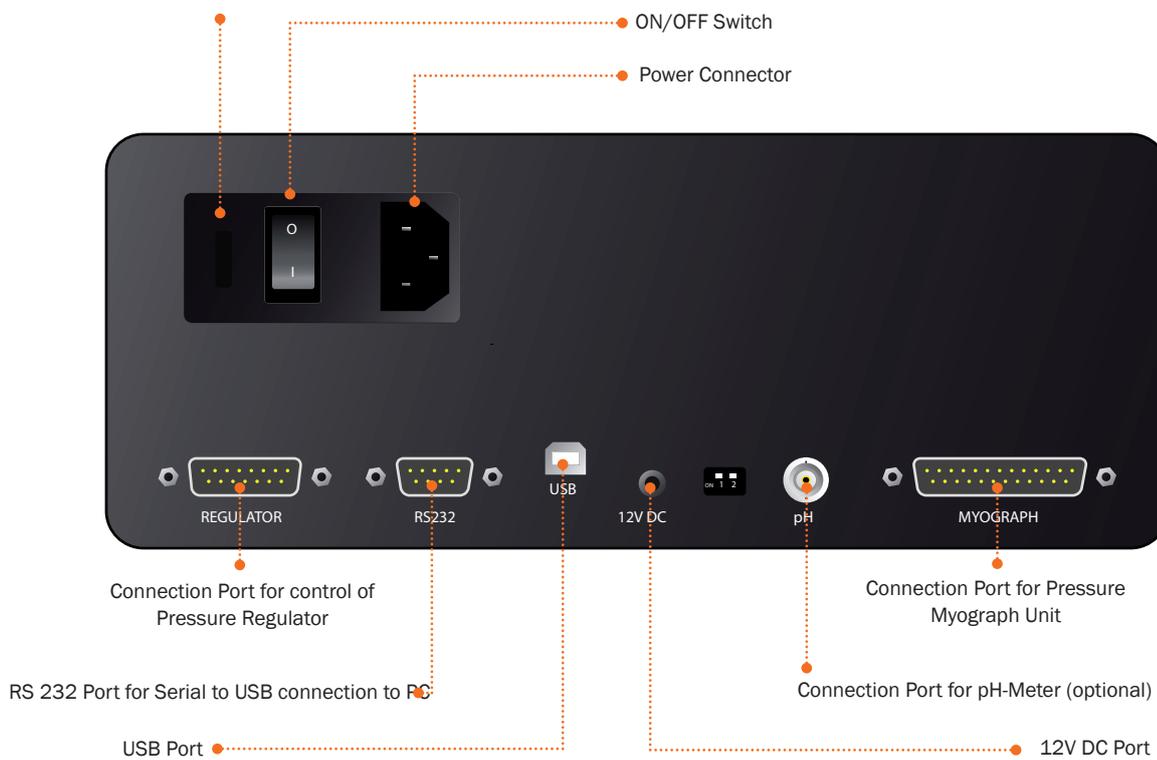
**Up and Down Arrow Keys** - Scroll up and down in the menus. If you are at the top of a menu, use ↑ to change to the previous main menu.

**Analogue Recorder Output** - Connection to external data acquisition system (optional).



**Figure 1.3** Pressure Myo-Interface Front

115-230 V / 50-60 Hz Automatic Voltage Selector



**Figure 1.4** Pressure Myo-Interface Back

### 1.3 Pressure Regulator

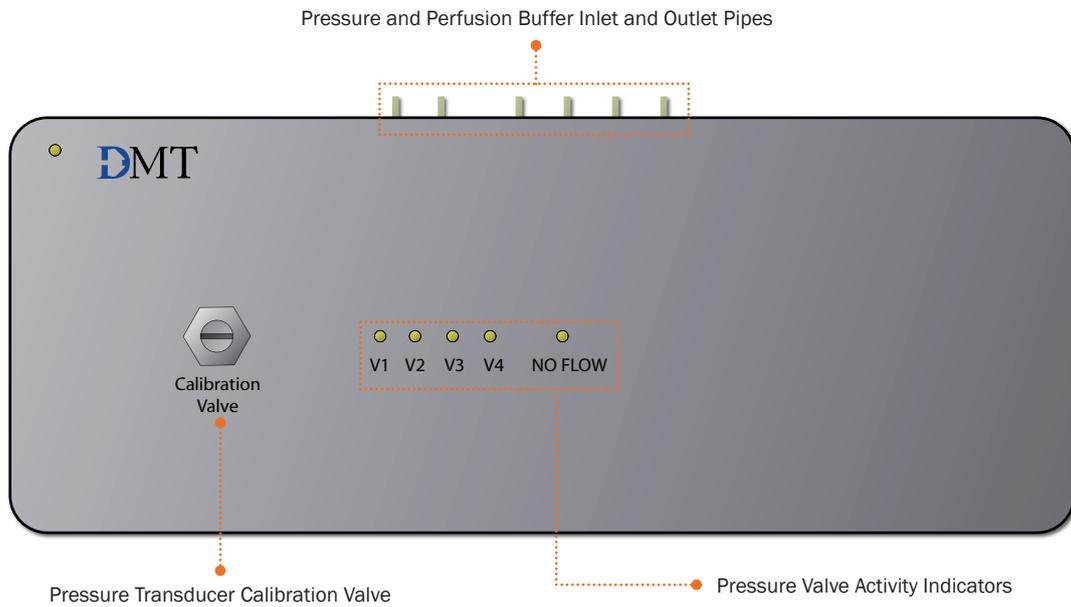


Figure 1.5 Pressure Regulator Front

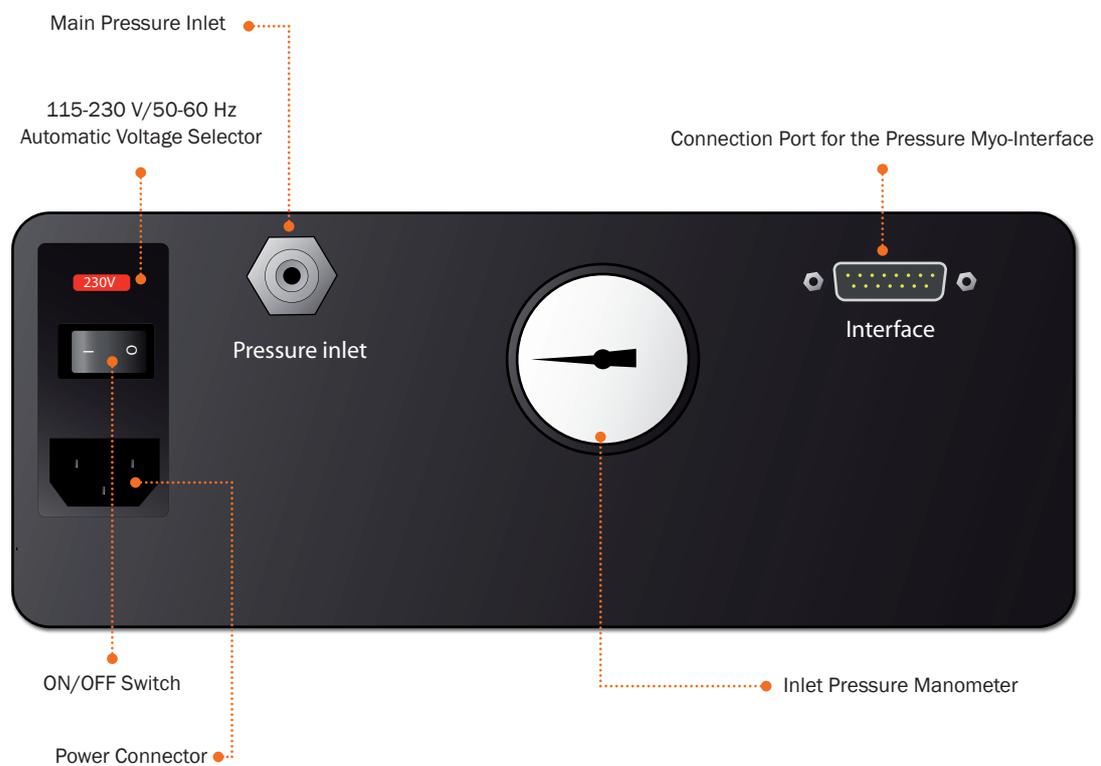
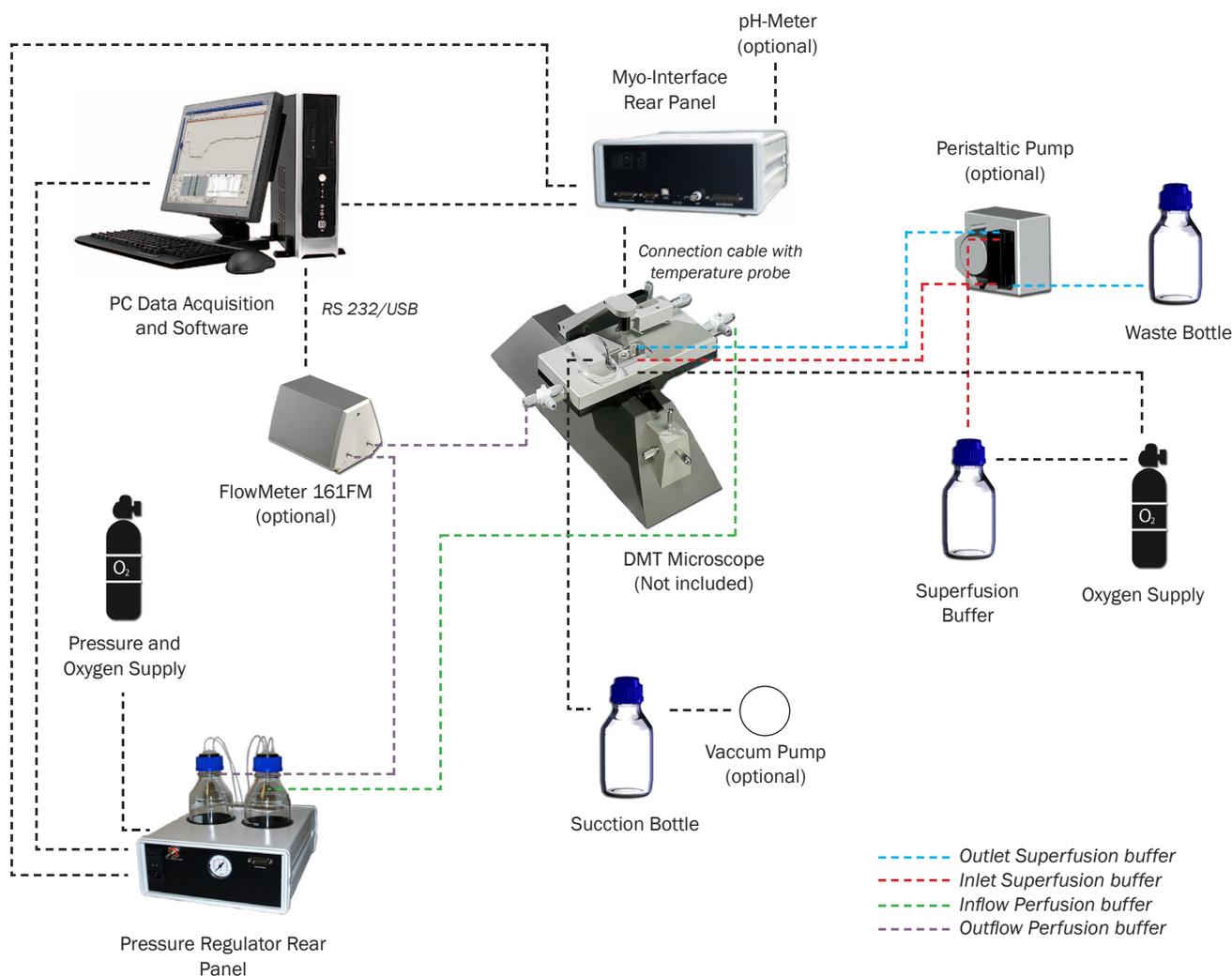


Figure 1.6 Pressure Regulator Back

## CHAPTER 2 - SETTING UP

### 2.1 Pressure Myograph System – Model 110P



**Figure 2.1** Example of the Complete Pressure Myograph System - Model 110P

Figure 2.1 above is a diagram of complete set-up for the Pressure Myograph System - 110P. The diagrams include optional equipment such as a peristaltic pump to enable superfusion flow and pH probes for measuring pH in the superfusion buffer. Also the diagram include the DMT Flowmeter Model 161FM. The Flowmeter enables flow measurements in the range of 1-500 $\mu$ l/min.

Figure 2.1 above is intended as an overview for the set up of the Pressure Myograph Systems followed by a detailed step-by-step description in the remaining sections of Chapter 2.

The Pressure Myograph System will work with a wide range of different microscopes; DMT Microscope, Zeiss Axiovert 40, Nikon TS-100F, Motic AE2000 or your own inverted microscope of choice.

#### **NOTE: THE MICROSCOPE IS ESSENTIAL FOR THE DIMENSIONAL ANALYSIS OF THE MOUNTED VESSELS.**

The DMT Microscope is a standard inverted microscope with a built-in digital CCD USB camera. The DMT Microscope is very useful and recommended in set-ups requiring standard image analysis.

The Zeiss Axiovert 40 is an inverted microscope, which provides the highest degree of flexibility in image analysis. The Zeiss Axiovert 40 and Motic AE 2000 enables the use of a wide range of different objectives and sophisticated fluorescence techniques.

Contact DMT Sales Department for further product information and prices on any optional equipment for the different available microscopes.

## 2.2 Setting up Step by Step

### NOTE:

**IF YOU HAVE PURCHASED A COMPUTER FROM DMT IN CONJUNCTION WITH YOUR PRESSURE MYOGRAPH SYSTEM THEN THE DMT VESSEL ACQUISITION SUITE (DMT VAS) HAS ALREADY BEEN INSTALLED ALONG WITH DRIVERS FOR THE DIGITAL CCD USB CAMERA. FOLLOW THE PROCEDURES IN SECTION 2.2.1 BELOW TO SET-UP THE PRESSURE MYOGRAPH SYSTEM.**

If you have **NOT** purchased a computer from DMT follow the procedures in the user manual “DMT VAS Manual - Core & Analysis Functions” to install DMT VAS on your own computer.

### 2.2.1 Setting up the complete Pressure Myograph System

This section describes how to connect the cables in the Pressure Myograph System as illustrated in figure 2.1 on previous page. Before connecting any of the myograph equipment, ensure that the USB Camera is connected to your PC and the DMT VAS is installed on your PC as described in the DMT VAS Manual.

### NOTE:

**BEFORE PROCEEDING WITH THE CONNECTION PROCEDURE, MAKE SURE THAT THE MYO-INTERFACE; THE PRESSURE REGULATOR, THE MICROSCOPE AND THE PC ARE SWITCHED OFF.**

#### 1. Myograph Unit – Myo-Interface Connection:

Connect the Pressure Myograph Unit to the Myo-Interface by using the grey 25-pin connection cable. The end of the cable with the temperature probe is used with the Pressure Myograph Unit.

#### 2. Myo-Interface – PC Connection:

Connect the Myo-Interface to the computer with the serial to USB cable on the back panel of the Myo-Interface to the USB port on the computer.

#### 3. Myo-Interface – Pressure Regulator Connection:

Connect the Myo-Interface to the Pressure Regulator by using the grey 15-pin connection cable.

Connect the power cord to the power inlet on the back panel of both the Myo-Interface and the Pressure Regulator.

#### 4. Pressure Regulator – Oxygen Supply Connection:

Connect the “Main Pressure Inlet” on the back panel of the Pressure Regulator to an adjustable oxygen supply using the “Atlas Copco Cablair hose” delivered with the Pressure Myograph System.

### IMPORTANT

**MAKE SURE THAT THE HOSE IS SECURELY FASTENED TO THE “MAIN PRESSURE INLET” USING THE HOSE CLIP.**

**THE PRESSURE FROM THE OXYGEN SUPPLY MUST NOT EXCEED 1.0 bar!**

#### 5. Microscope/Camera – PC Connection:

Connect the USB cable from the USB Camera to your PC.

#### 6. Myo-Interface – External Data Acquisition System Connection (optional):

If you have **NOT** obtained the DMT VAS then the four BNC ports on the Myo-Interface front enables connection of an external data acquisition system for recording the inlet pressure, the outlet pressure, the longitudinal force, the actual temperature and the pH value of the superfusion or perfusion buffer.

### NOTE:

**ONLY FOUR OF THE FIVE DATA CHANNELS CAN BE RECORDED SIMULTANEOUSLY.**

#### 7. Turn on the Power:

Turn the main power to the Myo-Interface and the Pressure Regulator on at the power switch. Turn on the computer and the Pressure Myograph System is now ready to be used with the DMT VAS.

### NOTE:

**A DETAILED GUIDANCE IN HOW TO CONNECT THE PRESSURE MYOGRAPH SYSTEM FOR AN EXPERIMENTAL SETUP IS DESCRIBED IN “CHAPTER 3 - THE PRESSURE MYO-INTERFACE” ON PAGE 16.**

### 2.2.2 Installation of DMT VAS and USB Camera

See the user manual “DMT VAS Manual - Core & Analysis Functions” and/or the Quick Guide for DMT VAS and IC Capture for installing the USB camera.

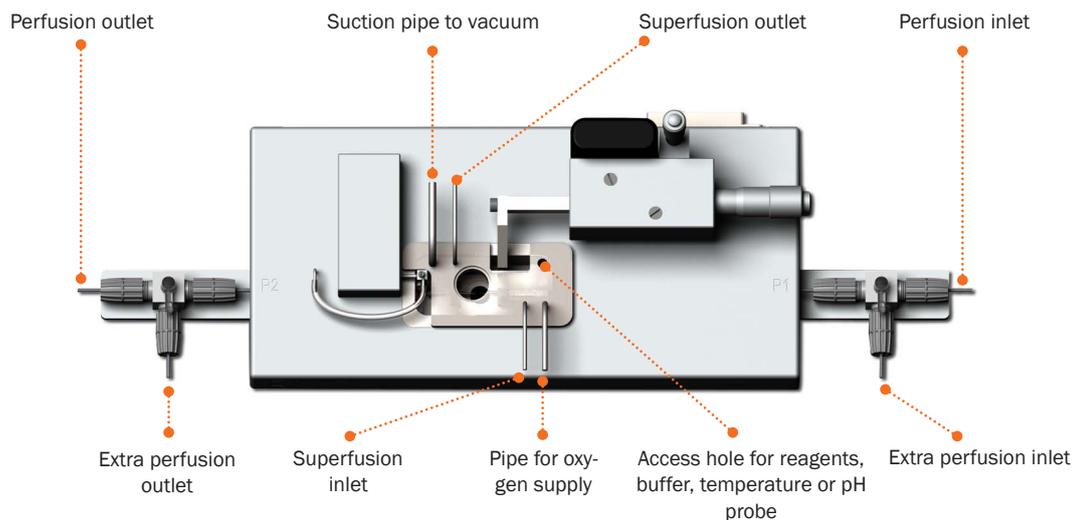
### 2.2.3 The first Weight and Pressure Calibrations

Prior to the shipment of the Pressure Myograph System, it has gone through two days of continuous testing including final weight and pressure calibrations. However in order to ensure that the myograph is working at highest performance, DMT recommends that new weight and pressure calibrations are performed before starting to use the Pressure Myograph System.

The weight and pressure calibration procedures are described in detail respectively in “4.1.3.1 Weight Calibration Procedure” on page 24 and “4.1.3.2 Pressure Calibration Procedure” on page 25.

### 2.2.4 External Pressure Myograph Unit Connections

This section describes how to connect the perfusion circuit, the superfusion circuit, oxygen supply, pressure supply and suction device.



**Figure 2.2** External Pressure Myograph connection

#### 2.2.4.1 Connecting Perfusion Flow

The perfusion flow is controlled by the Pressure Regulator via the Myo-Interface or DMT VAS. How to control the perfusion flow is described in detail in chapter “3.1.4 Pressure Menu” on page 18.

To connect the Pressure Regulator to the Pressure Myograph Unit use the silicone tube (internal diameter 1mm) delivered with the Pressure Myograph System. Connect the “perfusion inlet” at P1 on the Pressure Myograph Unit (see figure 2.1 on previous page) to the P1 outlet on the cap of the buffer bottle (see figure 2.1 on previous page). Connect the “perfusion outlet” at P2 on the Pressure Myograph Unit (see figure 2.1 on previous page and figure 2.2 above) to the P2 inlet in the Pressure Regulator Unit (see figure 2.1 on previous page).

#### NOTE I

**TO FILL THE TUBING AND AVOID AIR BUBBLES, MOUNT THE “RESISTANCE TUBE SHUNT” AS DESCRIBED IN CHAPTER “4.1.3.2 PRESSURE CALIBRATION PROCEDURE” ON PAGE 25. FILL THE SCHOTT BOTTLE WITH 250 ML BUFFER AND RUN THE FOLLOWING “FLUSH” PROGRAMME USING THE MYO-INTERFACE OR DMT VAS:**

P1 = 150mm Hg  
P2 = 70mm Hg

Enter the pressure values in the “Pressure Menu” and press F2 in the “Main Menu” of the Myo-Interface to start the “flush” programme. Run the programme until no air bubbles are visible in the perfusion circuit.

#### NOTE II

**NEVER FILL THE BUFFER BOTTLE WITH MORE THAN 250 ML OF BUFFER AS HIGHER VOLUMES MAKES PRECISE CONTROL OF THE PRESSURE MORE DIFFICULT.**

#### IMPORTANT

**NEVER RUN THE “FLUSH” PROGRAMME WITH A VESSEL MOUNTED IN THE PRESSURE MYOGRAPH CHAMBER AS THIS MAY CAUSE SERIOUS DAMAGE TO THE VESSEL.**

#### **2.2.4.2 Extra Perfusion In/Outlet**

The extra perfusion in and outlet on the Pressure Myograph Unit, 3-way valve makes it possible to add special agents or reagents to the perfusion circuit during an experiment. The small handle on top of the valves controls the flow directions through the 3-way valves at P1 and P2 as described in chapter “4.1.2 3-Way Valve Adjustments” on page 23.

#### **2.2.4.3 Connecting Superfusion Flow (Optional)**

Creating a superfusion flow in the Pressure Myograph Chamber requires an external peristaltic pump, which is not part of the standard Pressure Myograph System. Contact DMT for further information about recommended perfusion pump models, specifications and prices.

The superfusion circuit is connected to the Pressure Myograph Unit as illustrated in “Figure 2.1 Example of the Complete Pressure Myograph System - Model 110P” on page 12.

#### **2.2.4.4 Connecting Flowmeter – Model 161FM (Optional)**

The DMT Flowmeter – Model 161FM is optional for flow measurements in the range of 1 – 1500µl/min. The Flowmeter is easily combined with any DMT Pressure Myograph System as illustrated in “Figure 2.1 Example of the Complete Pressure Myograph System - Model 110P” on page 12.

Use of the FlowMeter makes it possible to control intravascular pressure, pressure gradient and flow conditions with high accuracy, which enables the performance of in vitro studies on vessel segments under conditions very close to those found in vivo. Application of the FlowMeter also enables measurement of flow rate as a parameter, as well as shear stress, Reynolds number and vascular resistance due to changes in vessel structure and function in various physiological and pharmacological studies.

The FlowMeter has to be added as a hardware in the DMT VAS program (see DMT VAS Manual). Besides calculating the flow rate, MyoFlow enables real time calculations of total flow and current drop volume. In addition MyoFlow features a real time flow graph showing flow rate development over time.

#### **2.2.4.5 Connecting Oxygen Supply**

Connect the “oxygen supply pipe” on the Pressure Myograph Chamber cover to an adjustable oxygen supply.

#### **2.2.4.6 Connecting Suction Device**

Connect the “large” pipe on the Pressure Myograph Chamber cover to a vacuum pump via a suction bottle and a vacuum valve as illustrated in “Figure 2.1 Example of the Complete Pressure Myograph System - Model 110P” on page 12. The internal diameter of the silicone tube used for this connection must be 2 mm. (a 2 mm tube is not included in the myograph system).

# CHAPTER 3 - THE PRESSURE MYO-INTERFACE

The DMT VAS control the Pressure Myo-Interface. However, the user **NOT** having DMT VAS; the following chapter will go through how to use the Myo-Interface without DMT VAS.

This chapter is a complete manual for the Pressure Myo-Interface. The chapter contains a step-by-step description of how to navigate in the menus and how to use the special features of the Pressure Myograph System, like the sequencer program.

## 3.1 Myo-Interface Manual

Figure 3.1 below is an overview of the menus in the Pressure Myo-Interface. The menus are divided into main menus and sub-menus. In the following sections, all menus are described in detail.

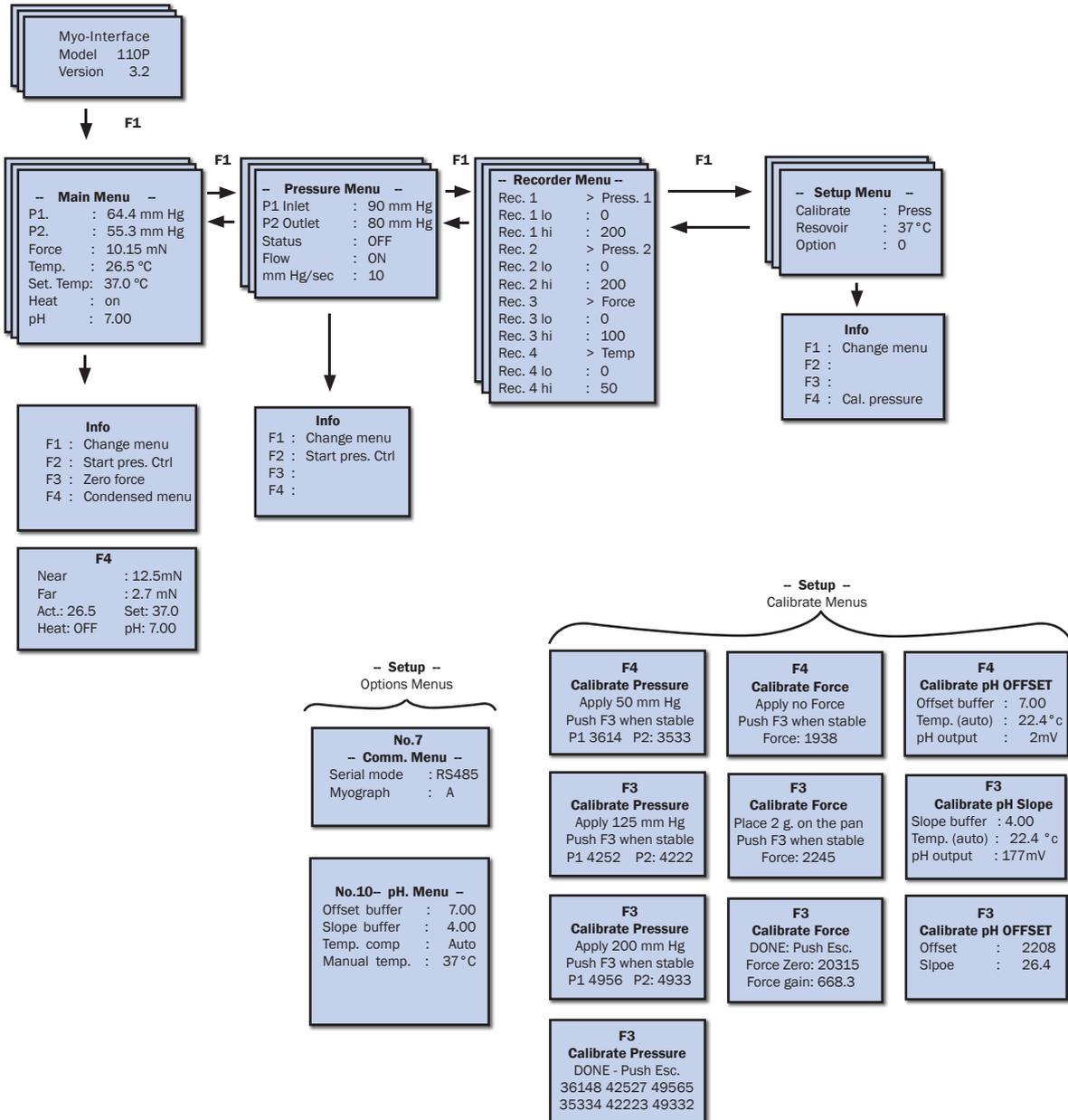


Figure 3.1 Menus in the 110P Myo-Interface

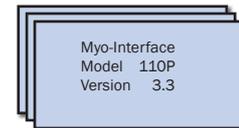
### 3.1.1 General Key Functions

↑ - ↓	Scroll up and down in the menus. Being in the top line of a menu, use ↑ to change to the previous main menus.
<b>Info</b>	Push this key in any menu to display all specific F-key functions.
<b>Numeric keys</b>	Enter data or setting values.
<b>Enter</b>	Confirm entered numeric values or change between available choices.
<b>Esc</b>	Delete incorrectly entered numeric values. In some submenus used to return to main menu.
<b>F1</b>	Change to the next menu.
<b>F2 – F4</b>	Have different but specific functions dependent of the active main menu.

### 3.1.2 Turn-on Message

Turning on the Pressure Myo-Interface, a message appears as shown to the right. Use the ↑ and ↓ keys to adjust the display contrast setting.

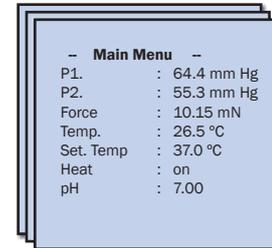
Press **F1** for proceeding to the Main Menu.



### 3.1.3 Main Menu

The Main Menu displays:

<b>P1:</b>	Inlet pressure reading in mm Hg
<b>P2:</b>	Outlet pressure reading in mm Hg
<b>Force:</b>	Longitudinal force reading in mN
<b>Temp.:</b>	Actual temperature probe reading in °C
<b>Set temp.:</b>	Heating temperature setting in °C
<b>Heat:</b>	Actual heating status (ON/OFF)
<b>pH:</b>	Actual pH reading (Optional)



Use the ↑ and ↓ keys to see all values as the display is only capable of showing four lines at the time.

#### Temperature setting:

To change the temperature setting, move the “Set. Temp” line to the top of the display. Use the numeric keys to enter a new temperature setting and press **Enter** to confirm.

#### Heating control:

To turn on heating, move the “Heat” line to the top of the display and use **Enter** to switch between on and off.

The Main Menu has the following functions:

**Info:** Press the **Info** key and the display shows the **F**-key options in the “Main Menu”.

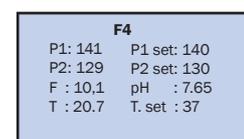
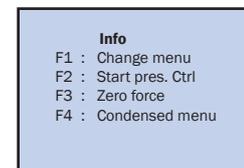
Press **Info** again to return to the “Main Menu” or one of the **F**-keys to proceed with one of these options.

**F1:** Press **F1** to proceed to the “Pressure Menu”.

**F2:** Press **F2** to start the pressure control programme. The settings of the pressure control programme are adjusted in the Pressure Menu.

**F3:** Press **F3** to zero the longitudinal force reading.

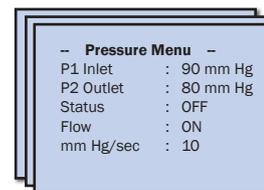
**F4:** Press **F4** for the display to show a condensed “Main Menu”, showing pressure inlet reading (P1), pressure inlet setting (P1 set.), pressure outlet reading (P2), pressure outlet setting (P2 set.), force reading (F), pH reading, Temperature reading (T) and temperature setting (T set.)



### 3.1.4 Pressure Menu

The “Pressure Menu” displays:

<b>P1 Inlet:</b>	Inlet pressure setting in mm Hg
<b>P2 Outlet:</b>	Outlet pressure setting in mm Hg
<b>Status:</b>	Activation of pressure control (ON/OFF)
<b>Flow:</b>	Activation of flow control (ON/OFF)
<b>mm Hg/sec:</b>	Setting of pressure changing gradient



-- Pressure Menu --	
P1 Inlet	: 90 mm Hg
P2 Outlet	: 80 mm Hg
Status	: OFF
Flow	: ON
mm Hg/sec	: 10

The “Pressure Menu” enables control and adjustment of pressures and flow.

**P1 Inlet** and **P2 Outlet** settings control the inlet and outlet pressure of the perfusion buffer. The pressure control is activated or deactivated in the Status line by choosing ON/OFF.

#### IMPORTANT:

**PLEASE NOTE THAT THE P1 INLET PRESSURE CANNOT BE SMALLER THAN THE P2 OUTLET PRESSURE. IF A P1 VALUE SMALLER THAN THE P2 VALUE IS ENTERED THE P1 VALUE WILL AUTOMATICALLY BE SET TO A VALUE EQUAL P2. FURTHERMORE IF A P2 VALUE LARGER THAN THE P1 VALUE IS ENTERED THEN THE P2 VALUE WILL AUTOMATICALLY BE SET TO A VALUE EQUAL P1. A SETTING WHERE P1 = P2 ACTIVATES THE “NO-FLOW” FUNCTION IN THE PRESSURE REGULATOR AS DESCRIBED IN “4.3.2 NO-FLOW SETTING” ON PAGE 28. THE FUNCTION WORKS TO PREVENT BACKFLOW.**

The perfusion flow can be activated or deactivated in the **Flow** line (ON/OFF).

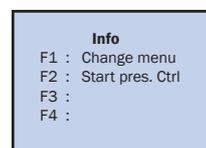
In the **mm Hg/sec** line it is possible to adjust and control the pressure-changing gradient.

The “Pressure Menu” has the following functions:

**Info:** Press the **Info** key and the display shows the F-key options in the “Pressure Menu”.  
Press Info again to return to the “Pressure Menu” or one of the **F**-keys to proceed with one of these options.

**F1:** Press **F1** to proceed to the “Sequencer Menu”.

**F2:** Press **F2** to start the pressure control programme.



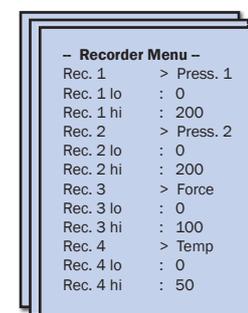
<b>Info</b>	
F1 :	Change menu
F2 :	Start pres. Ctrl
F3 :	
F4 :	

### 3.1.5 Recorder Menu

The Pressure Myo-Interface has four analogue output ports at the front panel for connection to a data recording system. Each of these output ports can be programmed to represent inlet pressure reading, outlet pressure reading, longitudinal force reading, temperature or pH-meter. The default settings are:

- Rec. 1:** P1 Inlet pressure output
- Rec. 2:** P2 Outlet pressure output
- Rec. 3:** Longitudinal force output
- Rec. 4:** Temperature output

The full-scale output on the recorder is one volt. In the “Recorder Menu” it is possible to change the value that corresponds to 0 V (lo) and 1 V (hi).

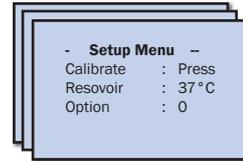


-- Recorder Menu --	
Rec. 1	> Press. 1
Rec. 1 lo	: 0
Rec. 1 hi	: 200
Rec. 2	> Press. 2
Rec. 2 lo	: 0
Rec. 2 hi	: 200
Rec. 3	> Force
Rec. 3 lo	: 0
Rec. 3 hi	: 100
Rec. 4	> Temp
Rec. 4 lo	: 0
Rec. 4 hi	: 50

### 3.1.6 Setup Menu

The “Setup Menu” displays:

**Calibrate:** Choose which sensor to be calibrated. (Pressure, force or pH)  
**Reservoir:** Temperature setting for perfusion buffer reservoir  
**Option:** The option line allows access to submenus using special codes



To change any of the settings in the “Setup Menu”, move the line to be changed to the top line of the display:

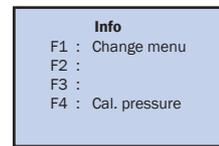
- Press **Enter** to change between calibration of pressure transducers, force transducer or pH in the calibrate line.
- Use the numeric keys to change the reservoir temperature setting and press **Enter** to confirm.
- Enter the specific access code and press **Enter** to go to the submenu.

The “Setup Menu” has the following functions:

**Info:** Press the **Info** key and the display shows the **F**-key options in the “Setup Menu”.

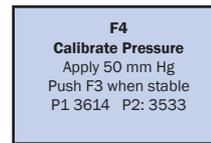
Press Info again to return to the “Setup Menu” or one of the **F**-keys to proceed with one of these options.

**F1:** Press **F1** to proceed to the “Main Menu”.



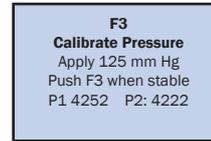
**F4: Pressure transducer calibration**

Choose **Press** in the calibrate line and press **F4** to enter the window shown to the right.

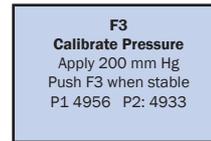


Apply a pressure of 50mm Hg on the Pressure Myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.

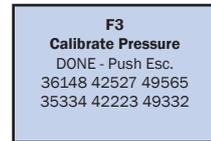
Apply a pressure of 125mm Hg on the Pressure Myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.



Apply a pressure of 200mm Hg on the Pressure Myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.



The calibration is now finished. The readings in the two bottom lines are the calibration parameters stored in the internal memory of the Myo-Interface. Press **ESC** to return to the “Setup Menu”.



For a more detailed instruction to the calibration procedure see chapter “4.1.3 Force and Pressure Calibration” on page 23.

## Force transducer calibration

Chose Force in the calibrate line and press **F4** to enter the window shown to the right.

Make sure that no force is applied on the force transducer and when the relative reading at the bottom line of the window appears stable, press **F3** to proceed.

Carefully place the 2-gram weight on the load pan and when the relative reading at the bottom line of the window appears stable, press **F3** to proceed.

The calibration is now finished. The readings in the two bottom lines are the calibration parameters stored in the internal memory of the Myo-Interface. Press **ESC** to return to the "Setup Menu".

For a more detailed instruction to the calibration procedure, see "4.1.3 Force and Pressure Calibration" on page 23.

## pH calibration (Optional)

Choose **pH** in the calibrate line and press **F4** to enter the window shown to the right.

The first line shows the value of the offset buffer (first buffer solution), which is always 7.00.

The second line shows the temperature of the buffer solution. The temperature is an important parameter in the calibration formula and is obtained automatically by placing the myograph temperature probe in the buffer solution.

The third line shows the output from the pH probe as raw data from the A-D converter.

Place the pH-meter electrode and temperature probe in the offset buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press **F3** to proceed.

In the first line is now shown the value of the slope buffer (second buffer solution), which is always 4.00.

Place the pH and temperature probes in the slope buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press **F3** to proceed.

The calibration is now finished. The parameters are stored in the internal memory of the Myo-Interface. Press **ESC** to return to the "Setup Menu".

### "pH" submenu (optional):

To open the special "pH" submenu, enter the code 10 in the option line in the "Setup Menu".

<b>Offset buffer:</b>	pH value of offset buffer
<b>Slope buffer:</b>	pH value of slope buffer
<b>Temp. comp:</b>	AUTO or MANUAL temperature compensation
<b>Manual temp:</b>	Manual temperature setting

The pH submenu contains the settings of the pH calibration procedure. Offset buffer and slope buffer are the pH values of the buffers used to calibrate the pH probe.

pH measurements are temperature dependent and in the "temperature compensation" line it is possible to choose between an AUTO or MANUAL temperature compensation. In the AUTO mode the temperature probe is used to measure the temperature of the calibration buffers. The temperature is then automatically used in the pH calibration. In the MANUAL mode, the temperature of the calibration buffers is measured with a thermometer and entered manually in the "manual temperature" line.

Press **F1** to return to the "Setup Menu".

```
F4
Calibrate Force
Apply no Force
Push F3 when stable
Force: 1938
```

```
F3
Calibrate Force
Place 2 g. on the pan
Push F3 when stable
Force: 2245
```

```
F3
Calibrate Force
DONE: Push Esc.
Force Zero: 20315
Force gain: 668.3
```

```
F4
Calibrate pH OFFSET
Offset buffer : 7.00
Temp. (auto) : 22.4 °c
pH output    : 2mV
```

```
F3
Calibrate pH Slope
Slope buffer  : 4.00
Temp. (auto)  : 22.4 °c
pH output     : 177mV
```

```
F3
Calibrate pH OFFSET
Offset       : 2208
Slope       : 26.4
```

```
No.7
-- pH. Menu --
Offset buffer : 7.00
Slope buffer  : 4.00
Temp. comp    : Auto
Manual temp.  : 37 °C
```

## CHAPTER 4 - PRESSURE MYOGRAPH MANUAL

Chapter 4 contains a complete explanation to all mechanical features of the Pressure Myograph System. This includes how to use the DMT Microscope and how to use, adjust and separate the Pressure Myograph Unit.

### 4.1 The Pressure Myograph Unit

The pressure myograph unit can be placed on the DMT microscope (as illustrated in figure 4.1 A below) or an inverted microscope (as illustrated in figure 4.1 B below). Press the black lever to lock the Pressure Myograph Unit on the DMT Microscope.



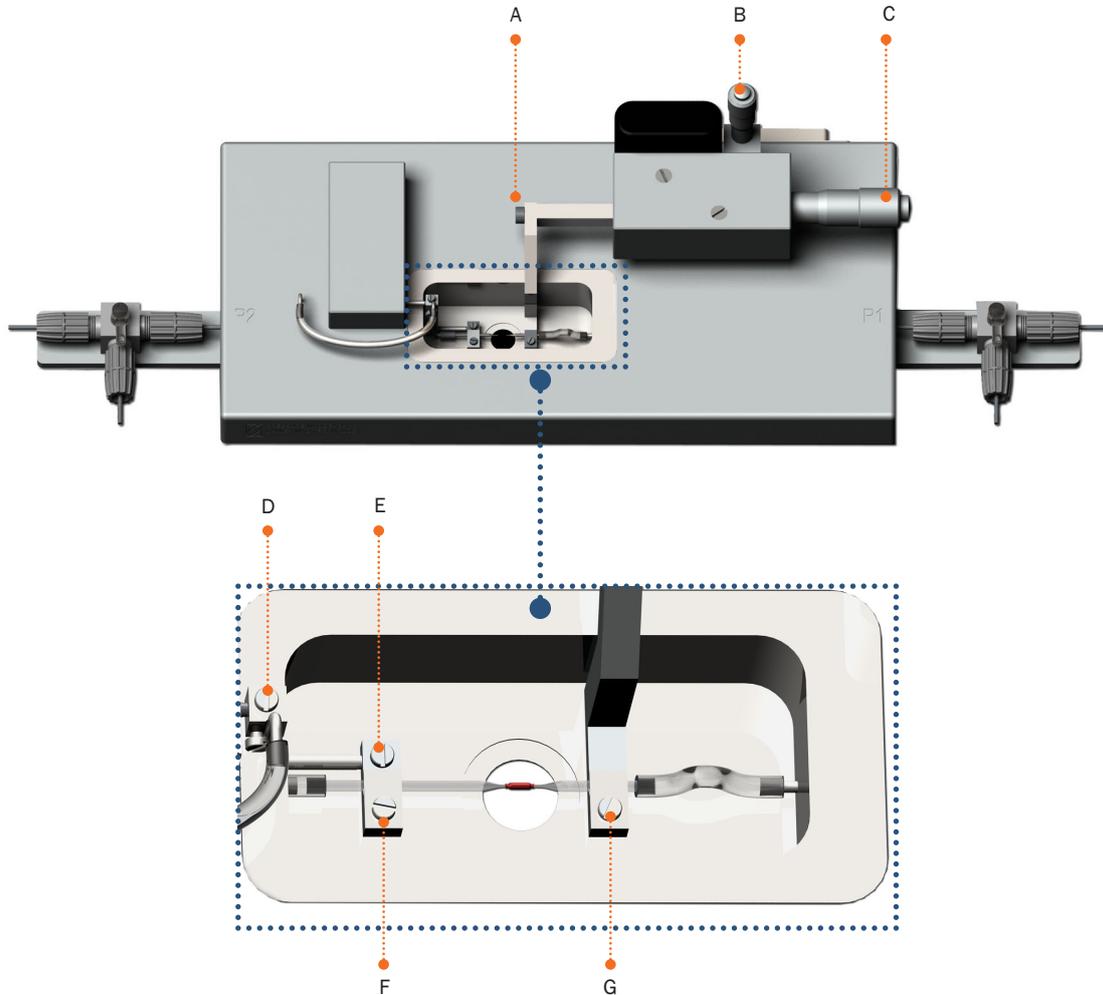
**Figure 4.1 A-B** DMT Microscope setup and Zeiss inverted Microscope setup

### 4.1.1 Adjustment of Glass Cannulas

Adjustment of the glass cannulas is divided into a pre-experimental alignment and general adjustments done during mounting of a blood vessel or when running an experiment.

#### General Adjustments:

General adjustments are performed using the two micropositioners on top of the Pressure Myograph Unit. The vertical micropositioner (figure 4.2 (B) below) is used to adjust the vertical position of the right glass cannula and thereby the vertical position of the mounted blood vessel. The horizontal micropositioner (fig. 4.2 (C) below) is used to define the length between the two glass cannulas and thereby the horizontal stretch of the mounted blood vessel.



**Figure 4.2** Adjustment of glass cannulas

#### Pre-Experimental Alignment:

To make the general adjustments as easy as possible during an experiment, it is important to make an alignment of the glass cannulas prior to the mounting of the blood vessel.

#### Left Glass Cannula:

Horizontal positioning of the left glass cannula is adjusted by carefully loosening screw D (figure 4.2 above). Vertical positioning of the left glass cannula is adjusted by carefully loosening screw E (figure 4.2 above). Longitudinal positioning of the left glass cannula is adjusted by gently loosening of screw F (figure 4.2 above).

#### IMPORTANT

**BE VERY CAREFUL NOT TO APPLY TOO MUCH FORCE (>100 GRAM) ON THE FORCE TRANSDUCER PIN WHEN ADJUSTING THE LEFT GLASS CANNULA. THE FORCE TRANSDUCER HAS A MECHANICAL PROTECTION IN ITS LONGITUDINAL DIRECTION OF MOVEMENT, BUT IS VERY VULNERABLE TO FORCE APPLIED ON THE FORCE TRANSDUCER PIN.**

**Right Glass Cannula:**

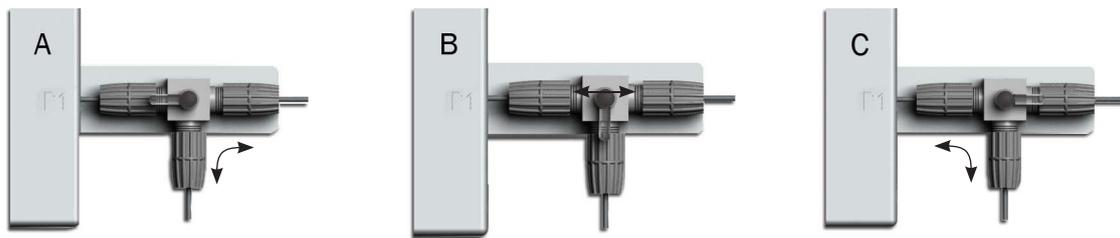
Horizontal positioning of the right glass cannula is adjusted by carefully loosening screw A (figure 4.2 on previous page). Vertical positioning of the right glass cannula is adjusted with the micropositioner C (figure 4.2 on previous page). Longitudinal positioning of the right glass cannula is adjusted by gently loosening of screw G (figure 4.2 on previous page).

**NOTE:**

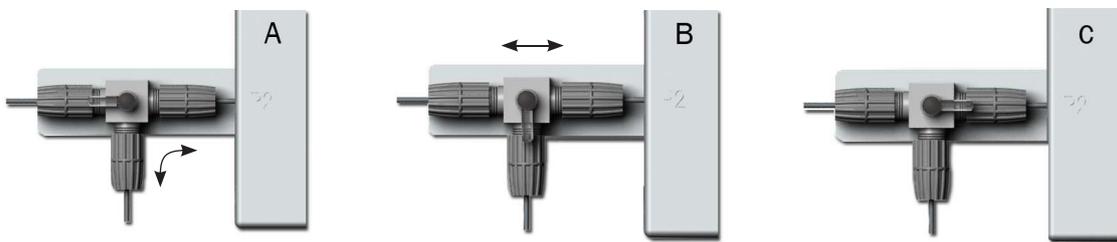
**BE CAREFUL NOT TO DAMAGE THE TIP OF THE GLASS CANNULAS DURING THE GENERAL ADJUSTMENT.**

**4.1.2 3-Way Valve Adjustments**

The 3-way valves on each side of the Pressure Myograph Unit (at P1 & P2) have three different settings to control the in- or outlet flow to the pressure transducers. The flow settings are illustrated in figure 4.3 and figure 4.4 below.



**Figure 4.3** Three-way valve flow settings at P1



**Figure 4.4** Three-way valve flow settings at P2

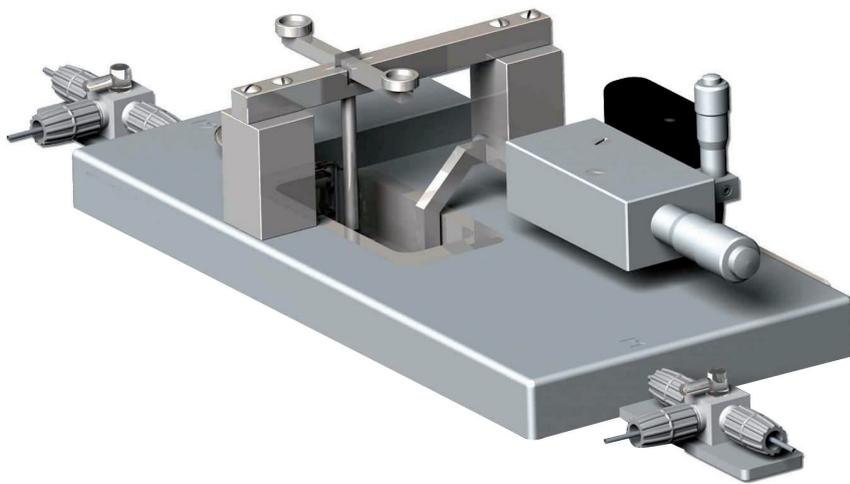
**4.1.3 Force and Pressure Calibration**

This section contains a complete explanation of how to perform the force (weight calibration) and pressure calibrations. As a part of the general maintenance of the myograph, DMT recommends that the myograph is force and pressure calibrated at least once every month. DMT also recommends that the myograph is force and pressure calibrated every time the system has been moved or has not been used for a long period of time.

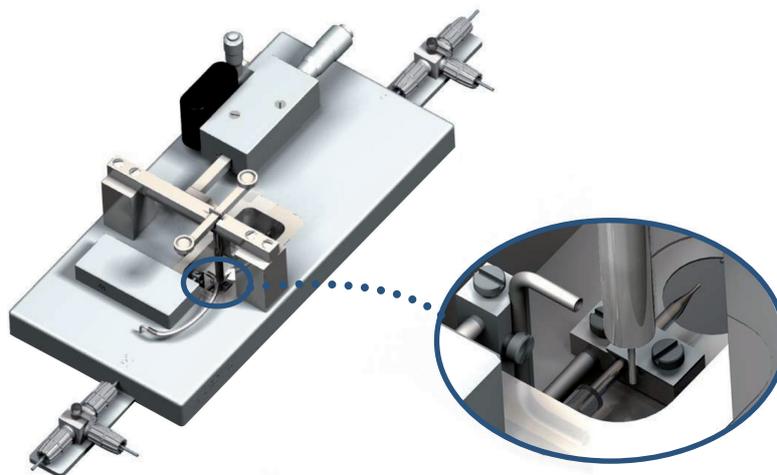
### 4.1.3.1 Weight Calibration Procedure

The section contains a complete step-by-step description of how to weight calibrate the force transducer.

1. Fill the myograph chamber with double distilled water and move the glass cannulas apart.
2. Place the calibration bridge, balance and weight on the myograph unit allowing it to be pre warmed together with the myograph unit. Turn on the heating in the “Main Menu” on the Myo-Interface.
3. After approximately 20-30 minutes the whole system will have reached the target temperature (normally 37°C). Place the warmed calibration bridge and balance on the myograph unit as illustrated in figure 4.5 below.
4. Make sure that the tip of the “transducer arm” on the balance is placed behind the glass cannula fixation plate as illustrated in figure 4.6 below. Carefully move the calibration bridge until the tip of the “transducer arm” is placed freely behind the glass cannula fixation plate, which means it does not touch the fixation plate.



**Figure 4.5** Weight Calibration Set up



**Figure 4.6** Illustration of how to fit the balance just behind the glass cannula fixation plate

5. Go to the “Setup Menu” on the Myo-Interface and choose Force to weight calibrate the force transducer Press **F4** to start calibration.
6. Make sure that absolutely no force is applied on the force transducer by checking that the tip of the “transducer arm” is not touching the glass cannula fixation plate. Also check that the relative force reading in the display is stable. Press **F3** to proceed with calibrating.
7. Carefully place the 2 g weight on the pan. The force applied on the force transducer should mimic the stretch created by the contraction of a mounted ring preparation. Wait until the relative force reading is stable. Press **F3** to finish the calibration.
8. Press **Esc** and go to the “Main Menu” on the Myo-Interface. The force reading on the Myo-Interface should now be very close to 9.81 mN. If the force reading is different from 9.81 mN then try to calibrate the force transducer once again starting with step 3.
9. After calibrating the force transducer, carefully remove weight, balance and calibration bridge. The Pressure Myograph System is now ready for use longitudinal force measurements.

```

-- Setup Menu --
Calibrate      : Press
Reservoir     : 37 °C
Option        : 0
  
```

```

F4
Calibrate Force
Apply no Force
Push F3 when stable
Force: 1938
  
```

```

F3
Calibrate Force
Place 2 g. on the pan
Push F3 when stable
Force: 2245
  
```

```

F3
Calibrate Force
DONE: Push Esc.
Force Zero: 20315
Force gain: 668.3
  
```

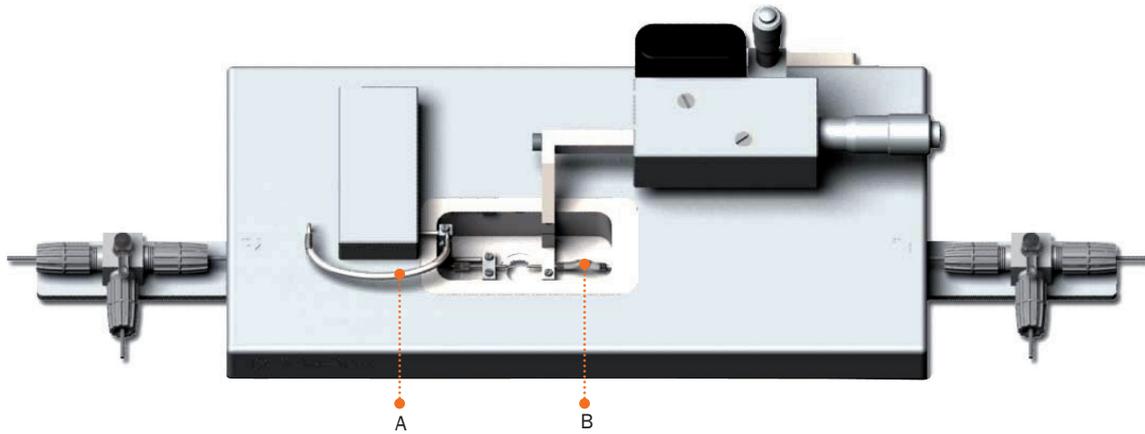
#### 4.1.3.2 Pressure Calibration Procedure

The setup to perform the pressure transducer calibration procedure is illustrated in figure 4.7 below. Carefully follow the procedure described below to pressure calibrate the Pressure Myograph System.

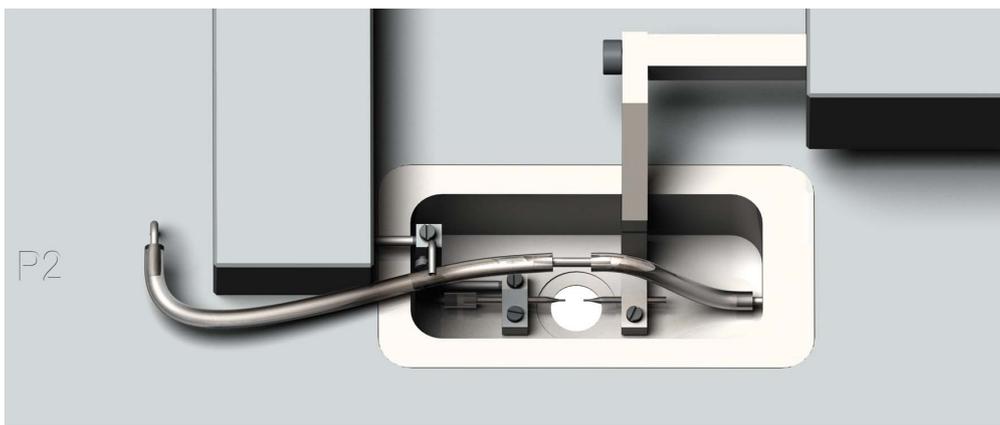


**Figure 4.7** Illustration of set up for pressure transducer calibration

1. Connect the Pressure Myograph Unit to the Pressure Regulator as to perform a standard pressure myograph experiment as illustrated in figure 4.7 above.
2. Connect the “Big Ben” Pressure Manometer to the 3-way valve at P1 on the Pressure Myograph Unit as illustrated in figure 4.7 above.
3. Inside the myograph chamber, on the right side, is a small stainless steel pipe, which via a small silicone tube is connected to the right glass cannula, see figure 4.8 (B) on next page. Carefully disconnect the silicone tube from the stainless steel pipe.
4. Carefully disconnect the silicone tube between the “outlet connecting pipe” and the “outlet pressure transducer connecting pipe”, see figure 4.8 (A) on next page.
5. Connect the enclosed “resistance tube shunt” to the stainless steel pipe inside the myograph chamber and the “outlet pressure transducer connection pipe”, as illustrated in figure 4.9 on next page.



**Figure 4.8** Disconnection of silicone tubes



**Figure 4.9** Mounting of "Resistance tube shunt"

6. Close the inlet from the pressure regulator to P1 by turning the handle on the 3-way valve towards the normal inlet way. The inlet to P1 now comes from the pressure manometer. See "Figure 4.3 Three-way valve flow settings at P1" on page 23 and "Figure 4.7 Illustration of set up for pressure transducer calibration" on page 25.
7. Close the "calibration valve" on the front of the Pressure Regulator by turning it clockwise.

**IMPORTANT**

**MAKE SURE THAT THE "CALIBRATION VALVE IS COMPLETELY CLOSED. IF THE "CALIBRATION VALVE" IS NOT COMPLETELY CLOSED IT WILL RESULT IN INCORRECT RESULTS AND UNRELIABLE PRESSURE READINGS AN SETTINGS DURING AN EXPERIMENT.**

8. Turn on the Pressure Regulator and the Myo-Interface. Use **F1** to go to the "Setup Menu" in the Myo-Interface. Bring the "calibrate line" to the top of the display by using the numeric keys and press Enter to choose between calibrating pressure, force or pH. Choose "calibrate pressure" in the calibrate line and press **F4** to enter the calibration menu.

9. Apply a pressure of 50mm Hg on the pressure myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.
10. Apply a pressure of 125mm Hg on the pressure myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.
11. Apply a pressure of 200mm Hg on the pressure myograph using the “Big Ben” pressure manometer. When the relative readings at the bottom line of the window appears stable, press **F3** to proceed.
12. The calibration is now finished. The readings in the two bottom lines are the calibration parameters stored in the internal memory of the Myo-Interface. Press **ESC** to return to the “Setup Menu”.
13. Carefully remove the “resistance tube shunt” and reconnect the tubing to the glass cannulas. Open the “calibration valve” on the Pressure Regulator by turning it counter clockwise.

**F4**  
**Calibrate Pressure**  
 Apply 50 mm Hg  
 Push F3 when stable  
 P1 3614 P2: 3533

**F3**  
**Calibrate Pressure**  
 Apply 125 mm Hg  
 Push F3 when stable  
 P1 4252 P2: 4222

**F3**  
**Calibrate Pressure**  
 Apply 200 mm Hg  
 Push F3 when stable  
 P1 4956 P2: 4933

**F3**  
**Calibrate Pressure**  
 DONE - Push Esc.  
 36148 42527 49565  
 35334 42223 49332

### IMPORTANT

**MAKE SURE THAT THE “CALIBRATION VALVE” IS COMPLETELY OPENED AFTER THE PRESSURE CALIBRATION. A HALF CLOSED “CALIBRATION VALVE” WILL MAKE IT VERY DIFFICULT TO CONTROL THE PRESSURE AND MAKE THE PRESSURE READINGS UNRELIABLE.**

#### 4.1.4 Replacement of Glass Cannulas

The mounted glass cannulas are easily replaced with new ones by carefully loosening the two screws F and G in “Figure 4.2 Adjustment of glass cannulas” on page 22. The recommended size for the new glass cannulas is 16 mm for the glass cannula connected to P1 (right side) and 14 mm for the glass cannula connected to P2 (left side).

#### 4.1.5 Checking the Force Transducer

The myograph force transducer is a strain gauge connected in a Wheatstone bridge. The force transducer is placed in a separate compartment on top of the pressure myograph unit. The separate compartment provides some mechanical protection for the force transducer but the transducer is still very vulnerable to applied forces exceeding 1 newton (100 gram) or fluid running into the transducer compartment due to insufficient greasing of the transducer pinhole.

This chapter describes how to check the force transducer for any kind of damage.

##### 4.1.5.1 Simple Force Transducer Check

###### Unstable readings:

1. If the force reading on the Myo-Interface appears unstable, then first check that the Myo-Interface and the Myograph Unit are properly connected through the 25-pin grey cable.
2. If the force reading still appears unstable, then perform a new weight calibration of the fore transducer as described in “4.1.3.1 Weight Calibration Procedure” on page 24.

During the weight calibration monitor the relative force reading values in the calibration menu on the Myo-Interface:

- If the value is 0 or above 6500, then the force transducer is broken and needs to be changed.
- If the reading is between 1 - 499 or 5000 – 6500 then contact Danish Myo Technology for further instructions.

If the message “OFF” is displayed in the force reading line in the Main Menu on the Myo-Interface then the force transducer is broken and needs to be replaced.

In case of any other problems related to the force transducer, please contact DMT for further instruction and advice.

### 4.1.6 Checking the Pressure Transducers

The Pressure Myograph Unit contains two pressure transducers (P1 and P2) based on Wheatstone bridge circuit. The transducers are placed inside the pressure myograph unit to protect them from mechanical damage. The pressure transducers are very vulnerable to applied pressures exceeding 300mm Hg, a pressure, which is easily exceeded if trying to flush the transducers using a syringe.

#### **IMPORTANT**

**DMT RECOMMENDS THAT THE MYO-INTERFACE IS CONNECTED AND TURNED ON, WHEN FLUSHING THE PRESSURE TRANSDUCERS, TUBING AND GLASS CANNULAS USING A SYRINGE. THIS ENABLES AN EXACT CONTROL OF THE PRESSURE APPLIED ON THE PRESSURE TRANSDUCERS, WHICH WILL PREVENT THEM FROM BEING DAMAGED. THE APPLIED FLUSHING PRESSURE SHOULD NOT EXCEED 150 mmHg.**

#### 4.1.6.1 Simple Pressure Transducer Check

1. If the pressure reading on the Myo-Interface appears unstable, then first check that the Myo-Interface and the Pressure Myograph Unit are properly connected through the 25-pin grey cable.
2. If the pressure reading still appears unstable, then perform a new pressure calibration of the pressure transducers as described in “4.1.3.2 Pressure Calibration Procedure” on page 25.

During the pressure calibration monitor the three relative pressure-reading values in the calibration menu on the Myo-Interface:

- If the value is 0 or above 6500 for one of the pressure transducers, then the pressure transducer is broken and needs to be changed.
- If the reading is between 1 - 499 or 5000 – 6500 then contact DMT for further instructions.

If the message “OFF” is displayed in either of the pressure reading lines in the Main Menu on the Myo-Interface then the pressure transducer is broken and needs to be replaced.

In case of any other problems related to the pressure transducer, please contact DMT for further instruction and advice.

## 4.2 Inverted Microscope

Pressure Myograph System set-ups require high flexibility in image analysis. DMT recommend to use inverted microscopes with high quality objectives and contrast features. DMT recommend the DMT Microscope, Zeiss Axiovert 40, Nikon TS-100F and Motic AE2000.

## 4.3 The Pressure Regulator

The Pressure Regulator controls the flow and pressure applied to the vessel mounted in the pressure myograph chamber via settings in the DMT VAS program or the Myo-Interface. This Chapter is intended as an overview of the Pressure Regulator to understand how it works to control flow and pressure settings.

### 4.3.1 Control of Flow and Pressure Settings

The Pressure Regulator consists of two individual circuits, an air circuit and a buffer circuit as illustrated in figure 4.10 on next page. The Pressure Regulator transfers the pressure and flow settings from the DMT VAS program or the Myo-Interface by individual control of the air pressure in the two buffer bottles.

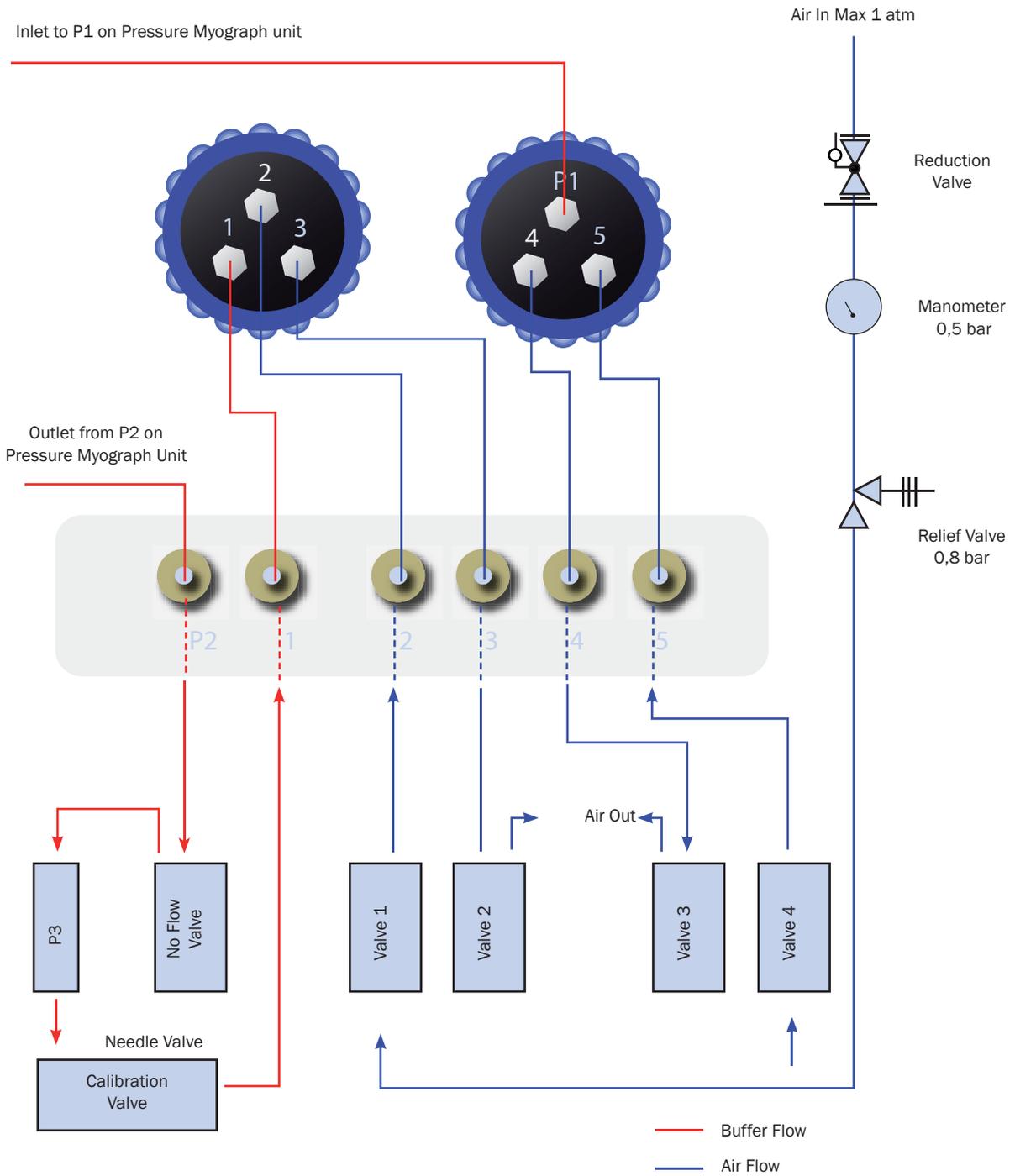
#### **IMPORTANT**

**NEVER FILL THE BOTTLE WITH MORE THAN 250 ML BUFFER AS HIGHER VOLUMES MAKES PRECISE CONTROL OF THE PRESSURE MORE DIFFICULT.**

### 4.3.2 No-Flow Setting

Activating the “No-Flow” setting in the Myo-Interface closes the “No-Flow Valve” in the diagram shown in figure 4.10 on next page. Closing the “No Flow Valve” serves to prevent any buffer back flow through the mounted vessel. Activating the “No-Flow” setting turns on the “No-Flow” indicator lamp on front of the Pressure Regulator.

Activating the “No-Flow” setting in the Myo-Interface deactivates the P2 pressure transducer and the P1 pressure transducer takes control of the pressure applied on the mounted vessel. At the same time the P3 pressure transducer is activated controlling the air pressure in the left bottle with buffer in figure 4.10 on next page. Activating P3 serves to prevent a huge pressure to build up in the left bottle with buffer, which may damage the mounted vessel when re-establishing the flow.



**Figure 4.10** Pressure Regulator Diagram

## 4.4 Pressure Myograph Maintenance and Cleaning

The Pressure Myograph System – Model 110P is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and that the instructions are followed at all times.

### 4.4.1 Pressure Myograph Chamber Pipes

To prevent the pipes from being blocked by buffer salt deposits after an experiment, use the chamber cover to remove the cleaning solutions. Afterwards, remove the cover from the myograph chamber and turn on the vacuum pump and vacuum valve for about 10 seconds. Wait to turn off the oxygen supply until turning off the vacuum pump. Wipe off any buffer remaining on the outside of the pipes using a piece of paper.

### 4.4.2 Force Transducer

The force transducer is one of the most delicate and fragile components of the Pressure Myograph System. Therefore careful handling of the force transducer is of most importance to prevent it being damaged.

The left glass cannula in the pressure myograph is connected to the transducer pin coming through a small hole in the transducer house located on top of the pressure myograph unit, as illustrated in figure 4.11 below. To prevent the buffer from running into the transducer house, the hole is filled with high vacuum grease.

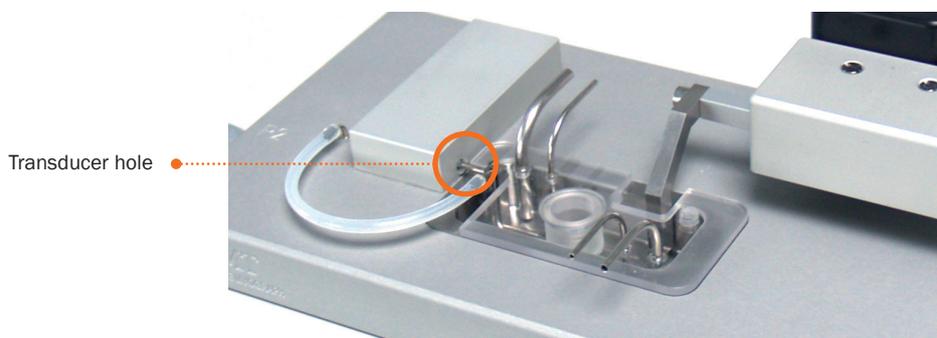
As a part of daily maintenance it is very important to inspect the greasing of the transducer hole before starting any experiment. Insufficient greasing causes damage and malfunction of the force transducer.

#### **IMPORTANT:**

**DMT RECOMMENDS THAT THE HIGH VACUUM GREASE SEALING UP THE TRANSDUCER HOLE BE CHANGED AT LEAST ONCE A WEEK (SEE FIGURE 4.11 BELOW).**

**DMT TAKES NO RESPONSIBILITIES FOR THE USE OF ANY OTHER KINDS OF HIGH VACUUM GREASE THAN THE ONE TO BE PURCHASED FROM DMT.**

**DMT TAKES NO RESPONSIBILITIES FOR ANY KIND OF DAMAGE APPLIED TO THE FORCE TRANSDUCERS.**



**Figure 4.11** Transducer pin hole sealed up with high vacuum grease

### 4.4.3 Pressure Transducer and Glass Cannulas

To prevent the pressure transducers and the tubing inside the pressure myograph unit from being blocked by buffer salt deposits after an experiment, DMT recommends that the system be flushed using the following procedure.

1. Disconnect the Pressure Regulator from the Pressure Myograph Unit. Keep the Pressure Myograph Unit connected to the Myo-Interface to enable continuous monitoring of the pressure readings from P1 and P2.
2. Fill a small syringe with 8% acetic acid and flush both pressure transducers and glass cannulas by connecting the syringe needle to the 3-way valve for the individual pressure transducers and glass cannulas using a small piece of silicone tube.

## IMPORTANT

**BE VERY CAREFUL NOT TO APPLY A TOO HIGH PRESSURE WHEN FLUSHING AS THIS MAY DAMAGE THE PRESSURE TRANSDUCERS. KEEP AN EYE ON THE PRESSURE TRANSDUCER READINGS ON THE MYO-INTERFACE WHEN FLUSHING AND MAKE SURE THAT THE PRESSURE DOES NOT EXCEED 150 mmHg.**

3. Flush the pressure transducers and glass cannulas as described in step 2. Repeat the procedure two to three times.

### 4.4.4 Pressure System Cleaning

1. After completing an experiment, remove the vessel mounted on the cannulas.
2. Remove buffer from the tissue chamber.
3. Turn off heat.
4. Dump any buffers in the bottles (both P1 and P2 bottles). P1 bottle is the bottle that starts with buffer. P2 bottle is the waste bottle. If looking at the pressure regulator from the front, P1 bottle is on the right and P2 bottle is on the left.
5. Put the bottles back in place and tighten the caps.
6. Make sure all the tubing is connected to the chamber and bottles.
7. Make sure that the system is NOT in No Flow (turn off No Flow if this function was used).
8. If using glass cannulas for small vessels, then insert the calibration shunt that came with your system to bypass the cannulas in the same fashion you would use the shunt to calibrate your pressure transducers.
9. If looking at the cap of the P1 bottle, the frits at the top where the tubing is connected should be clearly marked with "P1", "4", and "5". Detach the tubing from the top of the P1 bottle marked "5".
10. Attach the Big Ben manometer at the frit marked "5".
11. Using the Big Ben manometer, push the residual buffer out of the system. Using the Big Ben manometer will allow you to pressurize the system by knowing exactly how much pressure is being used without having to worry about blowing out the P1 and P2 pressure transducers. More importantly, this will protect the hidden P3 transducer in the pressure regulator.

**DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANSDUCERS!**

12. Once the buffer has been cleared from the system and into the waste bottle, fill the P1 bottle with double-distilled water.

**DO NOT FILL WITH MORE THAN 250 mL OF WATER.**

13. Re-attach the P1 bottle top, and make sure it is tight.
14. Using the Big Ben manometer that should still be attached to the "5" frit on top of the bottle, pressurize the bottle using the manometer. **DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANSDUCERS!** Water should be pushed from the bottle through the system. Do this for at least 5 minutes.
15. As an alternative, re-attach the tubing to the "5" frit on top of the P1 bottle. Make sure all the other tubing is connected in the system so that if flow is induced, the water from the P1 bottle will flow from the P1 bottle to the P2 waste bottle freely.
16. Induce flow by setting P1 on the interface to 200 mmHg and setting P2 to 50 mmHg. This should cause the water in the P1 bottle to flow through the system to wash the tubing and internal parts of the chamber and pressure regulator. If this method is used to clean the system, then run this flow program for at least 10 minutes.
17. Once the system has been flushed with water, empty the water from the P1 bottle.
18. Re-attach the cap to the P1 bottle, and using the Big Ben Manometer, push the water out from the system by attaching the Big Ben to frit "5" on the P1 bottle. Again, **DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANSDUCERS!**
19. Once the system is emptied, dry the system by continuing to push air through the lines. Push air through for at least 5 minutes.
20. Once air has been pushed through the system, detach the Big Ben manometer from the P1 bottle and reattach the appropriate tubing.

21. Detach the tubing from the regulator (NOT THE BOTTLES) where it is marked P2. This frit on the regulator is right next to the frit marked "1".
22. Connect the Big Ben manometer here, and push air through to make sure the inside of the regulator is dried. If buffer or water stays in this part of the regulator, it can cause problems that are not covered by warranty, and this step will guarantee that this part of the regulator stays clear.
23. If an 8% acetic acid rinse is performed, then repeat these steps and make sure that the system is thoroughly washed with double-distilled water again to remove the acetic acid. **An acetic acid wash is not needed after each use. Use this sparingly. If only buffer has been used in the perfusion line, then there is no need to ever use acetic acid to clean the tubing, internal buffer lines of the chamber, and inside the regulator.**

**IMPORTANT I**

**BE VERY CAREFUL NOT TO DAMAGE PRESSURE TRANSDUCER P3 WHEN FLUSHING THE BUFFER CIRCUIT. THERE ARE NO READINGS FROM P3 VISIBLE ON THE MYO-INTERFACE. DANISH MYO TECHNOLOGY THEREFORE RECOMMENDS THAT THE PRESSURE MYOGRAPH UNIT ALWAYS BE FLUSHED FIRST. IN THIS WAY IT IS POSSIBLE TO GET A FEELING OF HOW MUCH "FLUSHING PRESSURE" THAT IS EQUAL TO 150 mmHg.**

**IMPORTANT II**

**NEVER FLUSH THE AIR CIRCUITS (PORT NO. 2, 3 AND 4) ON THE PRESSURE REGULATOR WITH ANY KIND OF FLUID.**

**4.4.5 Cleaning Pressure Myograph Chamber and cannulas**

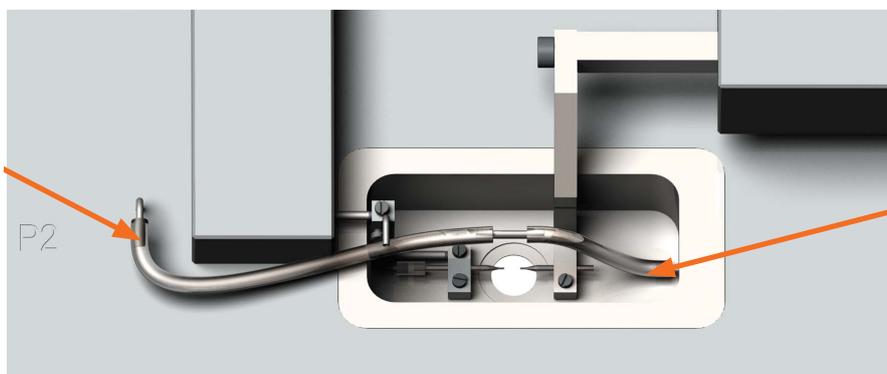
**DMT STRONGLY RECOMMENDS THAT THE MYOGRAPH CHAMBER AND SURROUNDINGS BE CLEANED AFTER EACH EXPERIMENT.**

After a "normal" experiment use the following procedure to clean the myograph chamber and supports:

The chamber and cannulas can be cleaned at the same time while the systems is being flushed if the flow program is used as described in step 15 above.

1. To clean the chamber, fill the chamber with double-distilled water and wash at least 3 times.
2. To clean the cannulas, one can use a syringe and needle. An 18-gauge needle with the sharp bevel removed works the best.
3. Fill the syringe with air.
4. To clean the cannulas, attach the syringe with needle at the point in the chamber indicated at figure 4.12 below.
5. Make sure the cannulas are not aligned. If they are still aligned, pushing the air into the cannulas will only cause the buffer or water to shoot into the cannula straight across from it.
6. Push the air from the syringe through the cannula. This should push out any buffer in the cannula.
7. Remove the syringe and fill with double-distilled water.

*Detach tubing here and insert end of blunted 18-gauge needle attached to syringe into tubing to clean P2 cannula.*



*Detach tubing here and insert end of blunted 18-gauge needle attached to syringe into tubing to clean P1 cannula.*

**Figure 4.12**

*By attaching the syringe at these point, the P1 and P2 pressure transducer is bypassed, and water or air can be pushed through the cannula as hard as the user likes.*

8. Re-attach as in step 4 above.
9. Push water through the cannula to wash.
10. Remove the syringe, remove the water from the syringe, and reattach the syringe as in step 4.
11. Push air through the cannula to dry.
12. Repeat steps 3 through 11 on the P2 cannula. Attach the syringe at the point in the chamber as indicated in figure 4.12 on previous page.

Using this cleaning procedure will minimize damage to the pressure transducers, will prevent clogs from occurring in the pressure regulator, and will minimize clogs in the cannulas.

**IMPORTANT!**

**ACETIC ACID MAY BE USED MORE LIBERALLY IN CLEANING THE CHAMBER AND GLASS CANNULAS. IT IS IMPORTANT TO THOROUGHLY WASH THE ACETIC ACID FROM THE CANNULAS, OTHERWISE PRECIPITATE WILL FORM IN THE CANNULAS AND CLOG THEM, REQUIRING THAT THE CANNULAS BE CHANGED MORE OFTEN.**

If the chamber still have salt build-up then continue with the following procedure:

1. Fill the myograph chamber to the edge with an 8% acetic acid solution and allow it to stand for a few minutes to dissolve calcium deposits and other salt build-up. Use a swab stick to mechanically clean all chamber surfaces.
2. Remove the acetic acid and wash the myograph chamber and glass cannulas several times with double distilled water.
3. If any kind of hydrophobic reagent have been used, which might be difficult to remove using step 1 and 2, then try incubating the chamber and glass cannulas with 96% ethanol or a weak detergent solution (e.g. Treepol).
4. To remove more resistant or toxic chemicals, incubate the myograph chamber and glass cannulas with 1M HCl for up to 1 hour. In exceptional cases incubate the chamber and supports with a up to 3M HNO<sub>3</sub> solution for about 15 minutes.
5. Wash the myograph chamber and glass cannulas several times with double distilled water.

**IMPORTANT NOTES:**

**BE VERY CAREFUL USING STEP 3 AND 4 REPEATEDLY AS STRONG REAGENTS CAN CAUSE EXTREME DAMAGE TO THE MYOGRAPH UNIT.**

**BE VERY CAREFUL NOT TO DAMAGE THE GLASS CANNULAS DURING THE CLEANING PROCEDURE.**

**AFTER CLEANING, ALWAYS CHECK THAT THE GREASING AROUND THE TRANSDUCER PIN IS SUFFICIENT TO KEEP OUT THE BUFFER SOLUTION FROM THE TRANSDUCER COMPARTMENT.**

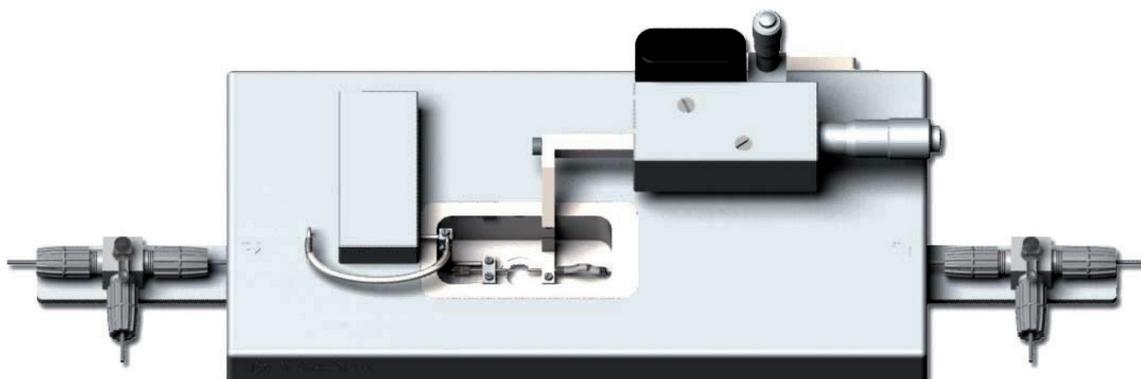
In cases of red or brown discolorations appearing on the chamber sides, the following cleaning procedure will work in most cases:

1. Incubate the myograph chamber for 30 minutes with 20µl of a 2mM T-1210 Tetrakis-(2-pyridylmethyl)-ethylenediamine solution dissolved in double distilled water.
2. Use a swab-stick to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.
3. Wash the myograph chamber several times with double distilled water.
4. Incubate the myograph chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a swab-stick.
5. Remove the ethanol solution and wash a few times with double distilled water. Incubate the myograph chamber with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.
6. Wash the myograph chamber several times with double distilled water.

## CHAPTER 5 - EXPERIMENTAL SETUP STEP-BY-STEP

### 5.1 Mounting protocol for small arteries

This section shortly describes the basic technique of mounting small vessels in the Pressure Myograph:



**Figure 5.1** Mounting of vessel in the Pressure Myograph Chamber

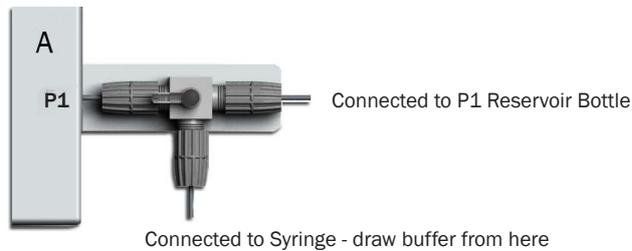
#### Preparation

1. Prepare your PSS. 60mM KPSS can be used to test contractions, and 16mM KPSS can be used to cause relaxations (usually 16mM KPSS only works in small arteries for relaxation).
2. Make at least 4 loops with nylon suture to use for securing the vessel to the mounting cannulas. Double loops are best and will prevent the loops from slipping loose once tightened.
3. Pre-heat PSS to 37°C, making sure the PSS is bubbled with carbogen (5% CO<sub>2</sub>, 95% O<sub>2</sub>) or PraxAir (5% CO<sub>2</sub>, 21% O<sub>2</sub>, balance N<sub>2</sub>) for at least 20 to 30 minutes.
4. If desired, rinse the mounting chamber and all tubing that sees buffer with ddH<sub>2</sub>O.
5. Fill the P1 reservoir bottle in the pressure regulator with pre-warmed PSS. DO NOT FILL MORE THAN 250mL. Keep the tubing from the bottle attached to port on top of the bottle marked P1 higher than the bottle, otherwise capillary action will draw buffer from the bottle and will leak all over the bench.

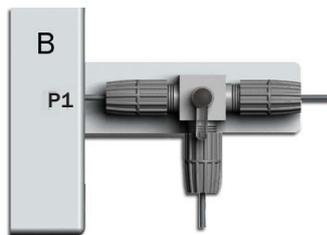
#### Mounting

1. Isolate small vessel of interest. If you want to learn more about dissection of mesenteric arteries, please request for the Dissection & Mounting video.
2. Clean the vessel as best as possible of perivascular fat and tissue.
3. Place the small artery into a small container of cold or room temperature PSS.
4. Place 10mL of cold or room temperature PSS in the mounting chamber.
5. Have a syringe with buffer attached to the P1 3-way valve, but the P1 and P2 3-way valves should be closed to the vessel.
6. Position the sutures appropriately on both cannulas so that they are ready for use when the vessel is mounted. Place 2 loops on each cannula in case one breaks or fails.
7. Carefully transfer the vessel from the small container to the mounting chamber. Using a large bore pipette tip and pipette or an eye-dropper, carefully suck up the isolated vessel and transfer it to the chamber. This will minimize handling and potential for damaging the vessel.
8. Continue to mount the vessel on the glass cannulas. Start with the P1 side. Once the P1 side of the vessel is mounted on the cannula, secure the vessel to the cannula by tightening the loops around the vessel onto the cannula.

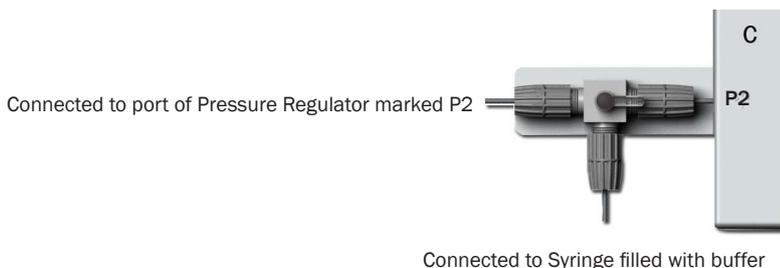
9. Make sure the vessel is mounted in the correct direction. Flow through the vessel is directional, so proximal and distal ends for flow will need to be noted somehow. Mount the proximal end to flow on the P1 cannula.
10. Once secured, gently rinse the vessel of any blood. Before washing the lumen, gently push some buffer from the syringe through the 3-way valve to the port that is not connected to the vessel so as to bleed out any air that might have been trapped in the valve.
11. Open the valve to the vessel and gently push buffer through to wash out any blood in the lumen. This will also help open the distal end if it is pinched shut. Close the P1 3-way valve so as to keep the vessel from collapsing.
12. Position the P1 cannula with attached vessel so as to facilitate mounting of the vessel on the P2 cannula.
13. Gently mount the vessel on the P2 cannula gently, without pulling it off the P1 cannula. Secure the vessel as described in step 7.
14. Once the vessel is secured on the P2 cannula, open the P1 cannula and gently push more buffer into the mounted vessel. The vessel should slightly fill and bulge from the small pressure-head. Close the P1 2-way valve to maintain a slightly pressurized vessel.
15. Transfer the mounting chamber to the microscope, and attach all the tubing.
16. Fill the tubing leading from the P1 reservoir bottle (marked P1 at the top of the bottle) with the buffer that has been pre-warmed and pre-bubbled. The easiest way to do this is attach a syringe to the P1 -3way valve as illustrated, and draw buffer from the bottle. The 3-way valve should be positioned the same way.



17. Once the buffer is drawn through the tubing, turn the 3-way valve as shown.

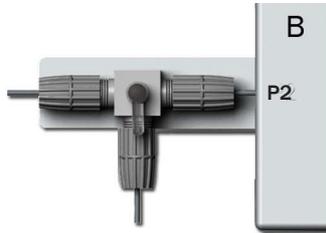


18. Disconnect the syringe and move it to the P2 side of the chamber, but **DO NOT EMPTY THE SYRINGE**. Attach to the 3-way valve as shown.



19. Use the buffer that was drawn from the bottle to charge the remainder of the system. The buffer should be pushed gently from the 3-way valve all the way to the waste bottle. When the buffer starts dripping in the waste bottle, stop pushing buffer. **BE CAREFUL WHEN CHARGING THIS PART OF THE SYSTEM. EXCESSIVE PRESSURE CAN DAMAGE THE INTERNAL 3<sup>RD</sup> HIDDEN TRANSDUCER IN THE PRESSURE REGULATOR.**

20. Close the 3-way valve as shown below.



21. The system is charged and ready.

### Equilibration

1. Turn on the heat, which should be preset to 37 °C.
2. Start bubbling the chamber with oxygen.
3. Set P1 to 20mmHg and P2 to 5mmHg. This will cause a flow gradient and perfuse the vessel with some of the newly charged buffer. Make sure that the charged lines **DO NOT** have air bubbles, otherwise endothelium will be stripped from the lumen. Perfuse for 2 to 3 minutes.
4. Set P1 and P2 pressure to 10mmHg.
5. Increase pressure by 10mmHg every 5 minutes until target pressure is reached. For cerebral arteries from rats, this may be 60 to 70 mmHg. For mesenteric resistance arteries, this would be 60mmHg.
6. After the first 20 minutes after starting the heat and pressurization, remove the old PSS and replace it with new PSS that should be pre-warmed and oxygenated.
7. Continue to pressurize the vessel.
8. After 40 minutes from the start of heat and pressurization, do another wash, as in step 6.
9. Once 60 minutes have passed, the Standard Start or "Wake-Up" Protocol can be initiated.

### Wake-up or standard-start protocol

1. Once the vessel is equilibrated, remove the old PSS and add 60mM KPSS to cause a contraction.
2. Allow the vessel to contract.
3. Wash the KPSS until baseline diameter is reached.
4. Repeat the KPSS contraction and washes.
5. An agonist such norepinephrine can be added (10-8M final in the bath) to cause a contraction. However, the vessel should also have developed some myogenic tone. The amount of tone will depend on the vessel used.
6. Once the vessel is contracted, Ach (10-5M final in the bath) can be added to assess endothelial function.
7. Wash the vessel 5 to 6 times over 20 to 30 minutes.
8. Vessel is now ready for experiments.

## 5.2 Buffer recipes

### 5.2.1 Physiological Saline Solution (PSS)

#### 1x PSS (1000ml):

##### Solution 1:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
NaCl	58.44	118.99	6.954
KCl	74.56	4.69	0.350
MgSO <sub>4</sub> - 7H <sub>2</sub> O	246.48	1.17	0.289
KH <sub>2</sub> PO <sub>4</sub>	136.09	1.18	0.161

##### Solution 2:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
CaCl <sub>2</sub> - 2H <sub>2</sub> O	147.02	2.50	0.368

##### Solution 3:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
NaHCO <sub>3</sub>	84.01	25.00	2.100
EDTA	372.24	0.03	0.010
Glucose	198.77	5.50	1.091

1. Dissolve the chemicals in approximately 100ml double distilled H<sub>2</sub>O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.
2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H<sub>2</sub>O to a final volume of 500ml.
3. Solution 3 is added to the graduated bottle, which afterwards is filled with additional double distilled H<sub>2</sub>O to a final volume of about 850ml.
4. Aerate the solution with carbogen (95% O<sub>2</sub> + 5% CO<sub>2</sub>) for about 20 minutes.
5. Solution 2 is added and the graduated bottle is filled with additional double distilled H<sub>2</sub>O to reach the final volume of 1000ml. Continue the carbogen bubbling until the pH of the buffer solution reaches 6.4.

#### 25x Concentrated PSS (1000ml)

##### Solution 1:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
NaCl	58.44	118.99	173.850
KCl	74.56	4.69	8.750
CaCl <sub>2</sub> - 2H <sub>2</sub> O	147.02	2.50	9.200

##### Solution 2:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
MgSO <sub>4</sub> - 7H <sub>2</sub> O	246.48	1.17	7.225
KH <sub>2</sub> PO <sub>4</sub>	136.09	1.18	4.025

##### Solution 3:

Chemical	MW (g/mol)	Conc. (mmol/l)	Conc. (g/l)
EDTA	372.24	0.03	0.250

1. Dissolve the chemicals for solution 1 in about 800ml double distilled H<sub>2</sub>O in a 1000ml graduated bottle. Dissolve the chemicals for solutions 2 and 3 in 75ml double distilled H<sub>2</sub>O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.
2. Solution 2 and 3 is added to solution 1 and the graduated bottle is filled with additional double distilled H<sub>2</sub>O to reach a final volume of 1000.0ml.

**Before use:**

3. Dilute the 25 x PSS stock solution 1:25 using double distilled H<sub>2</sub>O.
4. Add

**1.091 g/l Glucose**

**2.100 g/l NaHCO<sub>3</sub>**

5. Aerate the solution with carbogen (95%O<sub>2</sub> + 5%CO<sub>2</sub>) for at least 20 minutes. If necessary wait further for the pH of the buffer to reach pH 7.4.

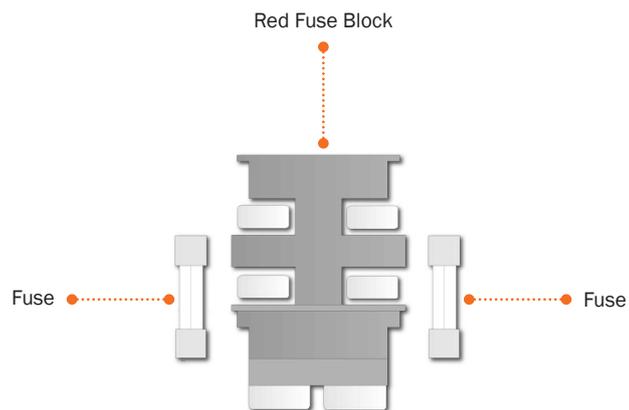
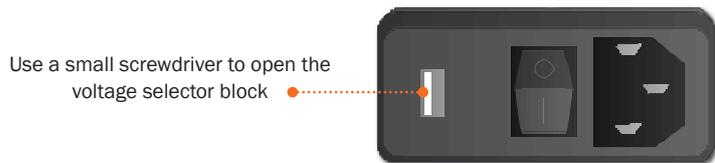
## APPENDIX 1 - FUSE REPLACEMENT

The main fuse of the myograph system is placed inside the power inlet on the Myo-Interface. If the fuse blows it is easily changed using the following procedure.

**IMPORTANT:**

**IF THE FUSE NEEDS TO BE CHANGED MAKE SURE THAT THE REPLACEMENT FUSE IS EQUAL TO THE ONE BLOWN.**

Specifications: T1,6A / 250V, 6.3 x 32mm



## APPENDIX 2 - SYSTEM SPECIFICATIONS

### Technical specifications

<b>Vessel size:</b>	>60 µm
<b>Vessel alignment:</b>	Manually X, Y & Z settings
<b>Mounting supports:</b>	Glass pipettes
<b>Chamber:</b>	Single
<b>Chamber volumes:</b>	Max. 10 ml (5 ml typical)
<b>Chamber material:</b>	Acid-resistant stainless steel
<b>Chamber cover:</b>	With pipes for suction/gassing/superfusion
<b>Force range:</b>	± 50 mN
<b>Force resolution:</b>	0.1 mN
<b>Pressure range:</b>	0 - 250 mmHg (requires external pressure source. app.1 ATO.)
<b>Pressure resolution:</b>	0.1 mmHg
<b>Pressure calibration:</b>	Manual
<b>Heating:</b>	Built-in
<b>Temp. range:</b>	Ambient - 50 °C
<b>Temp. resolution:</b>	0.1 °C
<b>Temp. probe:</b>	Included
<b>Weight calibration:</b>	Semi-automatic
<b>Analogue output:</b>	0-1.0V full scale (12 bit)
<b>Digital output:</b>	Serial interface - RS232/RS485
<b>Voltage:</b>	100 to 240 VAC (auto) 50/60 Hz
<b>Ambient temperature:</b>	15-30 °C
<b>Fluid capacity:</b>	250 ml
<b>Gas/superfusion ports:</b>	Built-in
<b>Valves:</b>	Safety and reduction

### Optional accessories

#### FlowMeter

- range: 15 µl/min to 1500 µl/min

#### Enable pH meter on the interface:

- range: pH 0 - 14

- temp. correction: 0 °C - 50 °C

**Peristaltic pump:** 2.5 - 50 rpm (for superfusion of the chamber)

## NOTES

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