ORGAN BATH SYSTEM – 820MO

USER MANUAL, VOL. 3.2

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Since then, changes may have been made to the software and hardware it
describes. New information may be supplied separately.

This documentation is provided with the DMT Organ Bath System – Model 820MO

Document Number: 820MO – UG2.2A

Every attempt is made to ensure an accurate information, misprints, construction- and specification changes can occur. Danish Myo Technology A/S reserves the right to alter/change content as required and without any notice.

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SAFETY

The Organ Bath System - 820MO has been designed for teaching and research only. It is not intended for clinical or critical life-care use and should never be used for these purposes. The 820MO also should not be used for the prevention, diagnosis, curing, treatment, or alleviation of disease, injury, or handicap.

CAUTION

- *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 EQUIPMENT OR DEVICES 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.*
- *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:*

Voltage	**Line plug according to standard**
110 - 125V	UL81 and CSA C22.2 No. 42
220 - 230V	CEE 7 page VII, SR section 107-2-D1/IEC 83, page C4
240V	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.

UNPACKING THE ORGAN BATH SYSTEM

Take a few minutes to carefully inspect your new Organ Bath System - 820MO for damage that may have occurred during handling and shipping. If you suspect any damage, please contact DMT immediately, and the matter will be pursued as soon as possible. If the packing material appears damaged, please retain it until a potential claim has been settled.

We recommend that you store the packing material for any possible future transport of the Organ Bath System. In case of transportation and the original packing material is unavailable, please get in touch with DMT for advice and packing instructions.

EC DECLARATION OF CONFORMITY

DMT A/S
Certify and declare that the following apparatus:

ORGAN BATH SYSTEM - MODEL 820MO

Restrictive use: Only for laboratory use.

Manufactured by:
DMT A/S
Rho 14
8382 Hinnerup
Denmark

Conforms with the essential requirements of the EMC Directive 2004/108/EC.
Based on the following specifications applied by:
EN 61326-1:2006
EN 61326-2-6:2006
EN 61326-2-6/Corr.:2007

And with the LVD Directive 2006/95/EC.
Based on the following specifications applied by:
EN 61010-1:2010
EN 61010-2-030:2010

General warnings regarding EMC:
Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these may interfere with the proper operation.

CHAPTER 1 - SYSTEM OVERVIEW

1.1 INTERFACE

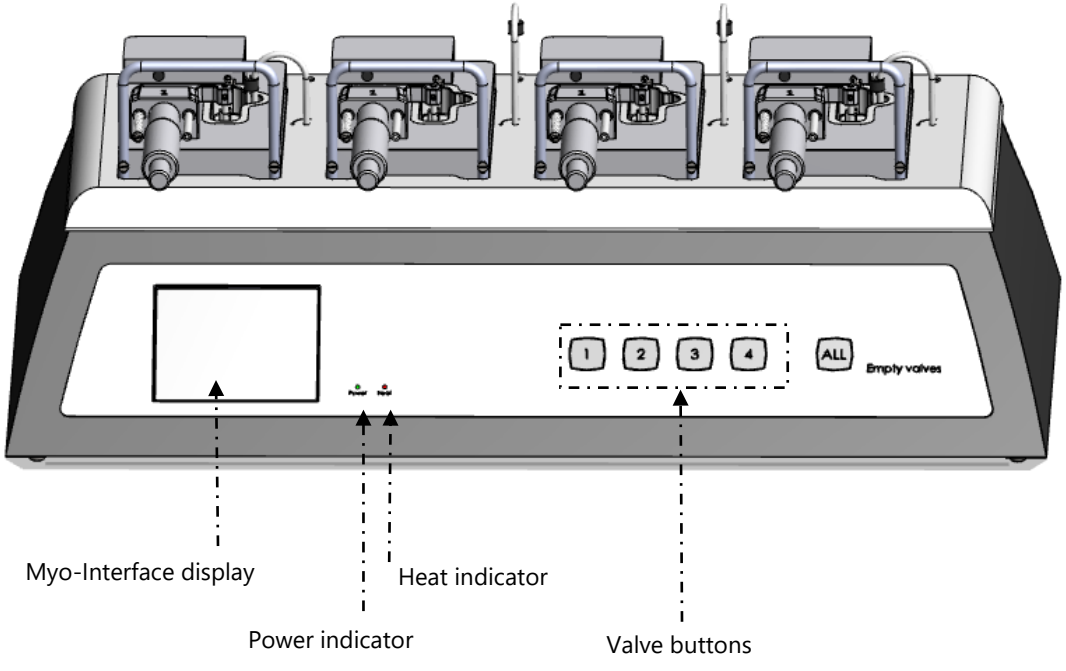


Figure 1.1 Interface front panel

1.2 INTERFACE REAR PANEL

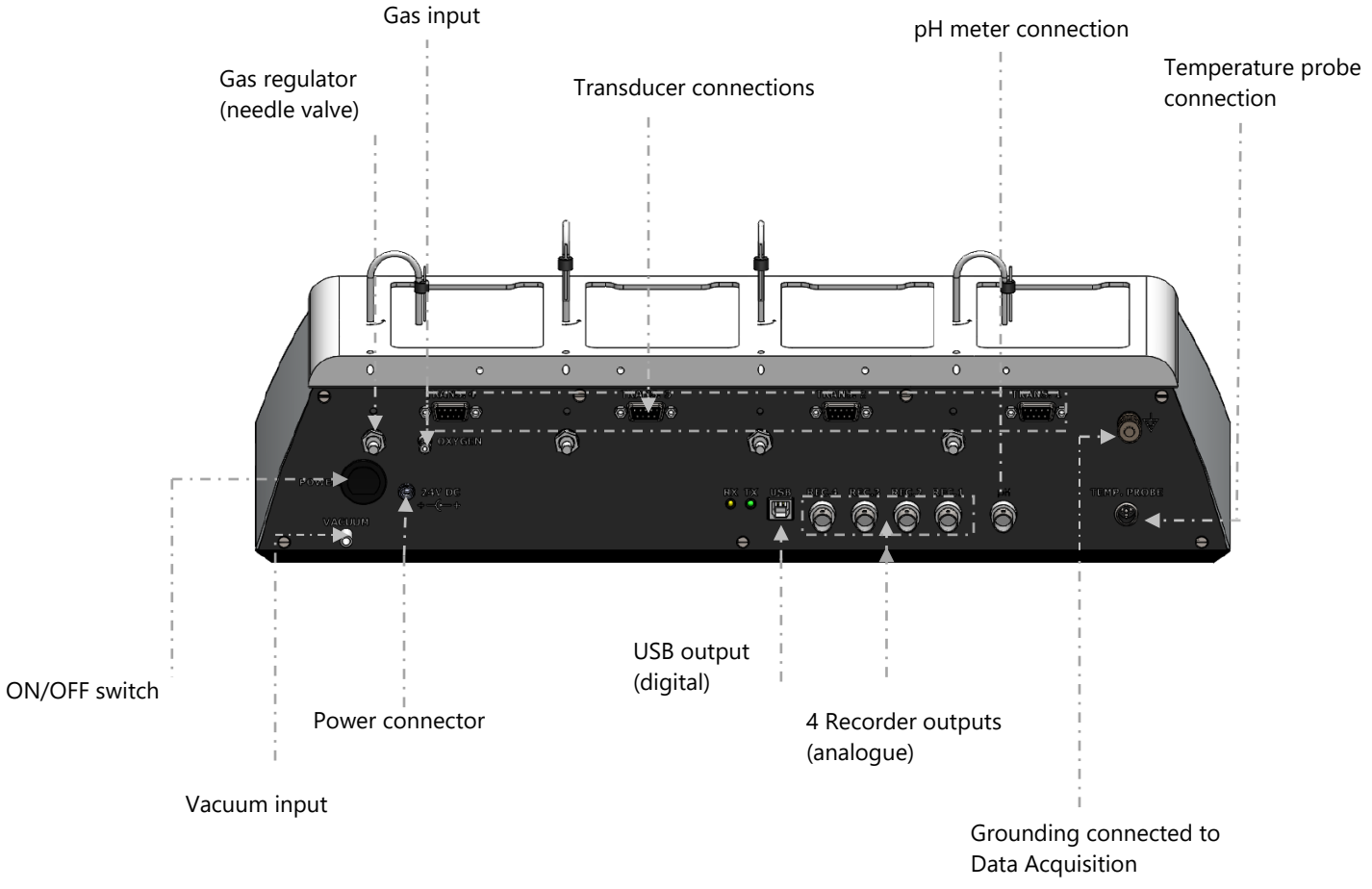


Figure 1.2 Interface Rear Panel

1.3 ORGAN BATH CHAMBER - 820MO

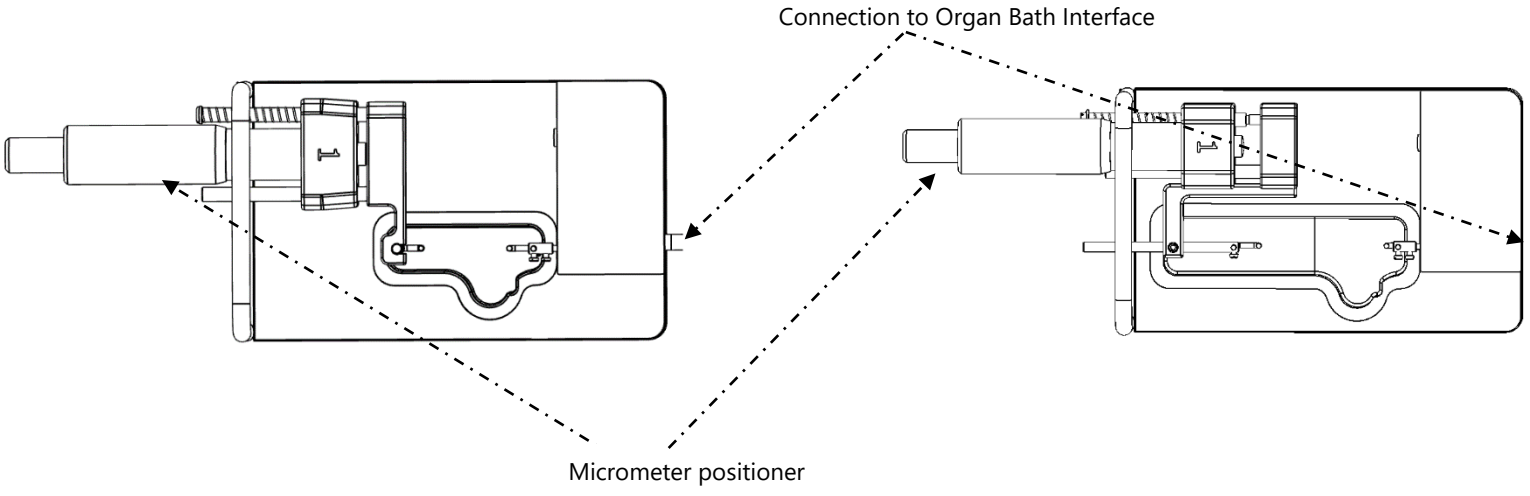


Figure 1.3 820MO Organ bath chamber unit and 820MO-XL Chamber Unit with mounting hooks

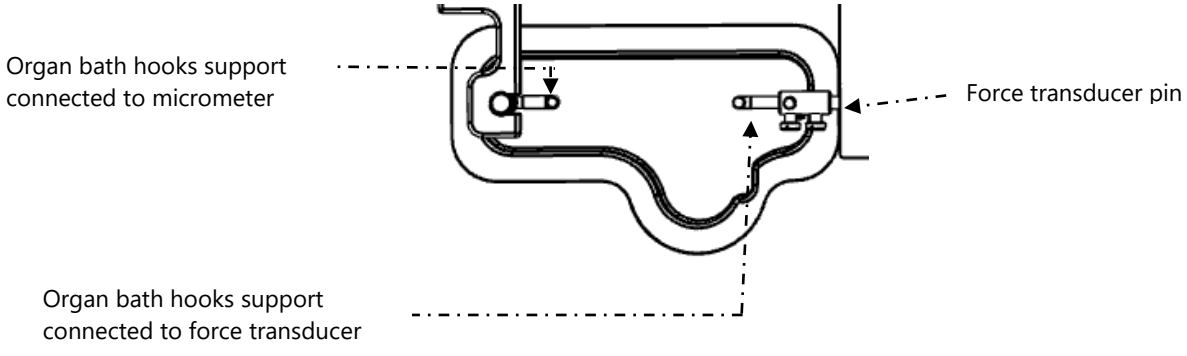


Figure 1.4 Close up of the 820MO myograph chamber unit

CHAPTER 2 - SETTING UP

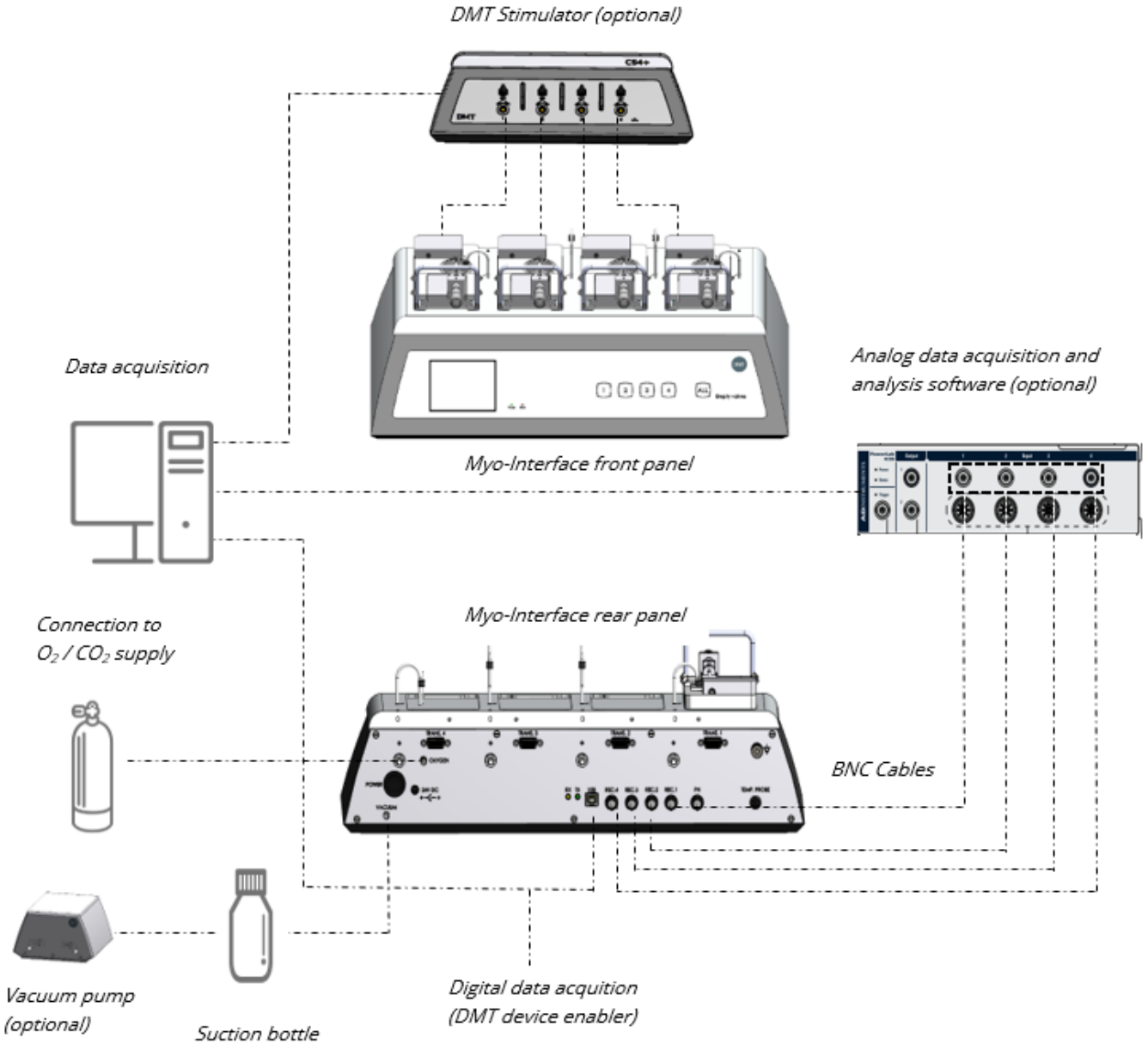


Figure 2.1 The complete Organ Bath System – 820MO

2.2 SETTING UP STEP-BY-STEP

This chapter contains a complete step-by-step description of setting up a complete organ bath 820MO system, as illustrated in Figure 2.1.

1. **Interface – PC Connection:**
   Data acquisition is possible either by connecting the 820MO Interface directly to a PC with LabChart Pro and the DMT Device enabler installed or through the BNC connectors, and BNC cables from the 820MO Interface e.g., to the Powerlab box and the Powerlab box is then connected using a USB cable to the computer with LabChart installed(optional).
   I. **Direct PC Connection:** Connect the 820MO Interface using the applied USB cable directly to the PC with LabChart Pro (version 8 or newer) and the DMT Device enabler installed.
   II. **PowerLab (Optional):** Connect the 820MO Interface to the PowerLab unit using BNC cables. Connect Rec 1on the Interface to Input 1on the Power Lab, Rec 2 to Input 2, etc. Connect the PowerLab unit to one of the USB-ports on the PC using the USB cable delivered with the PowerLab system.

2. **Oxygen Supply:**
   Connect the gas supply (95% O_2, 5% CO_2 or 21% O_2, 5% CO_2, balance N_2) with tubing running from the gas supply to the gas inlet MARKED “OXYGEN” on the back of the Interface. This stainless steel inlet pipe will supply oxygen to the chambers. The oxygen tubing needs to be inserted into the chamber in order to aerate the heated buffer. Needle valves on the back of the Interface can be adjusted to regulate the amount of bubbling that occurs. Turning the regulator clockwise increases the bubbling, while turning it counter-clockwise decreases the bubbling (see Figure 2.2). Each regulator has a locking device attached that can be used when the desired bubbling is achieved.

NOTE: THE NEEDLE VALVES NEED TO BE GREASED (USING THE GREASE FOR THE LINEAR SLIDES – THE BROWN GREASE) AND TURNED AT REGULAR INTERVALS TO PREVENT THEM FROM STICKING OR PERMANENTLY LOCKING.

3. **Vacuum Connection:**
   The system has a built-in manifold with separate valves that allow each chamber to be drained individually. After connecting the vacuum source at the back of the Interface, the vacuum pipes need to be inserted into the chambers for this feature to work properly. The pipes are inserted into the chamber by gently pulling

up on the curved part of the pipe, turning it 90° counter-clockwise and gently lowering it into the chamber, see Figure 2.2. A chamber can then be emptied by pressing the corresponding numbered button. Pressing the “all” button will drain all the chambers at the same time.

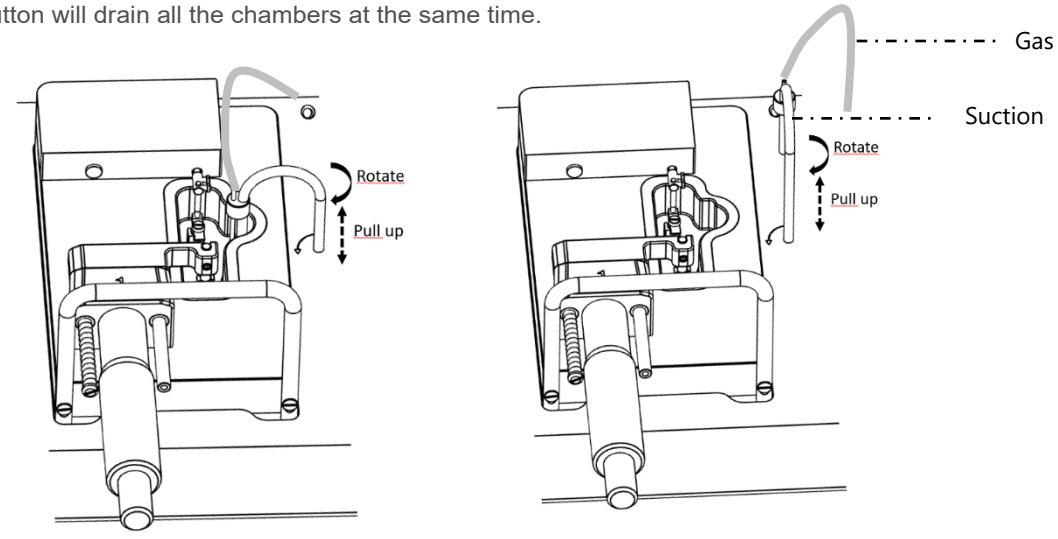


Figure 2.2 Oxygen supply and suction connection

NOTE: WHEN DRAINING THE CHAMBERS USING THE AUTOMATIC VACUUM FUNCTION, PRESS THE APPROPRIATE BUTTON FOR AN ADDITIONAL 3-5 SECONDS AFTER THE INITIAL EMPTYING. THIS WILL HELP DRAIN RESIDUAL BUFFER AND SOLUTIONS RETAINED IN THE TUBING AND VALVES.

4. **Chamber Covers**: The chamber covers will help maintain the temperature and other buffer conditions (gas tension, pH) fairly constant. Holes in the chamber covers serve different purposes, and they are illustrated in Figure 2.3A-B. The slots allow the covers to be placed over the chamber around the support arms and gas/ vacuum tubes.

Figure 2.3A - Chamber cover

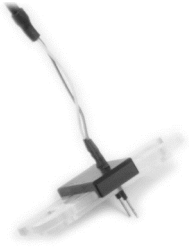


Figure 2.4B - Chamber cover with built-in electrodes (optional item)

5. **Initial Force Transducer Status:** Prior to the shipment of the Organ Bath 820MO System, it has gone through two days of continuous testing, including a final force transducer calibration. However, DMT recommends that a new force transducer calibration is performed before using the organ bath system for the first time. The force transducer calibration procedure is described in detail in the FORCE CALIBRATION sub-menu under SETTINGS, as explained in Chapter 3.

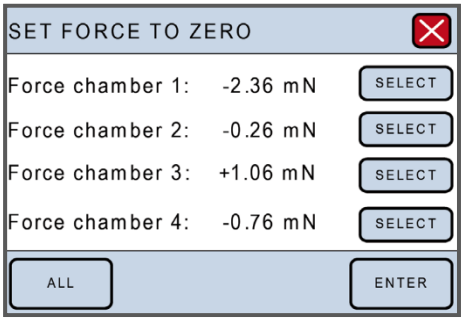
CHAPTER 3 - THE INTERFACE MENUS

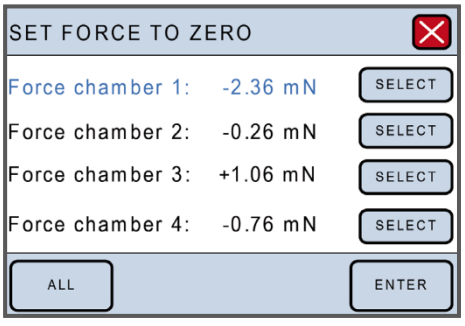
Chapter 3 is a complete manual for the 820MO Interface menus. This chapter contains a detailed description of how to navigate the touch-screen menus and how to use the special features of the 820MO myograph.

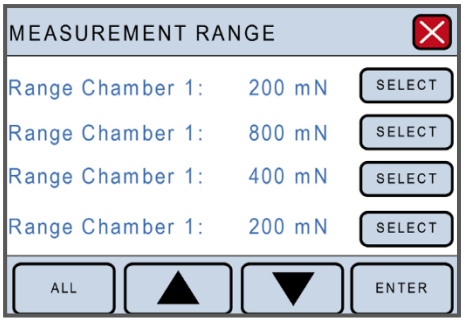
3.1 INTERFACE MENUS - NAVIGATION

Menus on the 820MO Interface are all accessible by a touch screen. To access a menu, simply touch the screen. A setting can be changed by touching the "SELECT" icon on the screen corresponding to the "SELECT" icon on the touch screen corresponding to the desired channel to be changed.

The line to be modified will turn blue, indicating that the Interface is waiting for input. When "ALL" is chosen, all lines corresponding to all 4 channels will turn blue. Changing the numeric value for the chosen parameter can be done by touching the up or down arrow keys.
Touch "ENTER" when the desired setting has been chosen. Touching the "X" will exit the menu and automatically take the user to the "ACTUAL FORCE" Display.



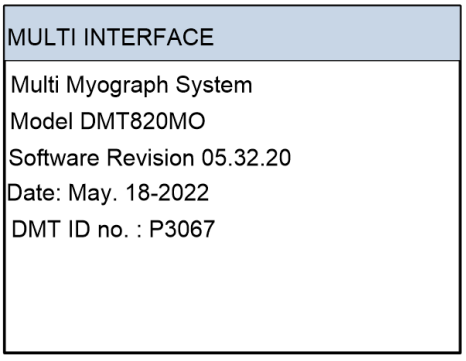




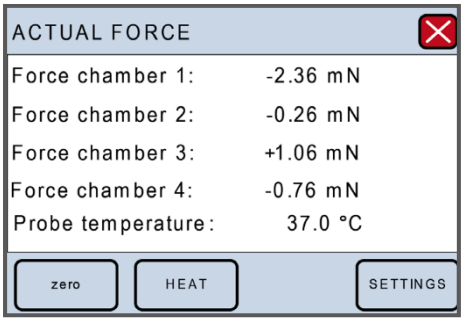
3.2 INTERFACE MENUS

POWER-UP SCREEN

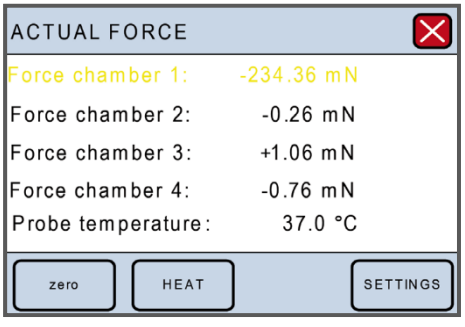
After turning on the 820MO Interface, an “Introduction” screen appears. The system is auto-calibrating the A/D converters while this screen is displayed.



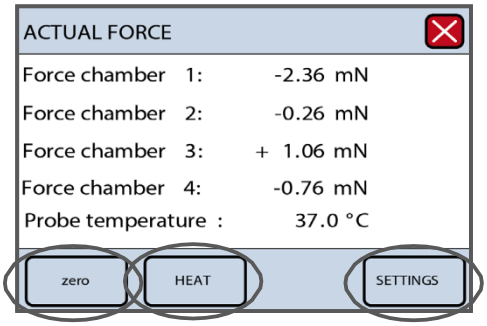
After a few seconds, the “ACTUAL FORCE” display will appear.



At any given time, if the force applied on any channel is out of range, the force reading for the overloaded channel will turn yellow as a warning.

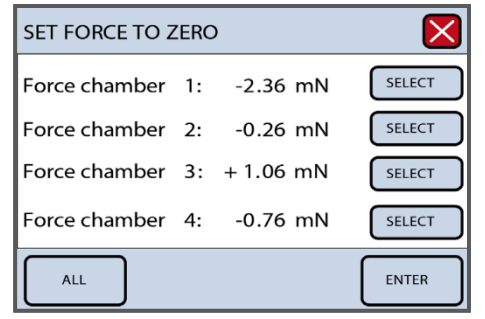


Three menus are accessible from the default “Actual Force” screen or display. These menus are Zero, Heat, and Settings.



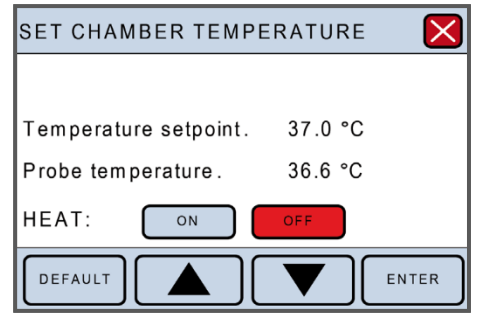
ZERO MENU

This menu is used to zero the output of the transducers. When using a data acquisition program like LabChart by AD Instruments ®, using this feature will reset the baseline of the chart traces without affecting the calibrations or physically changing any pre-load tensions placed on the mounted vessels. The channels can be changed individually by pressing “SELECT” or all at once by pressing “ALL.” Pressing “ENTER” will execute the zero function and return the user to the ACTUAL FORCE display.



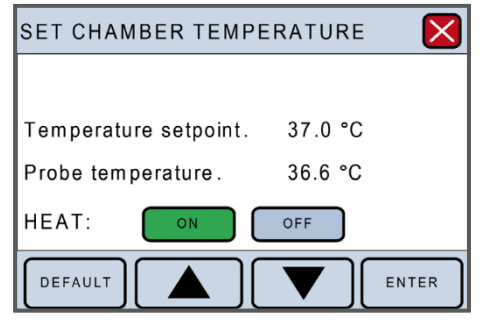
HEAT MENU

The heating unit and temperature are controlled from this menu. To turn the heat on or change the preset temperature for the system, access the temperature control menu. Pressing the “HEAT” key will enter the menu and allow the user to change the default system temperature, as well as turn the heat on or off. Pressing “DEFAULT” will automatically reset the temperature setpoint to 37°C. Manually change the temperature by pressing the up or down arrows.



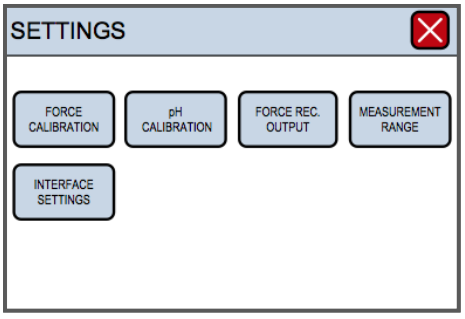
To turn the heat on, touch “ON” and the “ON” icon will turn green, indicating the heat has been turned on. The system will heat to the designated temperature setpoint.

Pressing the white “X” in the red box will send the user back to the “ACTUAL FORCE” display.



SETTINGS MENU

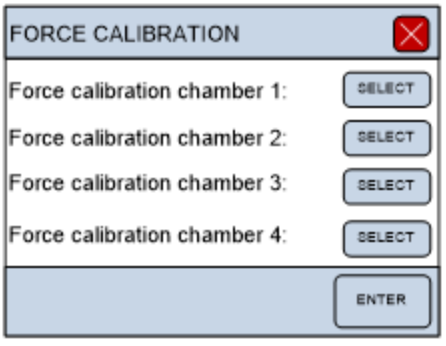
The “Settings Menu” contains several sub-menus that can be accessed to change functional aspects of the Interface. These sub-menus include:



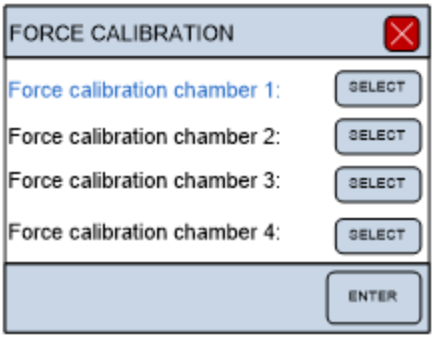
I. **Force Calibration**
II. **pH Calibration**
III. **Force REC. OUTPUT**
IV. **Measurement Range**
V. **Interface Settings**

1. Force Calibration

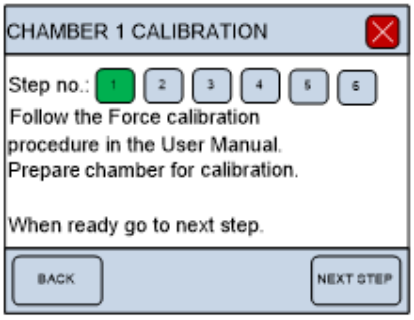
Entering the FORCE CALIBRATION sub-menu begins the transducer calibration procedure. Begin the calibration procedure by pressing “FORCE CALIBRATION” to enter the sub-menu. The sub-menu will list all 4 chambers for calibration.
(For more information and tips, please refer to Section 4.1- Calibration of the Force Transducer)



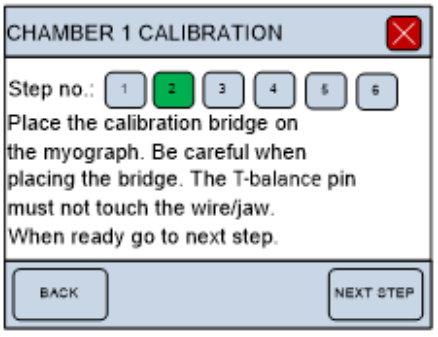
To begin the calibration, press “SELECT” for the chamber which calibration will be performed on. The text for the chamber to be calibrated will turn blue. Pressing “ENTER” will enter the 6-step procedure for calibrating the force transducer on the desired chamber.
The calibration procedure is listed in 6 individual steps and needs to be performed for each channel or transducer when calibrating the system.



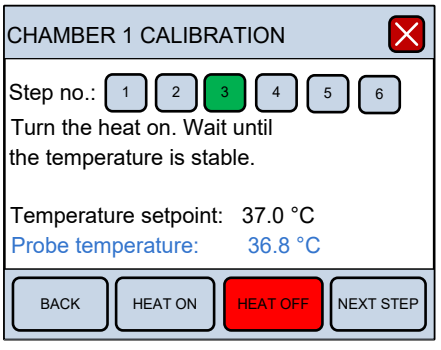
Step 1 involves setting up the chamber for calibration. Make sure the chamber contains the pins or clamps, depending on the type of tissue being studied. Fill the chamber with double-distilled water for the volume to be used experimentally. Press “NEXT STEP”.



Step 2 involves setting up the calibration kit appropriately for the actual weight calibration. Verify that the transducer arm pin does not touch the mounting pin for larger vessels or clamp for muscle, as instructed. The calibration pin should be as close as possible to the mounting pin or clamp without touching to get the most accurate calibration. Press “NEXT STEP” when the calibration kit has been properly placed.



Step 3 initiates the heating process for the chambers. For the calibration to be accurate, the transducers must be heated to the experimental temperature to be used to accommodate heat-induced expansion of the electronic parts in the transducer. Otherwise, inaccurate readings and transducer drift may occur, introducing large errors into the experiment.



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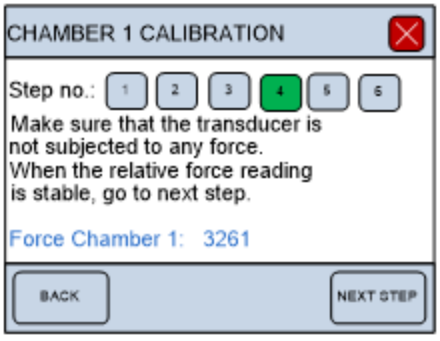
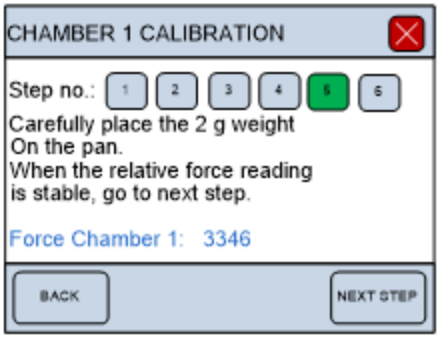
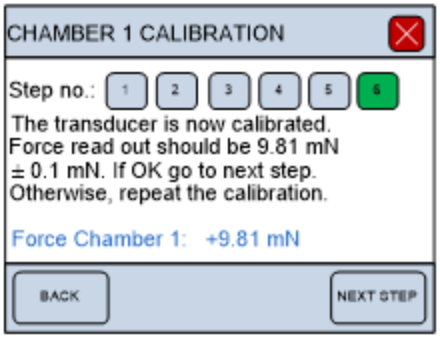
Step 4 is the first step in the actual weight calibration process. A 4-digit number will be displayed in blue at the bottom of the screen. If nothing has been perturbed during the heating process, the zero, 0 gram, or 0.00mN calibration should be stable as indicated by the 4-digit number, and "NEXT STEP" can be pressed after 30-40 seconds. If the 4-digit number is not stable, then wait until the number has stopped fluctuating before pressing "NEXT STEP".



Step 5 is the 2gram weight calibration. Place the 2gram weight in the pan closest to the transducer to simulate a mounted muscle pulling on the clamp attached to the transducer. Remember, a 2 gram weight in a 90° vector is cut in half, and the transducer will only detect 1 gram or 9.81mN of force. The weight placement should cause a positive increase in the 4-digit number. Wait at least 30 to 40 seconds for the applied force to stabilize before pressing "NEXT STEP". Once the 4-digit number has stabilized, press "NEXT STEP".



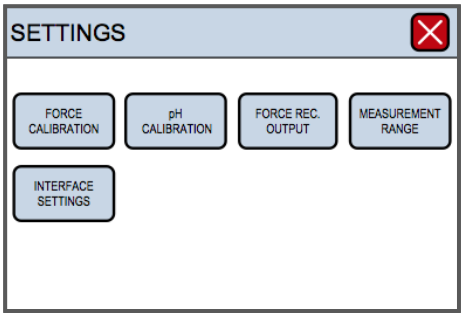
Step 6 is to verify that the calibration was performed correctly. The "Force Chamber 1" reading should be 9.81 ± 0.1mN. If the "Force Chamber 1" reading is off by more than 0.1mN, then remove the weight, press "BACK" to return to Step 4, and repeat the calibration process. If the "Force Chamber 1" reading is satisfactory, then press "NEXT STEP". Calibrate the other chambers in the same manner.



2. pH Calibration Menu (Optional)

The pH Module in the 820MO is locked by a code. If the lock screen appears, a code is needed from DMT to unlock the pH module.

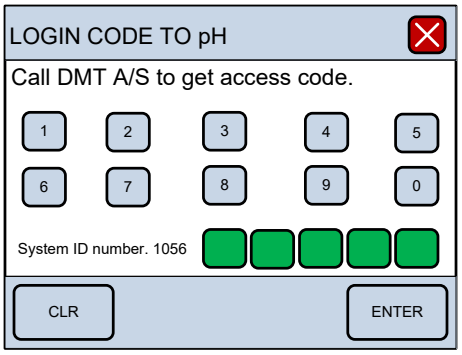
By ordering the pH sensor from DMT, the pH sensor is delivered with the unlocking code and a quick guide on how to do it. Entering the code will open the pH Calibration menu.



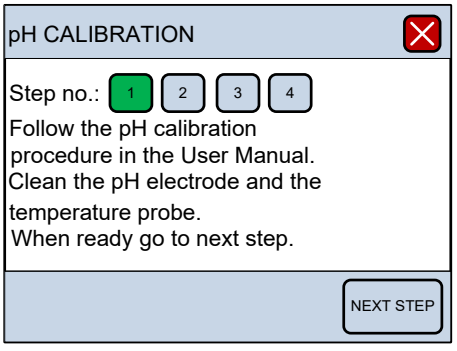
The 820MO system has a build-in pH meter and a pH-meter electrode plug-in port marked PH on the backside of the 820M interface.

The pH electrode can be ordered at DMT by contacting your sales representative or emailing sales@dmt.dk.

The pH calibration procedure is listed in 4 individual steps and needs to be performed one at a time.

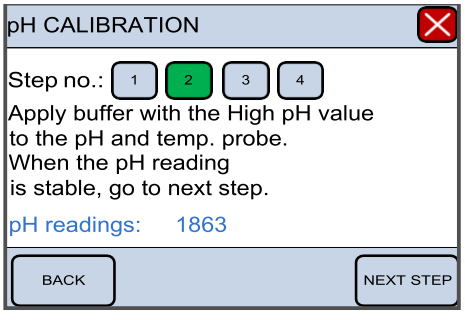


NOTE: BEFORE THE PH CALIBRATION IS PERFORMED BE SURE TO SELECT THE WAY THE PH ELECTRODE IS TO BE USED. SEE THE SUB- MENU PH SET-UP UNDER INTERFACE SETTINGS.

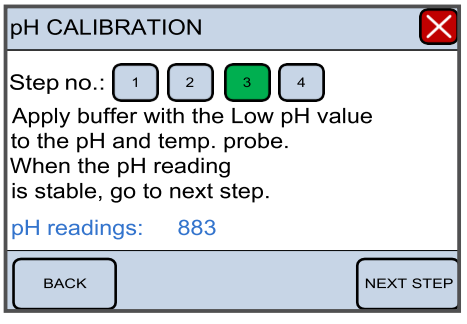


Step 1 - Step 1 involves cleaning the pH electrode and the temperature probe with double distilled water. When ready, Press NEXT STEP.

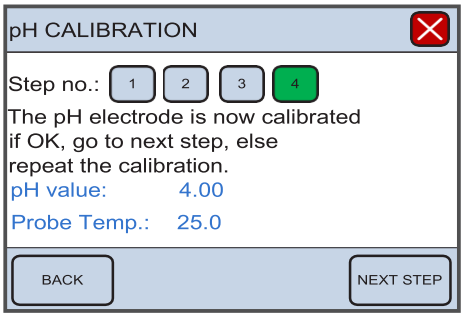
Step 2 - Place the pH electrode and temperature probe in the high buffer solution (here pH 7) and turn on stirring of the high buffer solution. When the relative pH output in the blue line is stable, go to NEXT STEP.



Step 3 - Place the pH electrode and temperature probe in the low buffer solution (here pH 4) and turn on stirring of the low buffer solution. When the relative pH output in the blue line is stable, go to NEXT STEP.

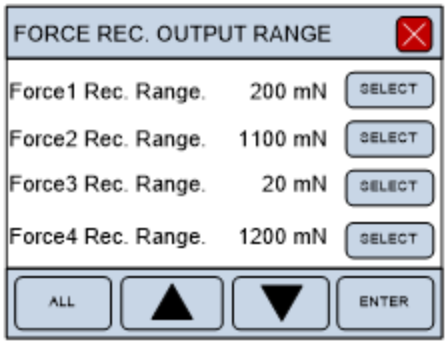


Step 4 - The calibration is now finished. The values in the two bottom lines are the actual pH and temperature reading.

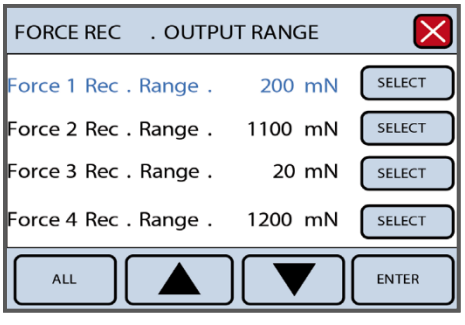


3. Force Rec. output (analog output only)

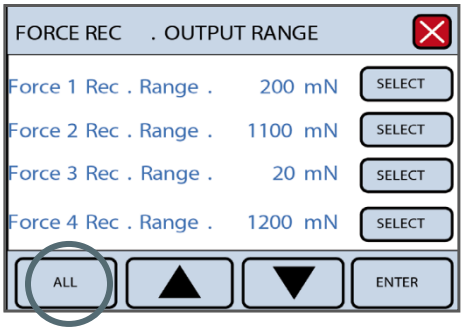
The FORCE RECORDING OUTPUT, or FORCE REC. OUT,sub-menu determines the upper limit for force sent through the BNC output connectors to e.g. Powerlab or a similar data acquisition system. The factory default setting for FORCE REC. OUT is 20mN, meaning that if the force of the mounted vessel exceeds 20mN, the force recorded in the data acquisition software will not record more than 20mN and will appear as a flat-line trace at 20mN even though the force readings on the Interface may exceed 20mN. Therefore, change the FORCE REC. OUT settings to an appropriate setting to capture any maximal response from the mounted tissue of interest. This value should not exceed the settings for the transducer range, which is defined by the sub-menu, MEASUREMENT RANGE, and is explained in the next section. As can be seen on the below screen figure, the Force Rec Output can be set to different values on different chambers.



The “SELECT” for a single chamber is used if different values are needed for the four chambers and changed by pressing the Up and Down arrow keys and stores in the interface setting by pressing ENTER.



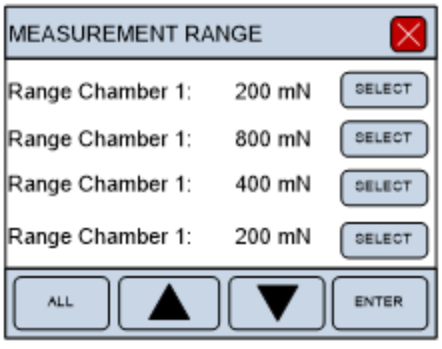
The “ALL” functions are used to change all four chambers Force Rec Output values at the same time. Pressing “ENTER” will store the numbers in memory for future experiments.



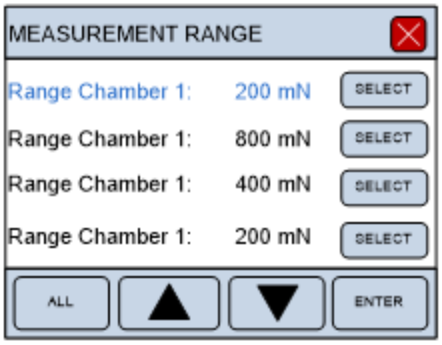
NOTE: FOR USERS USING THE ANALOG BNC OUTPUTS OF THE 820MO INTERFACE FOR DATA ACQUISITION ON A POWERLAB OR SIMILAR SYSTEM: ANYTIME THE FORCE REC OUTPUT IS CHANGED, A NEW 2-POINT CALIBRATION MUST BE PERFORMED IN E.G. LABCHART TO ENTER THE NEW VOLTAGE VALUES INTO THE DATA ACQUISITION SYSTEM BEING USED.

4. Measurement Range

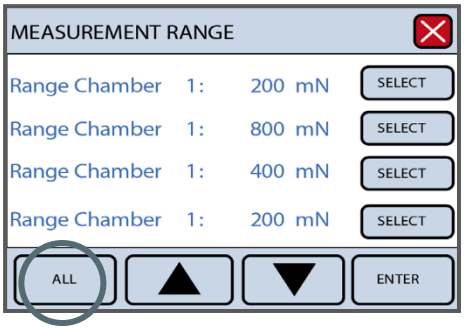
The factory setting is 200mN, but the transducer capacity can be changed to 400mN, 800mN or a maximum of 1600mN of force detection, depending on the contractility capacity of the mounted tissue used. As can be seen on the above screen image, the Measurement Range setting can be set to different values on the four chambers. If the Measurement Range is changed to e.g., 400mN then the Force Rec Out Put also has to be changed to 400mN. After this change, users of the analog signal from the BNC connectors must go into the data acquisition software and set 0V equals 0mN, and 2.5V equals 400mN. For DMT Device Enabler users, the only thing to do is to restart LabChart then the new settings are transferred to LabChart.



The “SELECT” for a single chamber is used if different values are needed for the four chambers and changed by pressing the Up and Down arrow keys and stores in the interface setting by pressing ENTER.



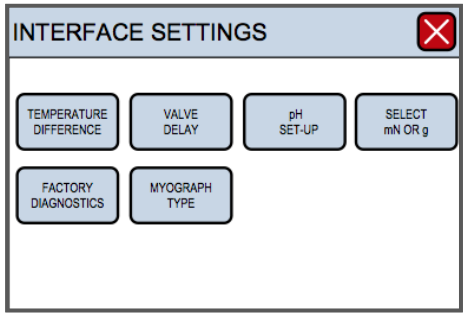
The “ALL” functions are used to change all four chambers Force Rec Output values at the same time. Pressing “ENTER” will store the values in memory for future experiments.



NOTE: FOR DMT DEVICE ENABLER USERS LABCHART HAS TO BE RESTARTED AFTER CHANGING THE MEASUREMENT RANGE VALUES OTHERWISE THE OLD SETTING WILL PERSIST IN LABCHART AND THE FORCE READINGS WILL NOT BE CORRECT.

5. Interface settings

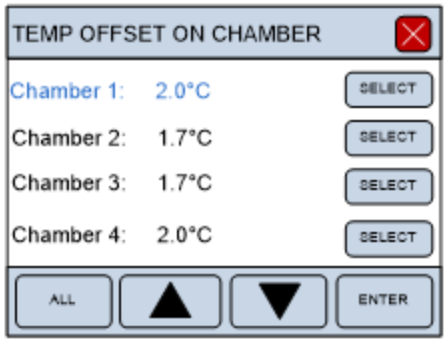
The INTERFACE SETTINGS sub-menu in SETTINGS has an additional six sub-menus. These six additional sub-menus are:



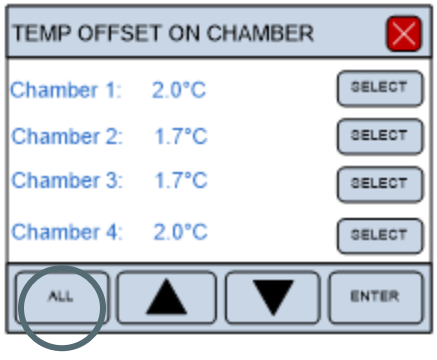
I. **Temperature Difference**
II. **Valve Delay**
III. **pH Set-up**
IV. **Select mN or g**
V. **Factory Diagnostics**
VI. **Myograph Type**

I. Temperature Difference

The TEMPERATURE DIFFERENCE function allows the user to fine-tune the temperature set point of the system. Although the temperature set point for the system can be set in the HEAT MENU, the actual temperature for the system may not heat to the exact defined set point. Therefore, the user can adjust the temperature of each chamber individually to fine-tune the temperature setting so that EXACT temperatures can be achieved for any particular chamber. This is referred to as a temperature offset (TEMP OFFSET ON CHAMBER). Use the thermometer connected to the backside of the 820MO Interface to check the actual temperature of the buffer in the chamber and use that information to adjust the offset temperatures.

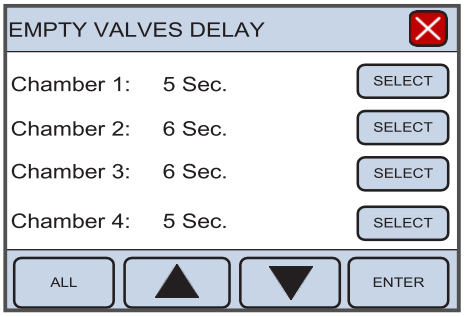


The “SELECT” and “ALL” functions are the same in this menu as previously described. Pressing “ENTER” will store the numbers in memory for future experiments.



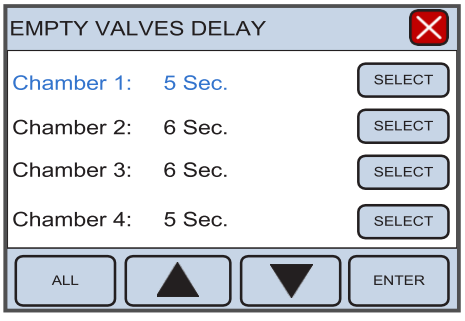
II. Valve Delay

Pressing “VALVE DELAY” in the INTERFACE SETTINGS sub-menu will allow the user to modify the time duration that the vacuum valves stay open for washes. Factory default is set at 1sec, but 1sec is usually not enough time to completely empty a chamber with even as small a volume of 5ml.



Pressing “ALL” will cause all the lines to turn blue, meaning all chambers can be modified at the same time. Again, the up and down arrow keys can be used to modify the length of time the vacuum valves stay open.

Pressing “SELECT” next to any given channel will cause the line selected to turn blue.

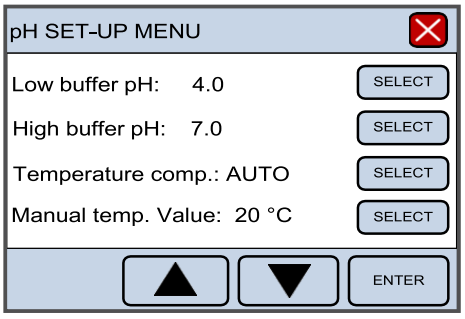


The up and down arrow keys can then be used to modify the length of time the vacuum valves stay open after the valves have been activated with the push buttons on the front panel of the Interface.

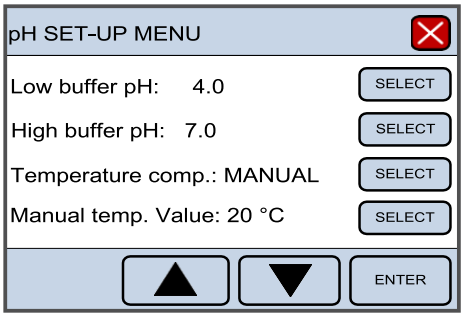
Pressing “ENTER” after modifying the value(s) for valve delay will lock in the number(s) and be retained in memory every time the system is turned on.

III. pH Set-up (Optional)

The temperature is an important parameter in the pH calibration formula and is obtained automatically if AUTO is selected in Temperature compensation function.

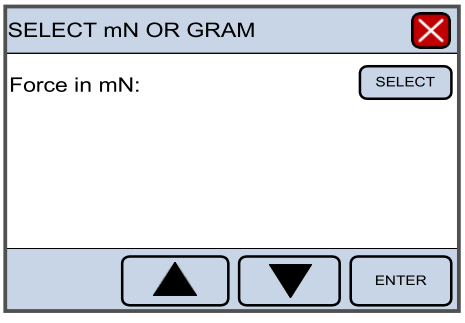


If Manual is selected, the manual temperature is used in the pH calibration formula, and the temperature probe is de-activated. In the Manual mode, the temperature of the calibration buffers is measured with a thermometer and entered manually in the Manual Temp. value line.



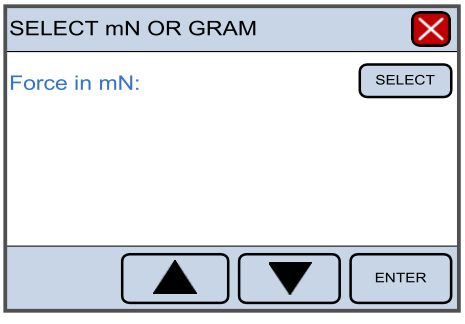
IV. Select mN or g

The contraction force can be shown in gram or in mN on the touch screen, and for LabChart Pro and DMT device enabler users, the selected force unit will be shown automatically in LabChart as well. For users of Powerlab or similar systems, the analog signal from the BNC connectors still has to make the unit conversion in the appropriate data acquisition system to get the force in either gram or mN.

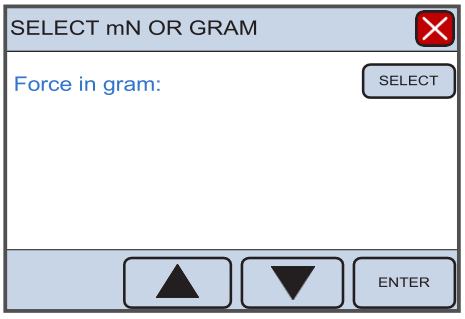


To change the Force units, press SELECT.
The Force in mN will turn blue.

Press the arrow key to change the force unit between mN and gram.



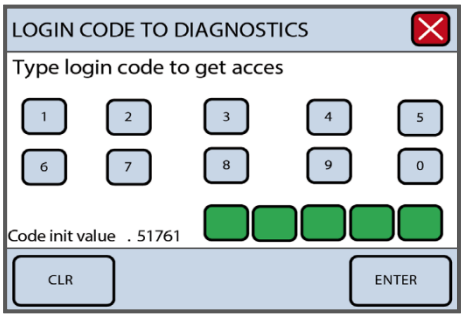
When the wanted force, unit is shown press ENTER to select this force unit. The color of the text will turn black when it is saved in the system.



NOTE: FOR DMT DEVICE ENABLER USERS (NO POWERLAB) IT IS VERY IMPORTANT TO RESTART LABCHART PRO AFTER CHANGING THE FORCE UNITS OTHERWISE THE FORCE UNITS WILL NOT BE TRANSFERRED TO LABCHART.

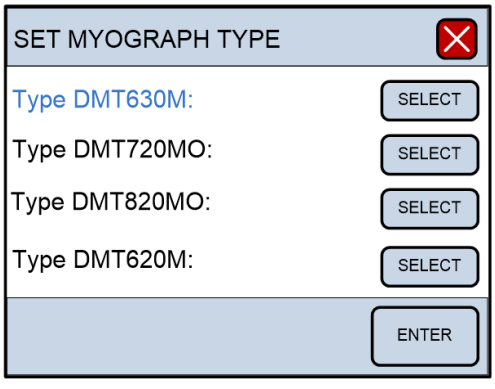
V. Factory Diagnostics

Entering FACTORY DIAGNOSTICS will display the LOGIN CODE TO DIAGNOSTICS window. This window is for trained technicians and used for diagnostics and troubleshooting purposes. The general user will not have access to this window. Entering the proper 5-digit pin number, however, will allow the trained technician access to Diagnostics panels that will provide information during a malfunction or mechanisms to change other settings controlled by the onboard computer.



VI. Myograph Type

The Interface of the 820MO system is identical to the interfaces of the DMT 620M Multi Myograph and 630MA Automated Multi Myograph system. The difference between these three systems is the chambers. This makes it possible to combine the three different types of chambers on this Interface. There are small differences in the menus of these four systems and to shift between these different menus the user can load these into the Interface using the myograph Type menu.



In this menu, the active myograph menu is marked by bold letters. To change to one of the other systems, press SELECT, and the text line will turn blue, and then press ENTER. The text line will not be in bold letters indicating that this systems menu structure is selected. Turn off the power of the system and turn it ON again. Now the system will load the menus of the select myograph system into the Interface and is now ready for use. It is important to make a new weight calibration and make sure that the Measurement Range is correct. If the device enabler is not used then also check the Force Rec Output values.

CHAPTER 4 - THE ORGAN BATH CHAMBER UNIT

Chapter 4 contains a complete explanation of how to calibrate and maintain the 820MO organ bath to ensure the equipment is always performing at peak performance.

4.1 CALIBRATION OF THE FORCE TRANSDUCER

As a part of the general maintenance of the myograph, DMT recommends that the myograph be weight-calibrated at least once a month. The myograph should also be weight-calibrated every time the Interface has been moved. Although lab benches are all supposedly perfectly horizontal, small differences in lab bench pitch can affect the calibration of the system. The myograph also should be calibrated if the system has been idle for longer than a month. A step-by-step procedure is included in the FORCE CALIBRATION sub-menu under SETTINGS, as explained in “Chapter 3 - The Interface Menus”.

FORCE TRANSDUCER CALIBRATION PROCEDURE

This section contains step-by-step instructions to calibrate the force transducer.

NOTE: EVERY TIME A FORCE CALIBRATION IS PERFORMED THE MEASUREMENT RANGE IS SET TO DEFAULT 200MN. SET MEASUREMENT RANGE AFTER THE FORCE CALIBRATION.

CALIBRATION PROCEDURE IN CALIBRATED MODE

1. Move the mounting supports apart. Fill the chamber with distilled water or buffer. Use the same volume that will be used during the experiments.
2. Set up the calibration kit (bridge and balance) on one of the myograph chambers as illustrated in “Figure 4.1 - 4.2. Turn the heat on as discussed. The system takes about 20 to 30 minutes to reach 37°C. For skeletal muscle preparations, most likely experiments will be performed at room temperature. It is recommended that the system temperature be set to 25 to 27°C so that experiments are performed at a constant temperature,

depending on the temperature regulation in the room the myograph is set up in.

3. Obviously, lower temperatures take less time, and higher temperatures take more time to reach. Make sure adequate time is allowed so that calibration can be performed at the temperature at which the experiments will be performed. Placing the calibration kit and weight on the chamber allows them to warm up to the experimental target temperature. No need to bubble the chambers while waiting for the system to heat up.
4. When the system reaches target temperature, adjust the calibration kit so that the tip of the transducer arm is as close to the mounting support on the transducer side as possible without touching. One way to do this is to use the following technique. Start with the calibration kit in place so that the transducer arm of the bridge with the pans is not touching any part of the mounting support. Go to the main menu displaying the forces, and zero the channel being calibrated, so the force reads zero. Slowly and gently slide the calibration kit forward toward the micromanipulator so that the transducer arm rests on the mounting support, creating a force reading on that channel. Carefully slide the calibration kit back toward the transducer slowly until the force reads zero or very close to zero. At this point, as soon as the force reads zero, the transducer arm will be properly placed for weight calibration.
5. Go to the FORCE CALIBRATION sub-menu of the SETTINGS menu on the Interface to begin the actual transducer calibration. The process that is described above is reiterated in 6 steps once the FORCE CALIBRATION sub-menu is initiated.

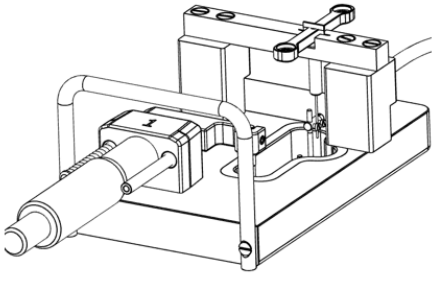


Figure 4.1

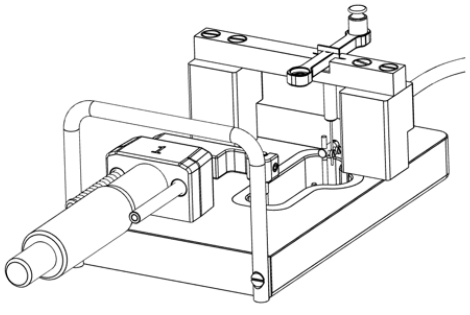


Figure 4.2

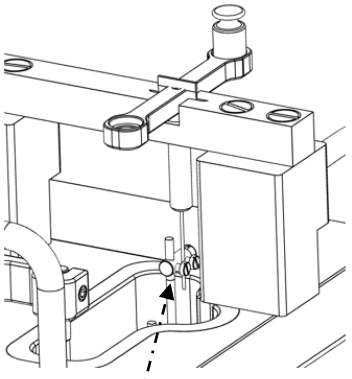


Figure 4.3

The tip of the balance arm is positioned behind the screw as shown here without touching

Figure 4.1 - 4.3 Weight calibration kit shown in place on a single organ bath chamber with mounting hooks

4.2 CHECKING THE FORCE TRANSDUCER

The myograph force transducer is a strain gauge connected to a Wheatstone bridge. The force transducers for each chamber are housed in a separate, protective compartment (see figure 4.3 below). While the protective cover offers some mechanical protection for the force transducers, they are still very vulnerable to applied forces exceeding 1 Newton (100 grams) or fluid running into the transducer compartment due to insufficient greasing of the transducer pinhole (see “Figure 4.6 Close-up of transducer pin from outside”).

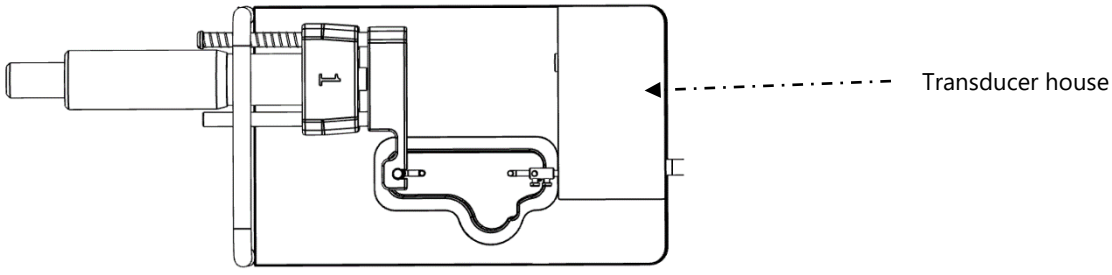


Figure 4.3 Illustration of the transducer house

If the force readings on the Interface appear unstable or noisy, then first check that the chambers are connected properly to the Interface and that the chambers are plugged all the way into the Interface.

If the force reading(s) are still unstable or noisy, then perform a new calibration of the force transducer as described in” Chapter 3 - The Interface Menus” and 4.1 Calibration of the force transducer”.

During the new calibration, monitor the relative force reading values in the FORCE CALIBRATION sub-menu on the Interface (Steps 4 and 5 of the calibration procedure):

If the value is 0, a single digit, or above 6500, then the force transducer is broken and needs to be replaced.

If the message “OFF” is displayed on the main page of the Interface even though the chamber is plugged in at the rear of the Interface, then the force transducer is broken and needs to be replaced. In addition, if the force reading(s) appear yellow in color, cannot be reset to zero, AND the transducer cannot be recalibrated, then the force transducer is broken and needs to bereplaced.

If any other problems related to the force transducer are encountered, please contact DMT for advice or further instructions.

4.3 FORCE TRANSDUCER REPLACEMENT

If the force transducer breaks and needs to be replaced, follow this step-by-step replacement procedure carefully:

1. Remove the pin/clamp from the transducer pin coming out of the transducer house.
2. Disconnect the Organ Bath Chamber from the Interface.
3. Turn the Organ Bath Chamber upside down and remove the transducer housing by loosening the two screws (A+B) as illustrated in figure 4.4.

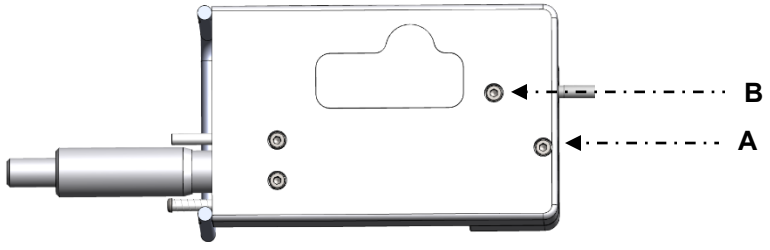


Figure 4.4 - The 2 screws that secure the transducer house to the chamber

4. The replacement transducer will be shipped with the new transducer inside a new transducer house.
5. Place a VERY small amount of vacuum grease (clear or whitish grease) around the bottom inside of the transducer housing to seal the transducer housing when put back in place. An arrow in figure 4.5 indicate the place that the grease needs to be applied.
6. Carefully realign the transducer housing with the new transducer on the Organ Bath Chamber and reinsert the Allen screws through the bottom of the Organ Bath Chamber.
7. Tighten the screws and place some vacuum grease from the outside around the transducer pin that protrudes from the transducer housing. Make sure that the hole is completely sealed to prevent buffer solution or water from entering the transducer housing and damaging the new force transducer. Arrow in figure 4.6 indicate the place that the grease needs to be applied.

IMPORTANT:
CALIBRATE THE NEW FORCE TRANSDUCER BEFORE PERFORMING A NEW EXPERIMENT, AS DESCRIBED IN "CHAPTER 3 - THE INTERFACE MENUS" AND "4.1 CALIBRATION OF THE FORCE TRANSDUCER".

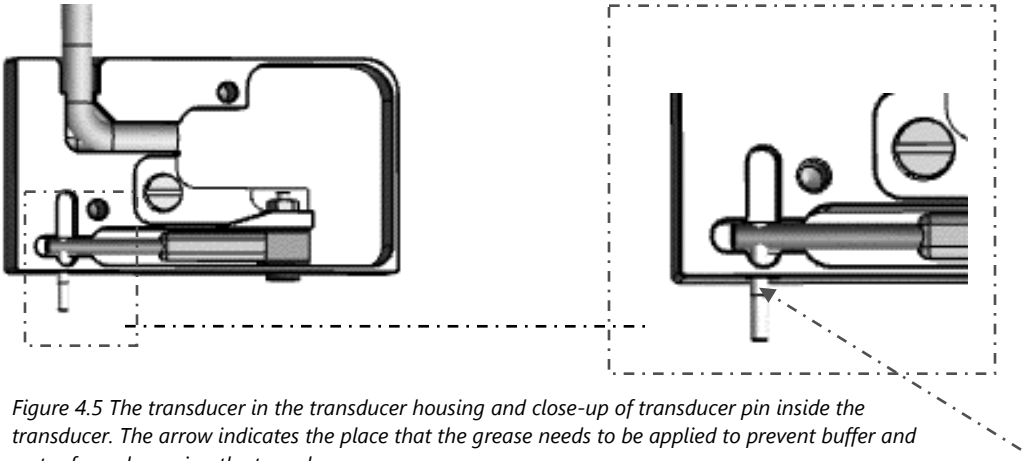


Figure 4.5 The transducer in the transducer housing and close-up of transducer pin inside the transducer. The arrow indicates the place that the grease needs to be applied to prevent buffer and water from damaging the transducer.

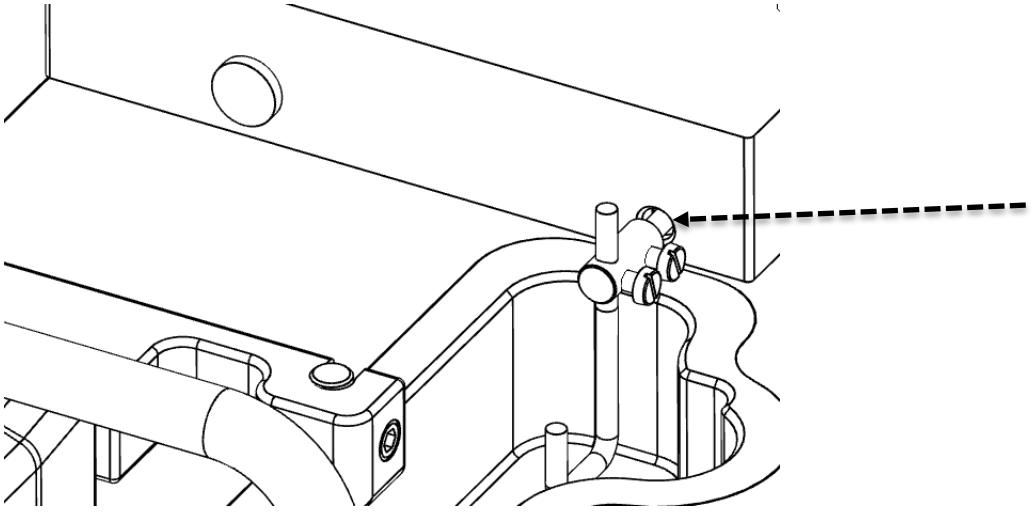


Figure 4.6 Close-up of transducer pin from outside. The arrow indicates the place that the grease needs to be applied to prevent water and buffer leaking into the transducer housing and damaging the transducer.

4.4 ORGAN BATH MAINTENANCE

The Organ Bath System - 820MO is a very delicate and sophisticated piece of research equipment. DMT recommends that the following sections are read carefully and that the instructions are followed at all times.

ORGAN BATH CHAMBER TUBING

To prevent the tubing from becoming blocked with buffer salt deposits after an experiment, remove the chamber cover from the Organ Bath Chamber. Fill the chamber with distilled water and turn on the vacuum and press the vacuum valve for about 10 seconds by holding down the valve button(s) down. Repeat this at least two times. Press the vacuum valve for about 10 seconds by holding the valve button down to empty chamber and tubes. Turn off the vacuum and gas supply. Remove any water or buffer remaining in the chamber or on the tubing using absorbent paper.

FORCE TRANSDUCER

The force transducer is the most delicate and fragile component of the organ bath system. Extreme care must be used when handling or touching the force transducers.

As a part of daily maintenance, inspect the grease around the transducer pin extending from the transducer housing pinhole before starting any experiment. Insufficient grease in this area will allow buffer and water to enter the transducer housing and cause damage to the force transducer.

IMPORTANT:

- *DMT RECOMMENDS THAT THE HIGH VACUUM GREASE SEALING THE TRANSDUCER PINHOLE IS CHECKED AND SEALED AT LEAST ONCE A WEEK, ESPECIALLY IF THE ORGAN BATH IS USED FREQUENTLY.*
- *DMT TAKES NO RESPONSIBILITIES FOR THE USE OF ANY OTHER KINDS OF HIGH VACUUM GREASE OTHER THAN THE ONE AVAILABLE FROM DMT.*
- *DMT TAKES NO RESPONSIBILITIES FOR ANY KIND OF DAMAGE APPLIED TO THE FORCE TRANSDUCERS.*

MICROMANIPULATORS

Check the micropositioner for grease at least once a month. There are 3 main parts that will need to be greased on the micropositioner, those parts being the micropositioner screw thread and the 2 slide bars to the left and right of the micropositioner screw thread. In case of insufficient lubrication, grease the micropositioner with the "Grease for Linear Slides" included with your system. Apply the linear slide grease in the areas indicated by the arrows in figure 4.6 below.

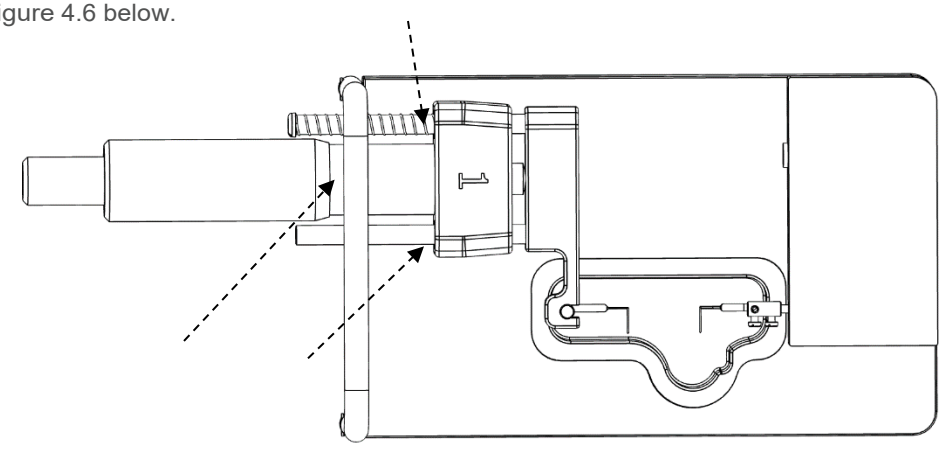


Figure 4.6 The areas where linear slide grease may be applied for smooth micropositioner movement

4.5 CLEANING THE ORGAN BATH

DMT strongly recommends that the organ bath chambers and surrounding areas are cleaned after each experiment.

At the end of each experiment, use the following procedure to clean the organ bath chambers and supports:

1. Fill the organ bath chamber to the edge with an 8% acetic acid solution and allow it to work for a few minutes to dissolve calcium deposits and other salt build-up. Use a cotton-tipped applicator to mechanically clean all chamber surfaces. Be careful not to apply too much force to the force transducer side mount.
2. Remove the acetic acid and wash the organ bath chamber and supports it several times with double distilled water.
3. If any kind of hydrophobic reagents has been used which might be difficult to remove using steps 1 and 2, then try incubating the chamber and supports with 96% ethanol or a weak detergent solution (i.e. 0.1% triton-100).
4. To remove more resistant or toxic chemicals, incubate the organ bath chamber and supports with 1M HCl for up to 1-2 minutes. In exceptional cases, incubate the chamber and supports with no stronger than a 3M HNO3

solution for about 1-2 minutes.

5. Wash the organ bath chamber and supports several times with double distilled water.
6. If acids such as 1M HCl and 3M HNO_3 are used to clean the chambers, make sure ALL surfaces are thoroughly dried after copious washes with double distilled water. Any residual acid will cause corrosion of the stainless steel chamber and/or mounting supports.

IMPORTANT:

- *BE VERY CAREFUL USING HCL OR HNO3 BECAUSE THESE ACIDS MAY CAUSE EXTREME DAMAGE TO THE STAINLESS STEEL CHAMBERS AND SUPPORTS, AS WELL AS POSE A SAFETY HAZARD TO THE USER. DO NOT USE BLEACH TO CLEAN THE CHAMBERS. REPEATED USE OF CHLORINATED SOLUTIONS SUCH AS BLEACH AND HCL WILL CAUSE DAMAGE TO THE STAINLESS STEEL PARTS OF YOUR ORGAN BATH SYSTEM. AVOID USING THEM IF AT ALL POSSIBLE.*
- *AFTER CLEANING, ALWAYS CHECK THAT THE GREASE AROUND THE TRANSDUCER PIN IS SUFFICIENT TO KEEP THE BUFFER AND WATER FROM ENTERING THE TRANSDUCER HOUSING.*

If red or brown discolorations appear on the chamber sides or on the supports, the following cleaning procedure will work in most cases:

1. Incubate the organ bath chamber and supports for 2 minutes with 2mM T-1210 Tetrakis- (2-pyridylmethyl)-ethylenediamine solution dissolved in double distilled water.
2. Use a cotton-tip applicator to mechanically clean all the affected surfaces during the incubation period.
3. Wash the organ bath chamber and supports several times with double distilled water.
4. Incubate the organ bath chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a cotton-tip applicator.
5. Remove the ethanol solution and wash a few times with double distilled water. Incubate the organ bath chamber and supports with an 8% acetic acid solution for 2 minutes and continue the mechanical cleaning with a swab-stick.
6. Wash the organ bath chamber and supports several times with double distilled water.
7. Dry the surfaces using absorbent paper (i.e. Kim-Wipes) or cotton-tip applicators.

IMPORTANT:

IN EXCEPTIONAL CASES, THE SUPPORTS (CLAMP) MAY NEED TO BE REMOVED FROM THE ORGAN BATH CHAMBER AND CLEANED INDIVIDUALLY TO ASSURE PROPER CLEANING OF ALL SUPPORT SURFACES. NEVER SOAK THE SUPPORTS IN ANYTHING STRONGER THAN 8% ACETIC ACID FOR EXTENDED PERIODS OF TIME (I.E. SEVERAL HOURS OR OVERNIGHT).

CHAPTER 5 – MOUNTING SUPPORTS AND MUSCLE MOUNTING IN THE 820MO CHAMBER

Each chamber contains supports with mounting hooks, pins or clamps to facilitate the mounting of tissue in the organ bath chambers.

Below is a short description of how to mount a tissue ring preparation on the Mounting Pins of the 820MO system. DMT deliver the 820MO system with 200µm pins allowing the user to mount ring preparation with an internal diameter from 450µm and up. For large tissue ring preparation with the ability to make high force contractions the 200µm pins may be too thin. DMT can delivery mounting pins with the following thickness:

L-shaped mounting pins (Diameter): 100µm, 200µm, 250µm, 300µm and 400µm.

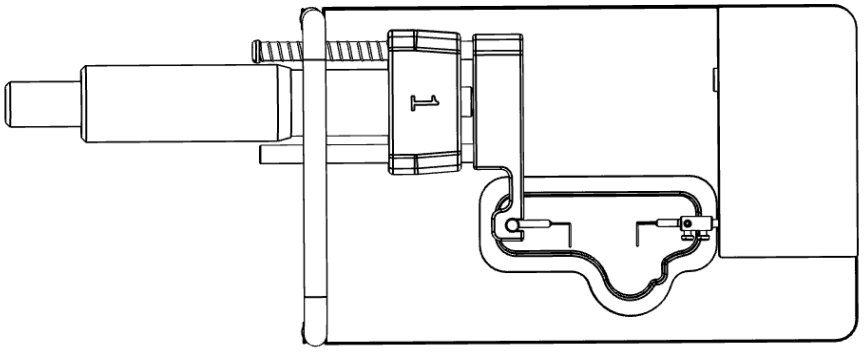
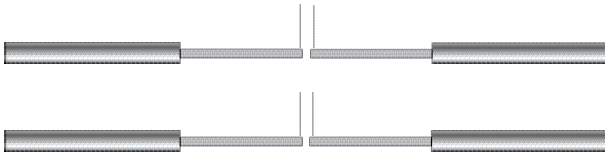


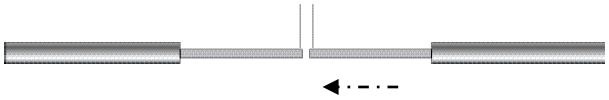
Figure 5.1. 820MO Chamber unit with Mounting Pins

1. Fill the chamber with a given buffer (5-7ml) (see appendix 1 for Buffer recipes). *DO NOT FILL THE CHAMBER TO THE EDGE OF THE CHAMBER.*
2. Make sure that the tissue Mounting pins are properly aligned.

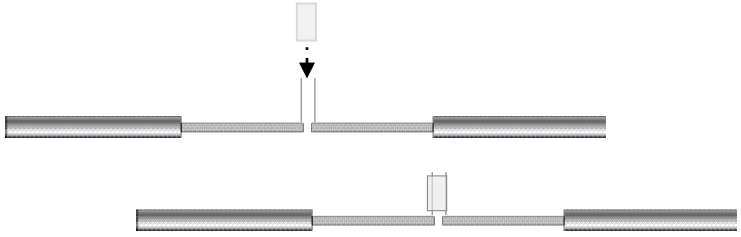


Properly aligned pins, seen from side and above

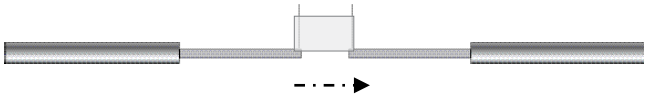
3. Use the micrometer to get the pins close together as possible without touching each other.



4. Slide your tissue ring preparation onto the two mounting pins.



5. Slowly move the pins apart using the micrometer until the force reading is increasing.



6. The tissue is now mounted and ready for a normalization protocol (see chapter 3.2) finding the optimal pre-load tension for the mounted tissue.

7. Place the micrometer at the position giving the optimal pre-load tension on the mounted tissue.

8. Continue with a standard start (see chapter 3.3).

9. The mounted tissue is now ready for experiments.

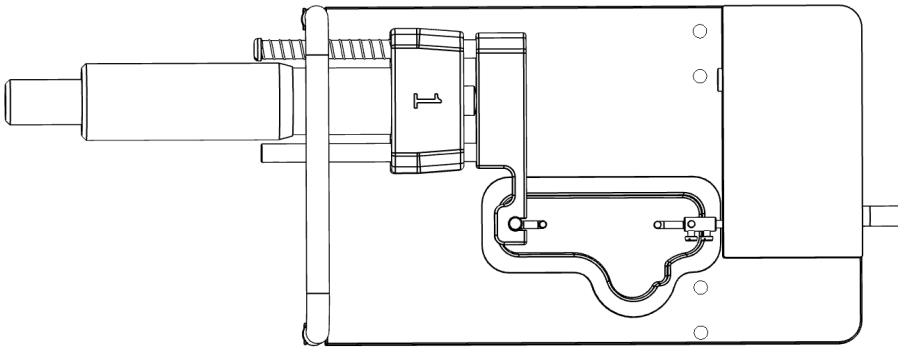
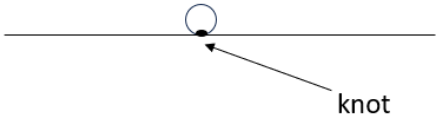


Figure 5.2. 820MO Chamber unit with Mounting Hooks

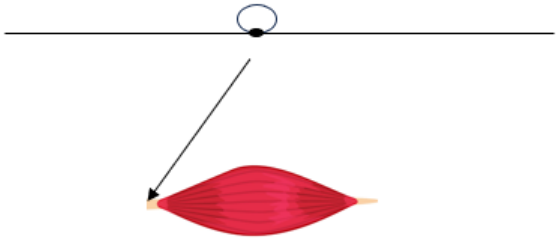
These hooks is for mounting of tissue to the hooks with suture delivered with the system. Below is an example of how to mount a muscle to the hooks with suture

8cm suture

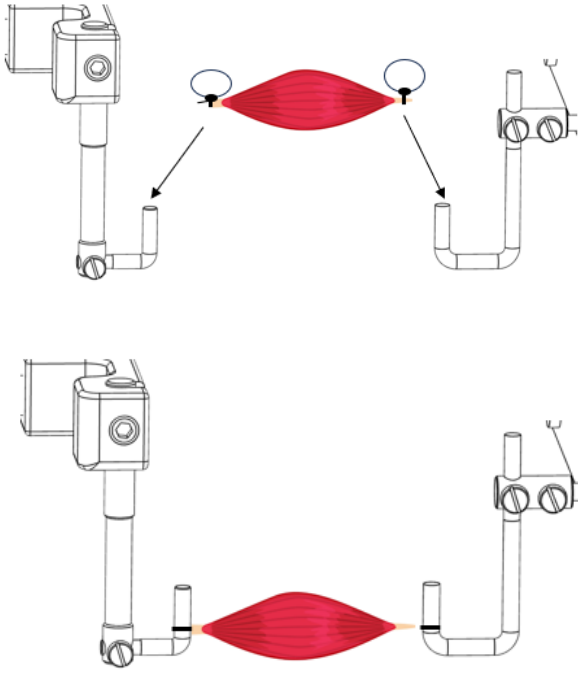
Tie a knot that generate a fixed loop fitting on the hooks. You can make a lot of these and save them until use



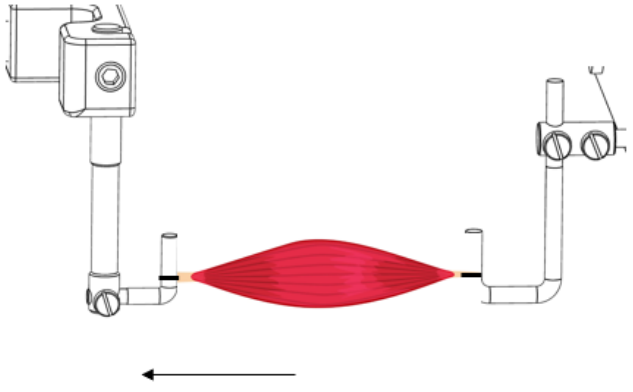
Tie one suture with the fixed loop to each ends of the muscle



Lower the loops onto the hooks



Stretch the mounted tissue to apply the optimal pre-load tension



3.2 NORMALIZATION – OPTIMAL PRE-LOAD TENSION

Artery tissue: DMT highly recommend the user to read the DMT Normalization Guide before performing any experiments on arteries. The Normalization guide can be downloaded on www.dmt.dk.

The importance of normalizing the preparation is three-fold:

1. Experiments with elastic preparations like vessels can only have meaning if they are performed under conditions where the size is clearly defined.

2. Clearly defined conditions are required in pharmacological experiments as the sensitivity of preparations to agonists and antagonists is dependent on the amount of stretch.

3. The active response of a preparation is dependent on the extent of stretch, which makes it important to set the preparation to an internal circumference or pre-load tension giving maximal response of a given tissue mounted in the 820MO chambers.

The aim of the normalization procedure is to stretch the mounted muscle segment to an optimal pre-load tension giving maximum response by muscle contraction. For arteries the.so-called normalized internal circumference (IC1): defined as a set fraction of the internal circumference (IC100) that a fully relaxed segment would have at a specified transmural pressure. For small rat arteries the target transmural pressure is typically 100mmHg = 13.3 kPa.

APPENDIX 1 - SYSTEM SPECIFICATIONS

SPECIFICATIONS:	
Vessel size	250µmm – 10 mm (rings) or 3mm - 19mm (muscle)
Chamber	Four individual chambers
Chamber material	Acid-resistant stainless steel
Chamber volume	Max. 8ml
Chamber suction	Manual or automatic, time controlled, user defined
Chamber cover	Supplied with connections for gassing
Chamber gassing	Individually controlled per chamber by needle valve
Force range	User selectable at ± 200/400/800/1600mN
Force resolution	0.1mN
Weight calibration	Semi-automatic
Heating	Built into chamber, independent of super fusion
Temperature range	Ambient temperature -50°C
Temperature resolution	0.1°C
Temperature probe	External
Output reading	Force (mN) or Grams (g)
Analogue output	Independently filtered or unfiltered (direct) 4-channel output at 2.5V full scale
Digital output	USB
Voltage	100-240 VAC (auto) 50/60Hz
Ambient temperature	15 -30°C

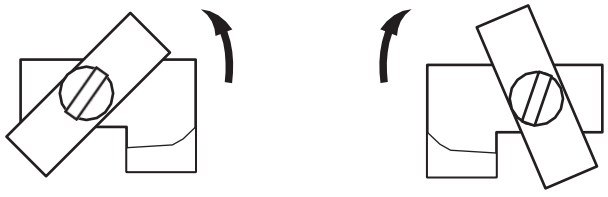
APPENDIX 2 - MOUNTING CLAMPS

Below is a short description of how to mount a tissue strips on clamps.These clamps have been designed to optimally "clamp" the tissue without slipping during a contraction. They are also designed to effectively "grab" tendon on whole striated muscle preparations without experiencing "slippage" during a whole striated muscle contraction.

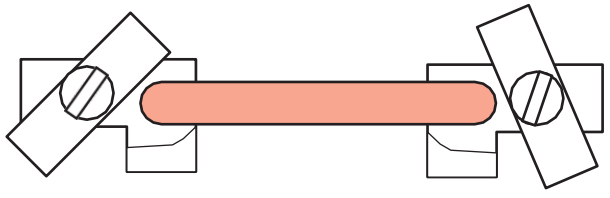
1. Starting position.

2. Loosen the screws on the mounting clamps.

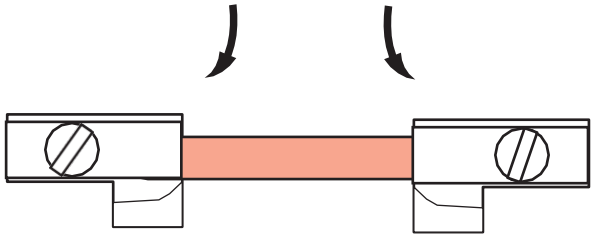
3. Turn the upper wings of the mounting clamps to expose the base of the support.



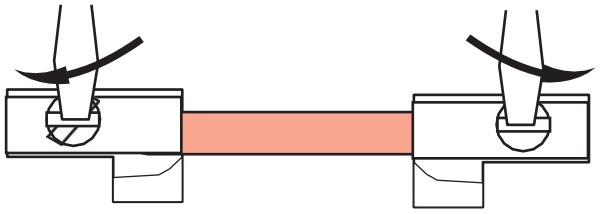
4. Place the muscle strip onto the base of the mounting clamp.



5. Turn the upper wings back into position so that the muscle strip is clamped between the wings and base of the mounting clamp.



6. Secure the screws.



IMPORTANT:

BE VERY CAREFUL WHEN TIGHTENING THE SCREW ON THE TRANSDUCER SIDE. EXCESSIVE PRESSING DOWN ON THE SCREW OR EXCESSIVE TORQUE FORCE WILL DAMAGE THE FORCE TRANSDUCER.

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