USER MANUAL, VOL.2.0

TISSUE BATH SYSTEM - 720MO

DMT

TISSUE BATH SYSTEM

MODEL 720MO

TRADEMARKS

CONTENTS

SAFETY

The Tissue Bath System - 720MO 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, or 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 system.
- 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 items that produce heat.
- Only use attachments and accessories specified by the manufacturer.
- Unplug this system 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
110–125 V	UL81 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.

NOTE:
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 61326-2-6:2005
	EN 61000-3-2.

Certified with the safety standards:

Directive 2006/95/EC:	EN 61010-1:2001
	EN 61010-1/Corr.1:2003
	EN 61010-1/Corr.1:2003
	EN 61010-2-101:2003

CERTIFICATE OF CONFORMITY

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

Tissue Bath System - Model 720MO

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

EN 61010-1:2001 EN 61010-1/Corr.1:2003 EN 61010-1/Corr.1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.
EN 61010-2-101:2003	Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
EN 61326-2-6:2005	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.
	With reference to regulations in the following directives: 2006/95/EC, 89/336/EEC

ABOUT THIS MANUAL

This manual contains a complete list of procedures that describe how to install, maintain and use the Tissue Bath System.

Chapter 1 provides an overview of the construction and basic features of the interface and the Tissue Bath unit.

Chapter 2 describes step-by-step instructions to set up a complete Tissue Bath System, including accessories.

Chapter 3 is a complete manual for the 720MO interface. This chapter describes, in detail, how to navigate the menus and how to use the special features of the Tissue Bath System - 720MO.

Chapter 4 contains procedures describing general and daily maintenance of the Tissue Bath unit; e.g. adjustment of supports, weight calibration of the force transducers and cleaning instructions.

Appendix contain system specifications.

UNPACKING THE TISSUE BATH SYSTEM

Take a few minutes to carefully inspect your new Tissue Bath System for damage which may have occurred during handling and shipping. If you suspect any kind of 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 possible claim has been settled.

We recommend that you store the packing material for any possible future transport of the Tissue Bath System. In case you need to transport and the original packing material is unavailable, please contact the DMT Sales Department for advice and packing instructions.

After unpacking your new Tissue Bath System, use the following list to check that the system is complete:

- 1 interface unit
- 4 chambers with mounted pin supports (200 µm)
- 4 chamber covers
- 1 external temperature probe
- 1 power cord*
- 1 calibration kit (including “bridge”, “balance” and 2 gram weight)
- 4 plastic funnels
- 1 tube of high vacuum grease
- 1 tube of grease for linear slides
- 2 Allen keys
- 1 small screwdriver
- 1 CD with user manuals for Tissue Bath Systems
- 1 CD with the manual “Procedures for investigation of small vessels using small vessel myograph”, by Professor M. J. Mulvany, Department of Pharmacology, Aarhus University, Denmark and the video “Dissection and mounting of small vessels in wire myographs”

** The shape of the AC plug varies by country; be sure that the plug fits the outlets for your location.*

CHAPTER 1 - SYSTEM OVERVIEW

1.1 Interface front panel

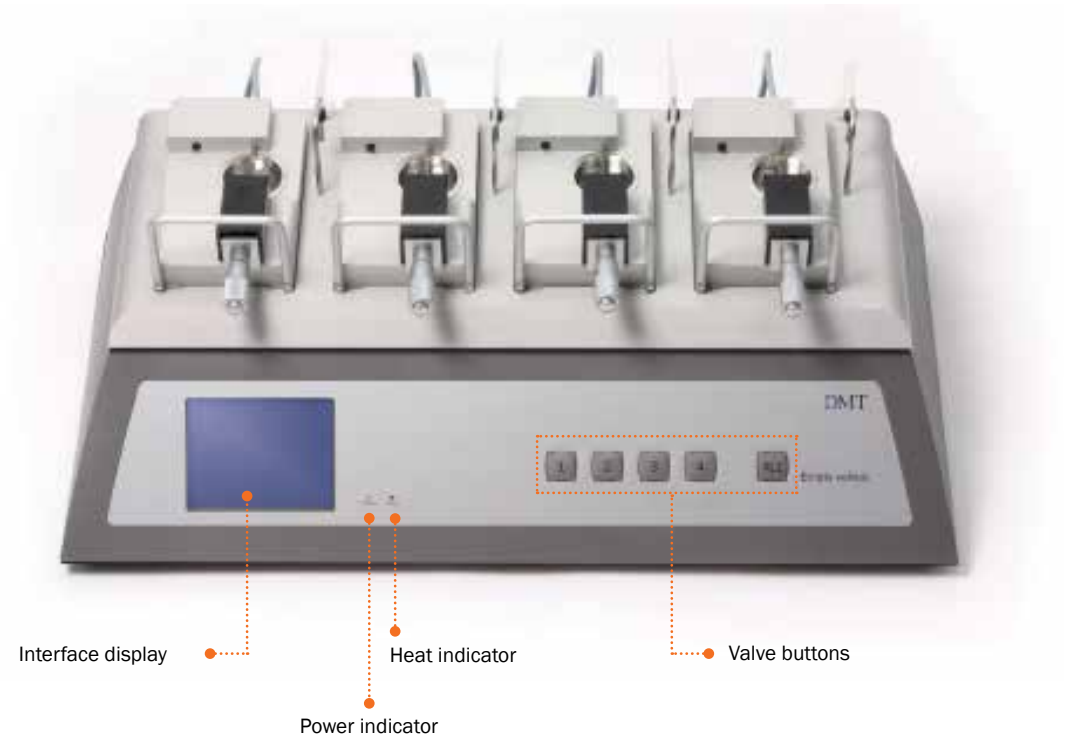


Figure 1.1 *Interface front panel with 720MO chambers*

1.2 Interface rear panel

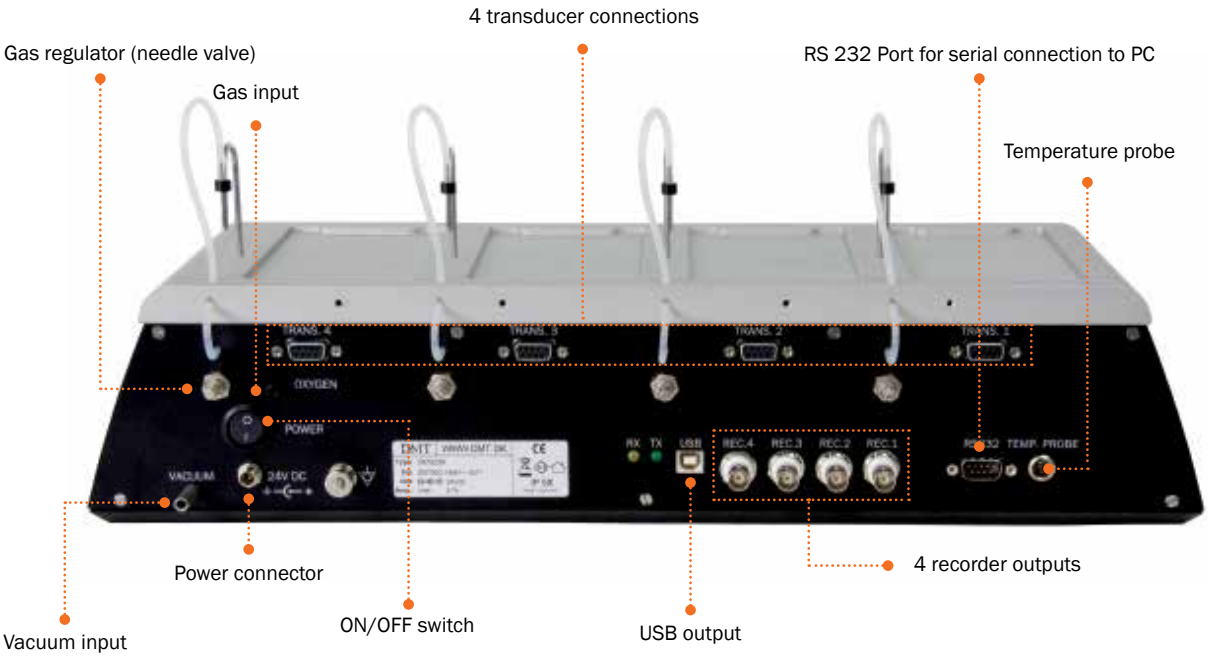


Figure 1.2 *Interface rear panel*

1.3 The Tissue Bath unit

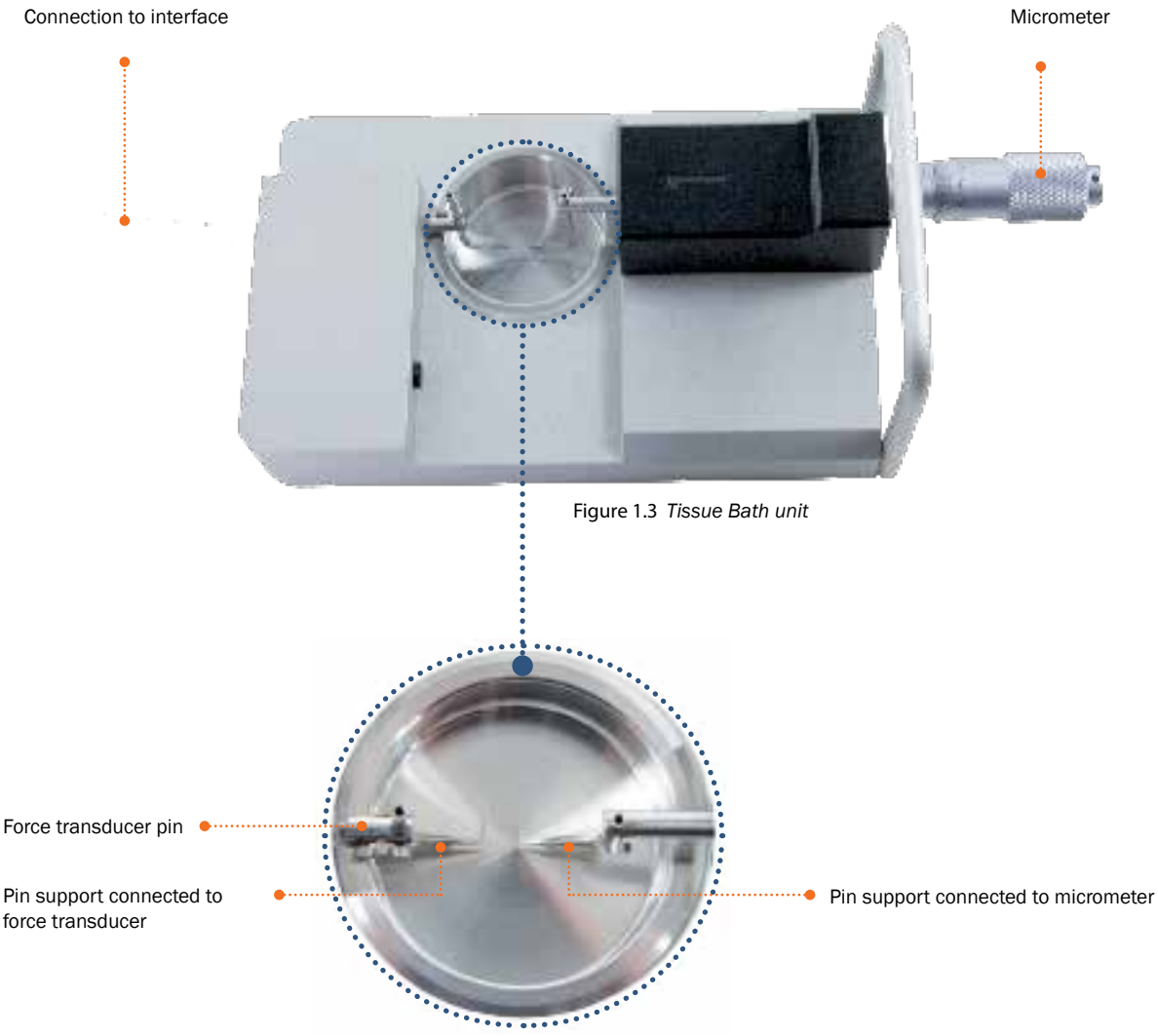


Figure 1.3 *Tissue Bath unit*

Figure 1.4 *Close up of Tissue Bath unit*

CHAPTER 2 - SETTING-UP

2.1 The complete Tissue Bath System - 720MO

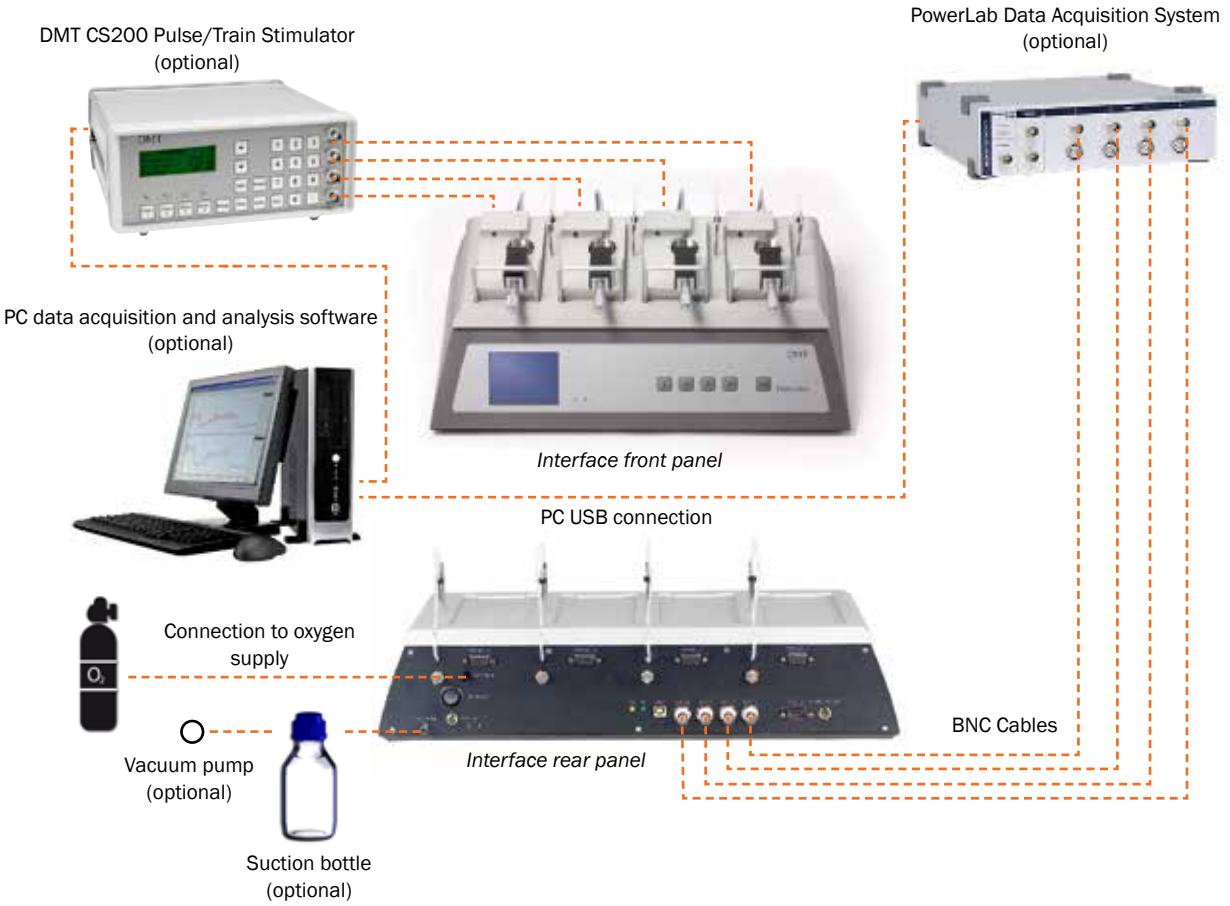


Figure 2.1 *The complete Tissue Bath System - 720MO*

2.2 Setting up step-by-step

This chapter contains a complete step-by-step description of how to set up a Tissue Bath System - 720MO as illustrated in figure 2.1 above.

1. Interface – PC connection
   Data acquisition is possible either by connecting the Interface directly to a PC or through a PowerLab data acquisition and analysis system (optional).

   I. Direct PC connection
   Connect the Interface to one of the COM-ports on the PC using a serial cable (cable not included).

   II. PowerLab (optional)
   Connect the Interface to the PowerLab unit using BNC cables. Connect Rec 1 on the Interface to Input 1 on the PowerLab, 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 on the back of the Interface. Oxygen is supplied to the chambers by tubing attached to the black stainless steel oxygen pipe. The oxygen and vacuum tubing need to be inserted into the chamber in order to aerate the heated buffer and emptying the chambers respectively. 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. Each regulator has a lock device attached that can be used when the desired bubbling is achieved (see “Figure 2.2 Oxygen supply and suction connection” on page 13).

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

3. Vacuum connection
   The system has a built-in drainage system with separate valves that allows each chamber to be emptied individually. After connecting the vacuum source to the vacuum pipe at the back of the Interface, the vacuum pipes need to be inserted into the chambers in order 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 (as illustrated in "Figure 2.2 below). A chamber can then be emptied by pressing the corresponding numbered button. Pressing the "all" button will empty all the chambers at the same time (see "valve buttons" in "Figure 1.1 Interface front panel with 720MO chambers" on page 10).

   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 SOLUTION 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 constantly. Holes in the chamber covers serve different purposes, and they are illustrated in Figure 2.3 below. The slots allow the covers to be placed over the chamber around the support arms and gas/vacuum tubes.

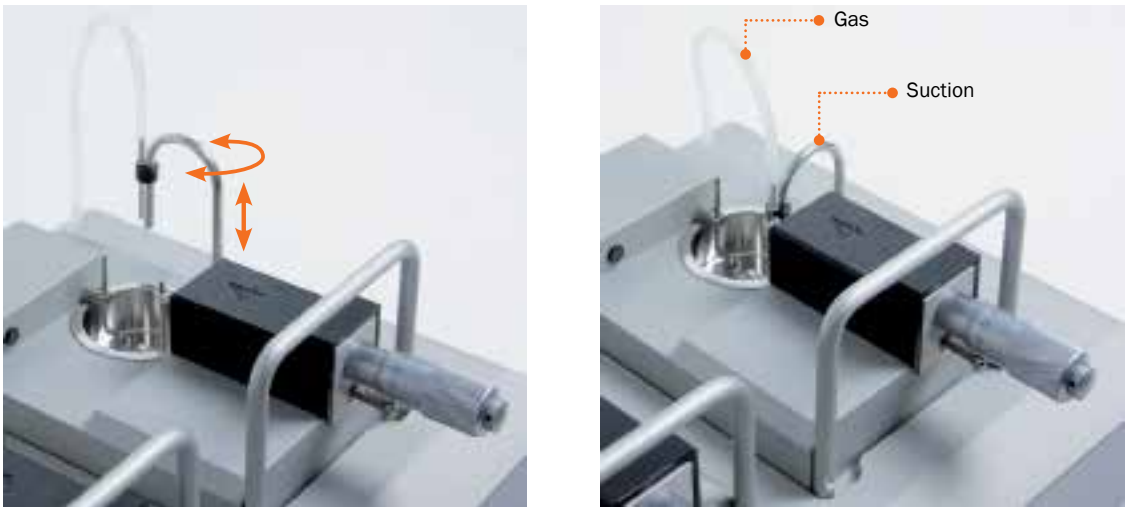


Figure 2.2 *Oxygen supply and suction connection*

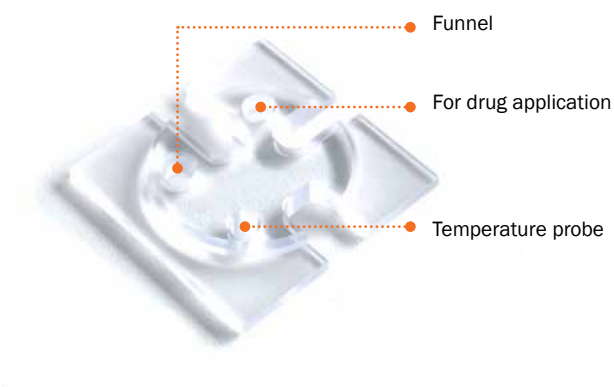


Figure 2.3 *Chamber cover*

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

2.3 The first weight calibration

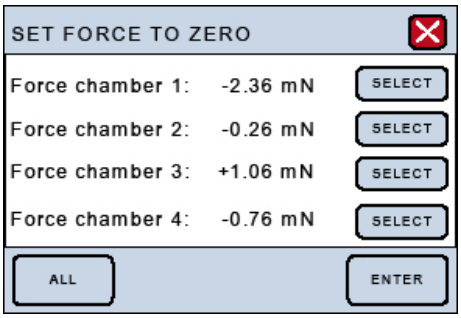
Prior to shipment the Tissue Bath System - 720MO has gone through two days of continuous testing, including a final force transducer calibration. However, DMT recommends that a new force transducer calibration is performed before using the Tissue Bath System for the first time. The force transducer calibration procedure is described in detail in the FORCE CALIBRATION sub-menu under SETTINGS, as explained in Chapter 3, on the next page.

CHAPTER 3 - THE INTERFACE MENUS

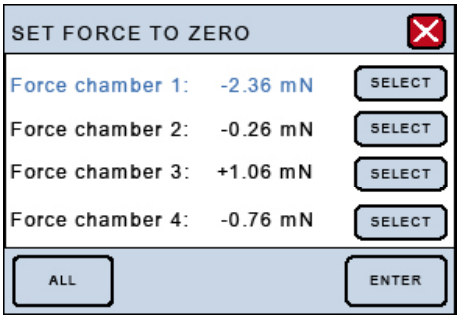
Chapter 3 is a complete manual for the 720MO interface. This chapter contains a detailed description of how to navigate the touch-screen menus and how to use the special features of the 720MO.

3.1 Interface menus – Navigation

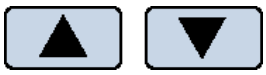
Menus on the 720MO interface are all accessible by a touch screen. To access a menu, simply touch the screen to access a menu. When a setting needs to be changed, the setting can be changed by pressing the "SELECT" icon on the touch screen corresponding to the desired channel to be changed.



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.

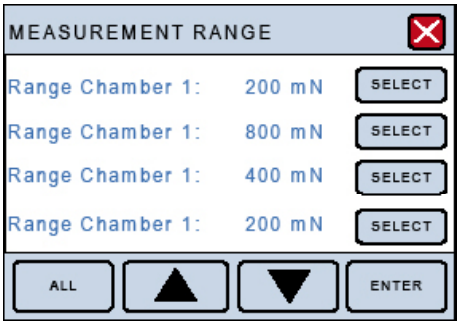


Changing the numeric value for the chosen parameter can be done by touching the up or down arrow keys.



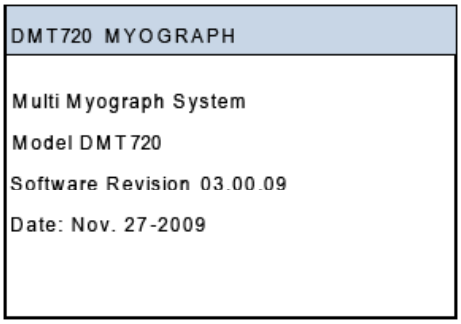
Once the desired setting has been chosen, pressing "ENTER" will lock the selection and be stored in memory.

Pressing the white "X" in the red box will exit that menu and take you automatically to the Actual Force Display.

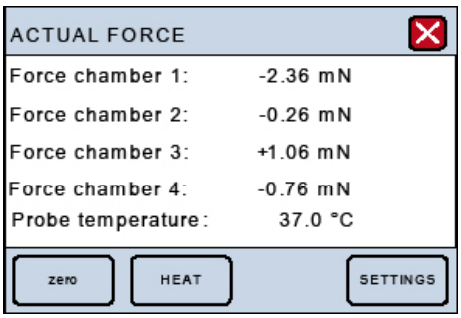


3.1.1. Power-up screen

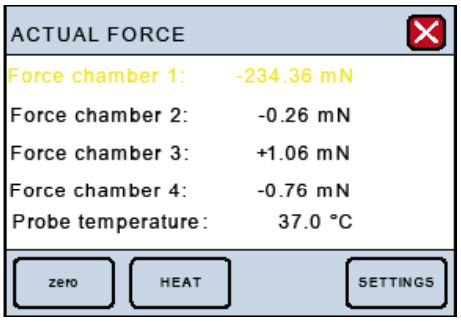
After turning on the 720MO interface, an "Introduction" screen appears. The system is auto-calibrating the A/D converters while this screen is displayed.



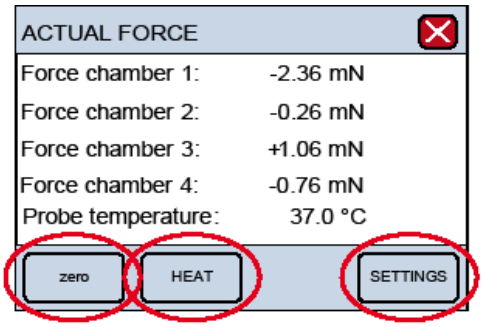
After a few seconds, the “ACTUAL FORCE” display will appear.



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.

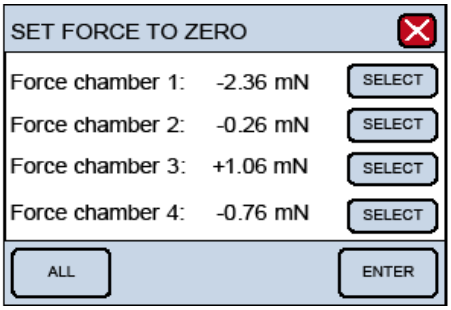


Three menus are accessible from the default “ACTUAL FORCE” screen or display. These menus are: Zero, Heat, and Settings.



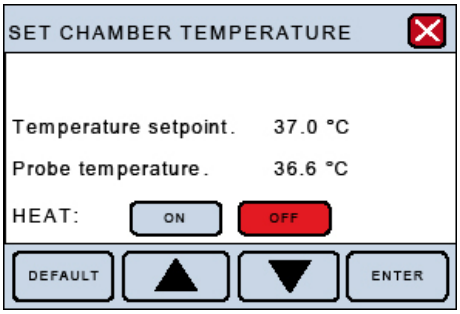
3.1.2 Zero menu

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.



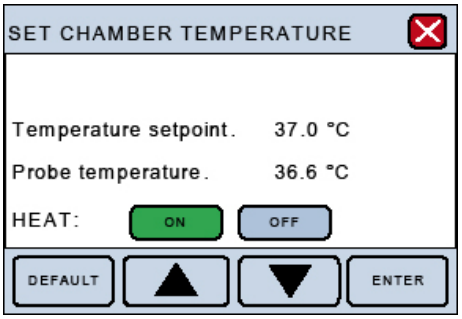
3.1.3 Heat menu

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. The system will heat to the designated temperature setpoint. A little red light to the right of the touch screen will turn on when the heat is turned on.

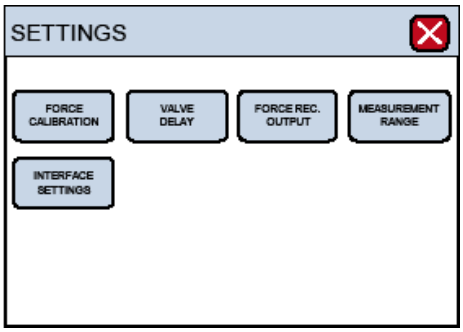
Pressing the white “X” in the red box will send the user back to the “ACTUAL FORCE” display.



3.1.4 Settings menu

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

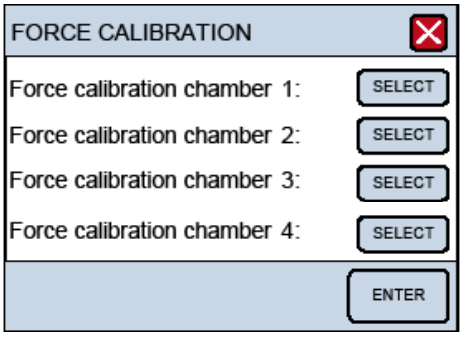
1. FORCE CALIBRATION
2. VALVE DELAY
3. FORCE REC. OUTPUT
4. MEASUREMENT RANGE
5. INTERFACE SETTINGS



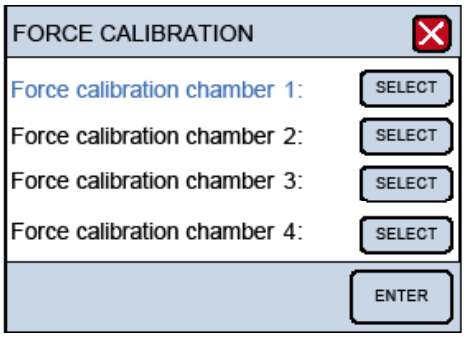
1. FORCE CALIBRATION

NOTE: EVERYTIME A FORCE CALIBRATION IS PERFORMED THE MEASUREMENT RANGE IS SET TO DEFAULT 200 mN. SET MEASUREMENT RANGE AFTER THE 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.

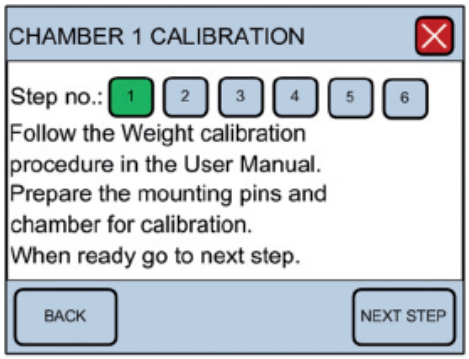


To begin the calibration, press “SELECT” for the chamber you wish to calibrate. The text for the chamber to be calibrated will turn blue. Pressing “ENTER” will start the 6-step procedure for calibrating the force transducer on the desired chamber.

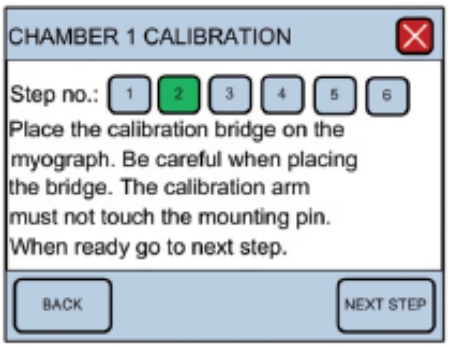


The calibration procedure is listed in 6 individual steps and needs to be performed for each channel or transducer when calibrating the system.

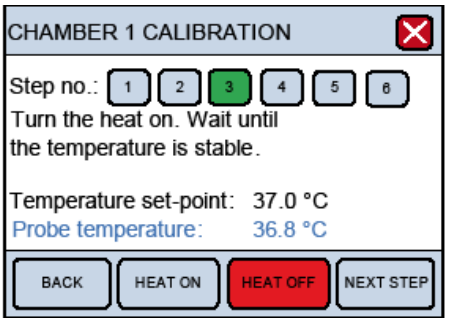
Step 1 involves setting up the chamber for calibration. Make sure the chamber contains the tissue holding pin. Fill the chamber with double-distilled water. Press “NEXT STEP”.



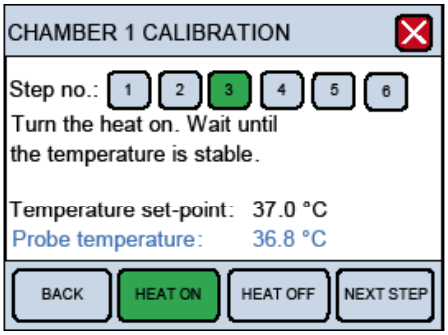
Step 2 involves setting up the calibration kit appropriately for the actual weight calibration. Verify that the tissue holding pin does not touch the calibration arm, as instructed. The calibration arm should be as close as possible to the tissue holding pin support without touching in order to get the most accurate calibration. Press “NEXT STEP” when the calibration kit is in position.



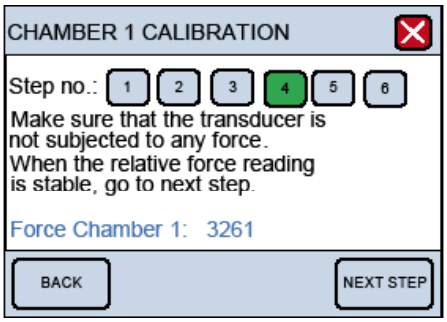
Step 3 initiates the heating process for the chambers. In order for the calibration to be accurate, the transducers must be heated to the intended experimental temperature to compensate for heat-induced expansion of parts in the transducer. Otherwise, inaccurate readings and transducer drift may occur.



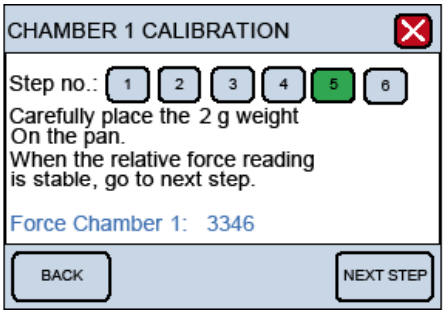
To start heating, press “HEAT ON”. Covering the chambers with the chamber covers will expedite the chamber heating. Place the temperature probe into the chamber for the first calibration to monitor when the chamber has reached the target temperature. Heating will take about 20 to 30 minutes for the chambers and transducers to come to 37 °C with the chamber covers in place. Once the chamber(s) are heated and have reached the target temperature, press “NEXT STEP”.



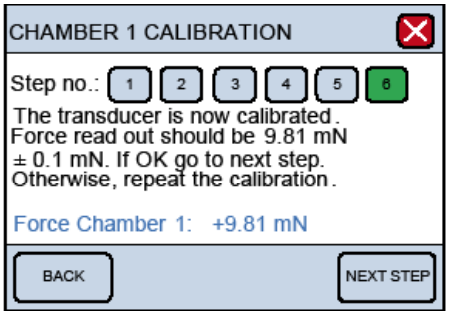
Step 4 is the first step in the actual weight calibration process. A 4-digit number will be displayed in blue at the bottom of the screen. If nothing has been disturbed during the heating process, the zero, 0 gram, or 0.00 mN calibration should be stable after 20-30 sec. indicated by the 4-digit number and “NEXT STEP” can be pressed at this time. Normally, the reading will be stable after 20-30 sec. If the 4-digit number is not stable, then wait until the number has stopped fluctuating before pressing “NEXT STEP”.



Step 5 is the 2 gram weight calibration. At this step, place the 2 gram weight in the pan closest to the transducer so as to simulate a vessel pulling on the tissue holding pin closest 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 9.81 mN of force. The weight placement should cause a positive increase in the 4-digit number. Wait at least 20-30 seconds for the applied force to stabilize. Normally, the reading will be stable after 20-30 sec. If the 4-digit number is not stable, then wait until the number has stopped fluctuating before pressing “NEXT STEP”.

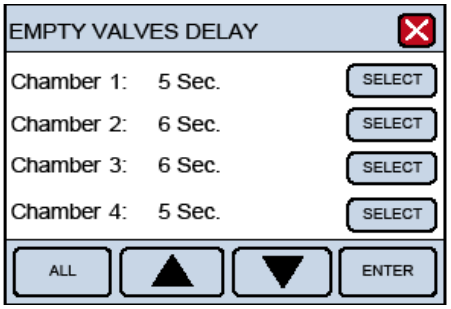


Step 6 is to verify that the calibration was performed correctly. The “Force Chamber 1” reading should be 9.81 ± 0.1 mN. If the “Force Chamber 1” reading is off by more than 0.1 mN, then remove the weight, press “BACK” to return to Step 4, and repeat the calibration process. If the “Force Chamber 1”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

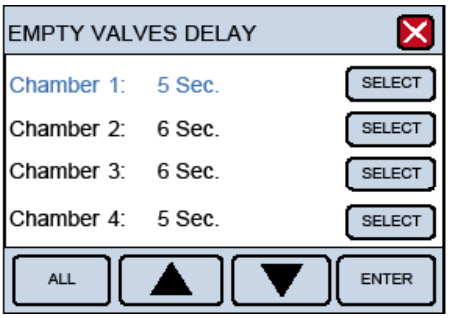


2. VALVE DELAY

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

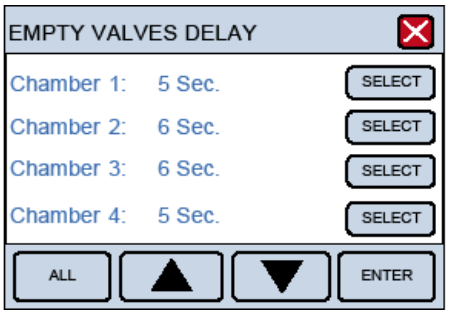


Pressing “SELECT” next to any given channel will cause the line selected to turn blue. The up and down arrow keys can then be used to modify the length of time the vacuum valves stay open after the valves have been activated with the “valve buttons” on the front of the interface.



Pressing “ALL” will cause all the lines to turn blue, meaning all chambers will 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 “ENTER” after modifying the value(s) for valve delay will lock in the settings. They will be retained in memory and used when the system is next turned on.

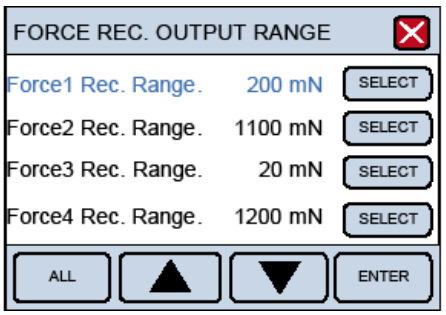


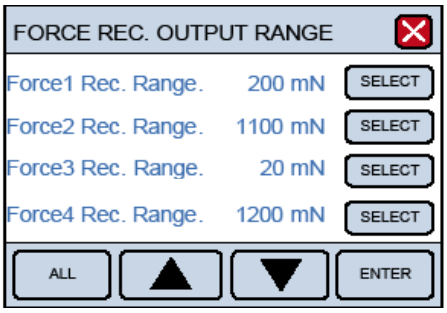
3. FORCE RECORDING OUTPUT

The FORCE RECORDING OUTPUT, or FORCE REC. OUT, sub-menu determines the upper limit for force sent from the BNC analogue outputs. This will only affect the data collected from the interface by a data acquisition system such as an AD Instruments PowerLab. The factory default setting for FORCE REC. OUT is 20 mN, meaning that if the force of the mounted vessel exceeds 20 mN, the force recorded by the data acquisition system will not record more than 20 mN and will appear as a flat-line trace at 20 mN, even though the force readings on the interface may exceed 20 mN. Therefore, change the FORCE REC. OUT settings to an appropriate setting to capture the maximum response from the vessel 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.

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

NOTE:
ANYTIME FORCE REC. OUT IS CHANGED, A NEW WEIGHT CALIBRATION ON THE TRANSDUCERS SHOULD BE PERFORMED, ENTERING THE NEW VOLTAGE VALUES INTO THE DATA ACQUISITION SYSTEM BEING USED.

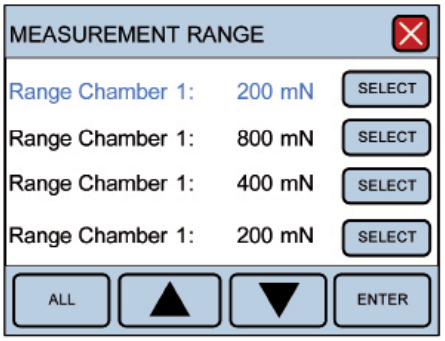


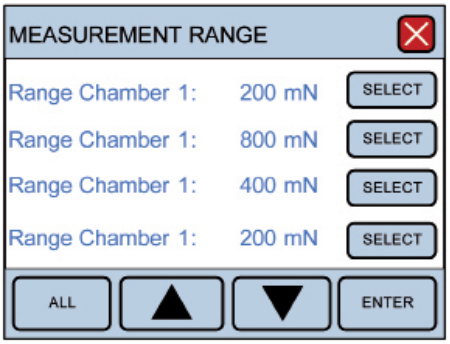


4. MEASUREMENT RANGE

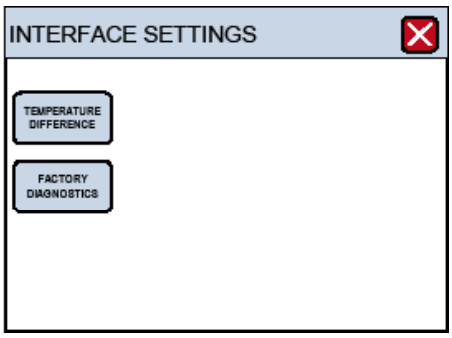
The MEASUREMENT RANGE sub-menu in SETTINGS determines the maximum force capacity of the transducer. The factory setting is 200 mN, but the transducer capacity can be changed to 400 mN, 800 mN or a maximum of 1600 mN of force detection, depending on the size of the vessel used.

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





5. INTERFACE SETTINGS

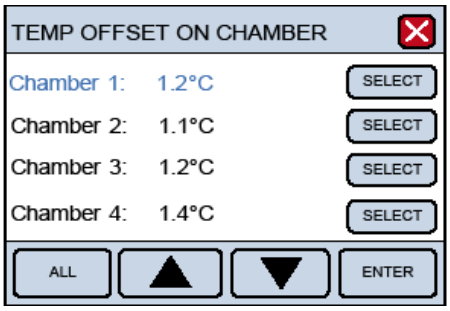


The INTERFACE SETTINGS sub-menu in SETTINGS has an additional 2 sub-menus. These 2 additional sub-menus are:

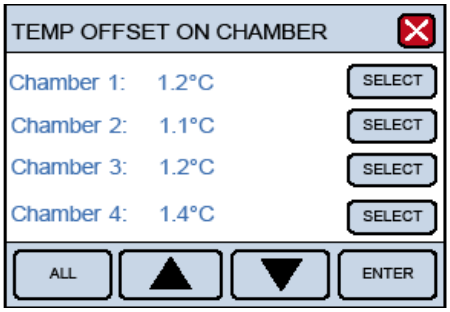
I. TEMPERATURE DIFFERENCE
II. FACTORY DIAGNOSTICS

I. TEMPERATURE DIFFERENCE

The TEMPERATURE DIFFERENCE function allows the user to finetune the temperature setpoint of the system. Although the temperature setpoint for the system can be set in the **HEAT MENU**, the actual temperature for the system may not heat to the exact defined setpoint. The exact temperature in the bath can be measured using the external temperature probe. 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).

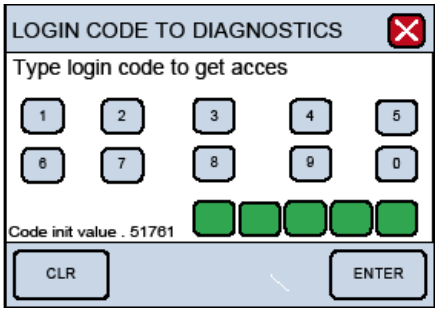


The "SELECT" and "ALL" functions are the same in this menu as previously described for the "VALVE DELAY" menu. Pressing "ENTER" will store the numbers in memory for future experiments.



II. 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.



CHAPTER 4 - THE TISSUE BATH UNIT

This chapter contains a complete explanation of how to adjust, calibrate and maintain the Tissue Bath System.

4.1 Changing and adjusting the mounting supports

Each chamber can accommodate mounting supports for larger segments (>500 µm). Because the mounting supports can be changed easily, experiments can be performed with different vessels of varying internal diameter from 500 µm up to 10 mm. Continuous use and repeated greasing of the transducer arm holes will cause some misalignment of the mounting supports. The mounting supports will need occasional adjustments.

Changing and adjustment of the supports is performed using the following step-by-step procedure.

NOTE:
THE TRANSDUCERS ARE FRAGILE AND SENSITIVE TO MECHANICAL STRAIN. BE VERY CAREFUL WHEN CHANGING OR ADJUSTING THE MOUNTING SUPPORTS!

Changing the supports (Figure 4.1):

1. Use the micrometer to separate the supports as far apart as possible.
2. Use the small screwdriver provided to gently loosen screw D on the support attached on the transducer side. Screw D is the screw on the support closest to the transducer.
3. Gently pull the support away from the transducer pin.
4. Loosen screw B on the micrometer side with the appropriate Allen key.
5. Pull the pin support away. NOTE: number the supports with the number of the chamber they were removed from using some kind of permanent marker. Store the supports in the provided plastic case. Numbering the supports will save time when the supports are changed again, limiting the amount of adjustments needed after each change.

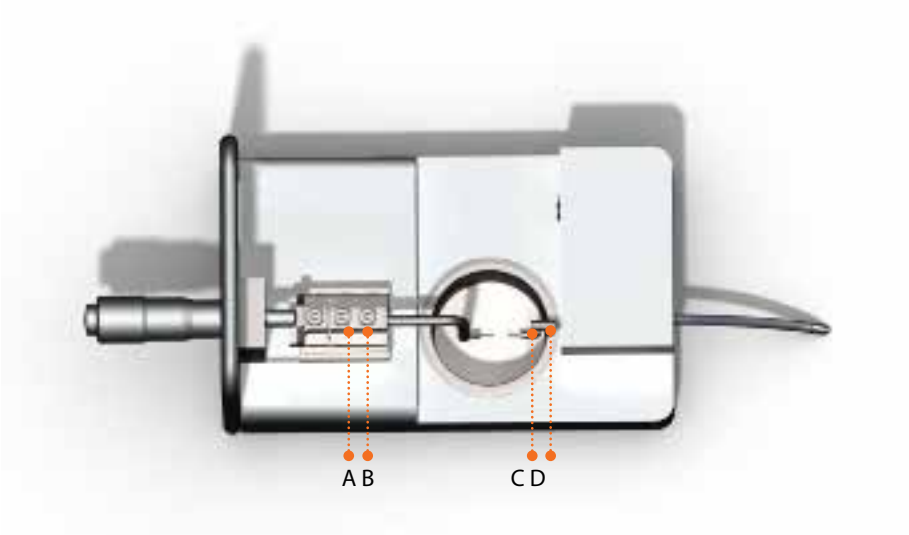


Figure 4.1 *Tissue Bath chamber - screws for changing supports and adjustment of the pins*

Fine-adjusting the pins for larger vessels (Figure 4.2 and 4.3):

1. Loosen screw A to move the micrometer-side arm holder sideways
2. Loosen screw B to move the micrometer-side pin toward or away from the transducer.
3. Loosen screw C to align the transducer-side tissue holding pin horizontally.
4. Loosen screws D and E to align the heights of the tissue holding pins vertically.

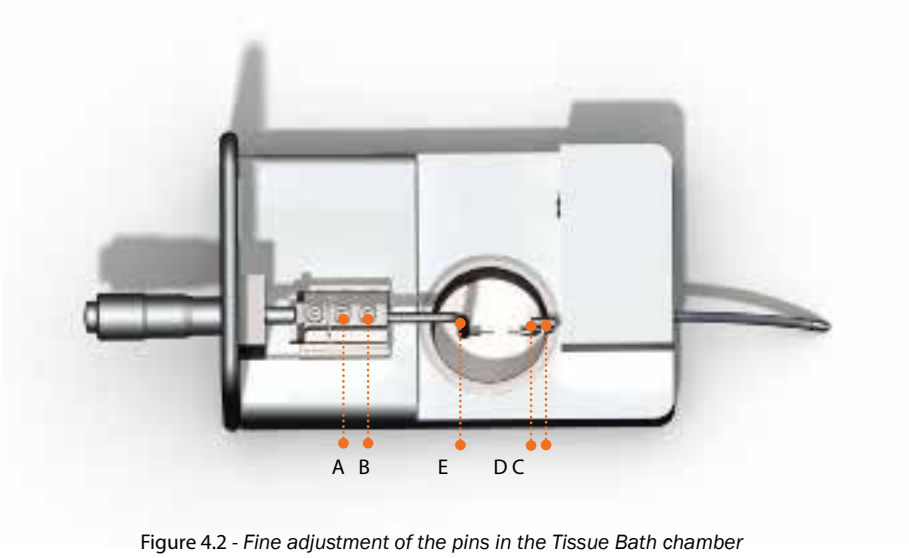


Figure 4.2 - *Fine adjustment of the pins in the Tissue Bath chamber*

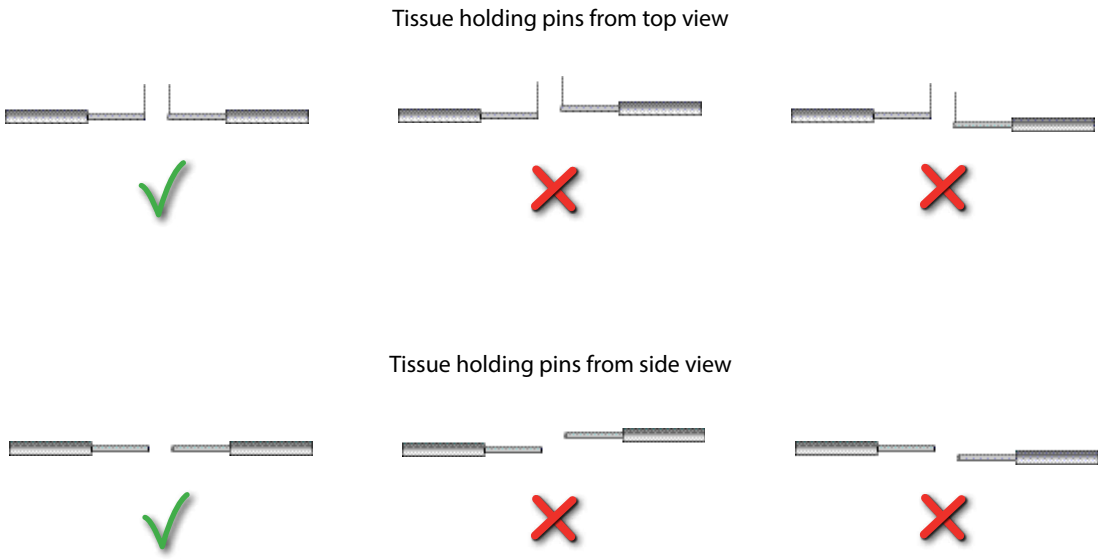


Figure 4.3 - *Illustrations of properly aligned tissue holding pins (depicted on the far left) and incorrectly aligned pins (depicted in the middle and far right).*

4.2 Calibration of the force transducer

As a part of the general maintenance of the Tissue Bath System, DMT recommends that the Tissue Bath System is weight-calibrated at least once a month. The Tissue Bath System 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 Tissue Bath System 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 in “3.1.4 Settings menu” on page 16.

Force transducer calibration procedure (Figure 4.4)

NOTE: EVERYTIME A FORCE CALIBRATION IS PERFORMED THE MEASUREMENT RANGE IS SET TO DEFAULT 200 mN. SET MEASUREMENT RANGE AFTER THE FORCE CALIBRATION.

This section contains step-by-step instructions to calibrate the force transducer and should be used in conjunction with the steps described in FORCE CALIBRATION sub-menu in “3.1.4 Settings menu” on page 16.

1. Move the tissue holding pins 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 Tissue Bath chambers as illustrated in Figure 4.4 below. Also place the weight on one of the chambers. Turn the heat on as discussed in “3.1.3 Heat menu” on page 15. The system takes about 20 to 30 minutes to reach 37 °C. 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. There is no need to bubble the chambers while waiting for the system to heat up.

3. When the system reaches target temperature, adjust the calibration kit so that the tip of the calibration arm is as close to the tissue holding pin on the transducer side as possible without touching, as illustrated in Figure 4.4 below. One way to do this is to use the following technique. Start with the calibration kit in place so that the tip of the calibration arm is not touching any part of the tissue holding pins. 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 micrometer so that the calibration arm rests on the tissue holding pin, creating a force reading on that channel. Carefully slide the calibration kit back toward the transducer slowly until the force reads zero. At this point, as soon as the force reads zero, the calibration arm will be properly placed for weight calibration.

4. 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, which is described in detail in FORCE CALIBRATION sub-menu in “3.1.4 Settings menu” on page 16.

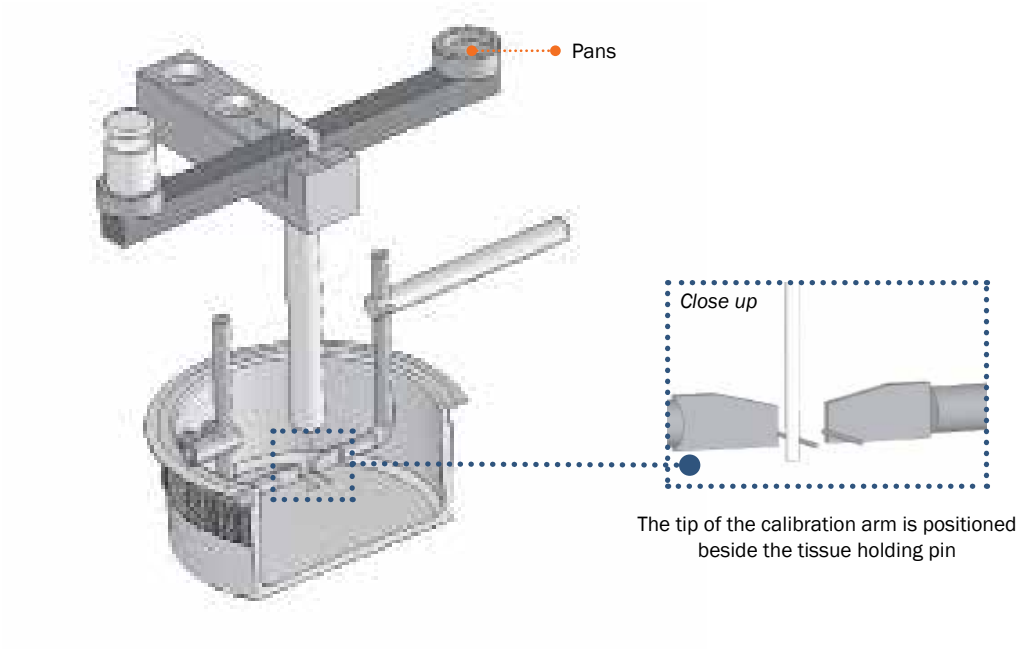


Figure 4.4 *Weight calibration kit shown in place on the chamber*

4.3 Checking the force transducer

The Tissue Bath force transducer is a strain gauge connected to a Wheatstone bridge. The force transducers for each chamber are housed in a separate, protective compartment (Figure 4.5 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.

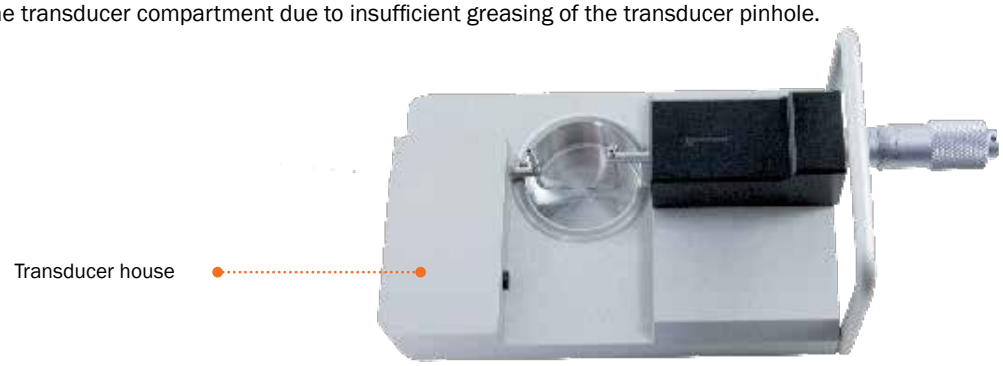


Figure 4.5 *Illustration of the proper transducer house*

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” on page 14 and “4.2 Calibration of the force transducer” on page 23.

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). The normal operating values for the force transducer during calibration should be between 3000 and 3500.

- If the value is 0, a single digit, or a three digit number, the force transducer is broken and needs to be replaced.
- If the value is less than 2000 or greater than 4500, the force transducer has been broken and needs to be shipped to DMT for further test and a temperature compensation.
- 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, 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, the force transducer is broken and needs to be replaced.

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

4.4 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 from the transducer pin coming out of the transducer house.
2. Disconnect the Tissue Bath chamber from the interface.
3. Turn the Tissue Bath chamber upside down and remove the transducer housing by loosening the two screws (A+B) as illustrated in “Figure 4.6 - The 2 screws that secure the transducer house to the chamber” on next page.
4. The replacement transducer will be shipped with the new transducer inside a new transducer house.
5. Place a small amount of vacuum grease (clear or whitish grease) around the bottom of the transducer housing to seal the transducer housing when put back in place, see ”Figure 4.7 - The transducer in the transducer housing and close-up of transducer pin inside the transducer” on the next page.
6. Carefully realign the transducer housing with the new transducer on the Tissue Bath chamber and reinsert the Allen screws through the bottom of the Tissue Bath chamber.
7. Tighten the screws and place some vacuum grease around the transducer pin that protrudes from the transducer housing (see “Figure 4.8 - Close-up of transducer pin from outside” on the next page). 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.

Figure 4.6 *The two screws that secure the transducer house to the chamber*

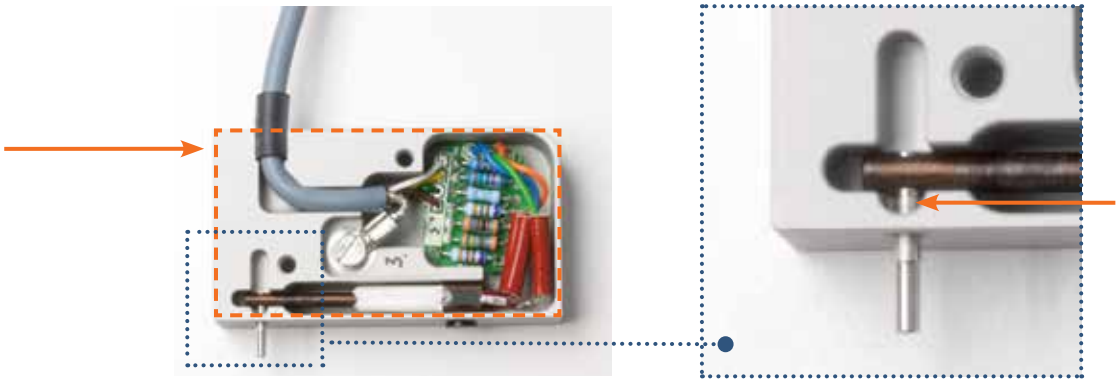


Figure 4.7 *Inside the transducer housing and close-up of transducer pin.*

The orange arrows and the dashed frame indicate the place that the vacuum grease needs to be applied to prevent water and buffer from damaging the transducer.

Figure 4.8 *Close-up of transducer pin from outside.*

The arrow indicate the place that the grease needs to be applied to prevent water and buffer from damaging the transducer.

IMPORTANT NOTE:
CALIBRATE THE NEW FORCE TRANSDUCER BEFORE PERFORMING A NEW EXPERIMENT, AS DESCRIBED IN "CHAPTER 3 - THE INTERFACE MENUS" ON PAGE 14 AND "4.2 CALIBRATION OF THE FORCE TRANSDUCER" ON PAGE 23.

4.5 Tissue Bath System - Maintenance

The Tissue Bath System - 720MO 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.

Tissue Bath chamber tubing

To prevent the tubing from becoming blocked with buffer salt deposits after an experiment, remove the chamber cover from the Tissue Bath chamber and turn on the vacuum and press the vacuum valve for about 10 seconds by holding down the valve button(s). 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 Tissue 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 NOTES:

DMT RECOMMENDS THAT THE HIGH VACUUM GREASE SEALING THE TRANSDUCER PINHOLE IS CHECKED AND SEALED AT LEAST ONCE A WEEK, ESPECIALLY IF THE TISSUE BATH IS USED FREQUENTLY.

DMT TAKES NO RESPONSIBILITY FOR THE USE OF ANY OTHER KINDS OF HIGH VACUUM GREASE OTHER THAN THE ONE AVAILABLE FROM DMT.

DMT TAKES NO RESPONSIBILITY FOR ANY KIND OF DAMAGE TO THE FORCE TRANSDUCERS.

Linear slides

Check the linear slides (under the black covers) for grease at least once a week. In case of insufficient lubrication, grease the slides with the “Grease for Linear Slides” included with your system. See figure 4.9 below.

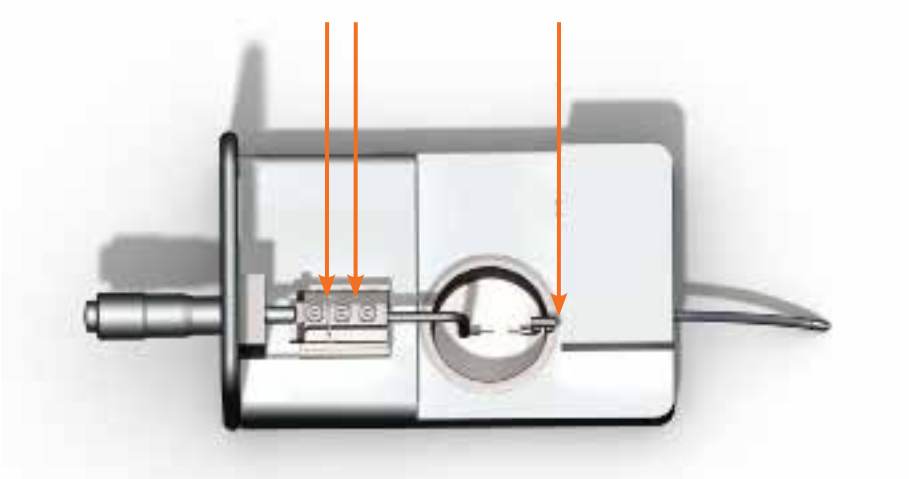


Figure 4.9 *The areas where linear slide grease may be applied for smooth micropositioner movement*

Cleaning the Tissue Bath

DMT strongly recommends that the Tissue Bath chambers and surrounding areas are cleaned after each experiment.

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

1. Fill the Tissue 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.

2. Remove the acetic acid and wash the Tissue Bath chamber and supports several times with double distilled water.

3. If any kind of hydrophobic reagents have 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 Tissue Bath chamber and supports with 1M HCl for up to 1 hour. In exceptional cases, incubate the chamber and supports with no stronger than a 3M HNO_3 solution for about 15 minutes.

5. Wash the Tissue 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 pins.

IMPORTANT NOTES:

BE VERY CAREFUL USING HCL OR HNO_3 BECAUSE THESE ACIDS MAY CAUSE EXTREME DAMAGE TO THE STAINLESS STEEL CHAMBERS AND SUPPORTS. 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 TISSUE 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:

7. Incubate the Tissue Bath chamber and supports for 30 minutes with 2mM T-1210 Tetrakis- (2-pyridylmethyl)-ethylenediamine solution dissolved in double distilled water.

8. Use a cotton-tip applicator to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.

9. Wash the Tissue Bath chamber and supports several times with double distilled water.

10. Incubate the Tissue Bath chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a cotton-tip applicator.

11. Remove the ethanol solution and wash a few times with double distilled water. Incubate the Tissue Bath chamber and supports with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.

12. Wash the Tissue Bath chamber and supports several times with double distilled water.

13. Dry the surfaces using absorbent paper or cotton-tip applicators.

IMPORTANT NOTES:

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

APPENDIX 1 - SYSTEM SPECIFICATIONS

Technical specifications

Tissue size:	500 µm up to 10 mm as rings
Chamber:	Four individual chambers
Chamber material:	Acid-resistant stainless steel
Chamber volume:	Max. 8 ml
Chamber suction:	Manual or automatic, time controlled, user defined
Chamber cover:	Supplied with connections for gassing
Chamber gassing:	Individually controlled per chamber by needle valves
Force range:	User selectable at ± 200/400/800/1600 mN
Force resolution:	0.1 mN
Micropositioners:	Manually operated
Weight calibration:	Semi-automatic
Heating:	Built into chamber, independent of superfusion
Temp. range:	Ambient temp. - 45 °C
Temp. resolution:	0.1 °C
Temp. probe:	External
Output reading:	Force (mN)
Analogue output:	Independently filtered 4-channel output at 2.5V full scale
Voltage:	100 to 240 VAC (auto) 50/60 Hz via external power supply
Ambient temp.:	15-30 °C

Optional accessories

Automatic Buffer Filler System - 625FS
Chamber cover for field stimulation
Combined pulse & train generator - CS200

NOTES