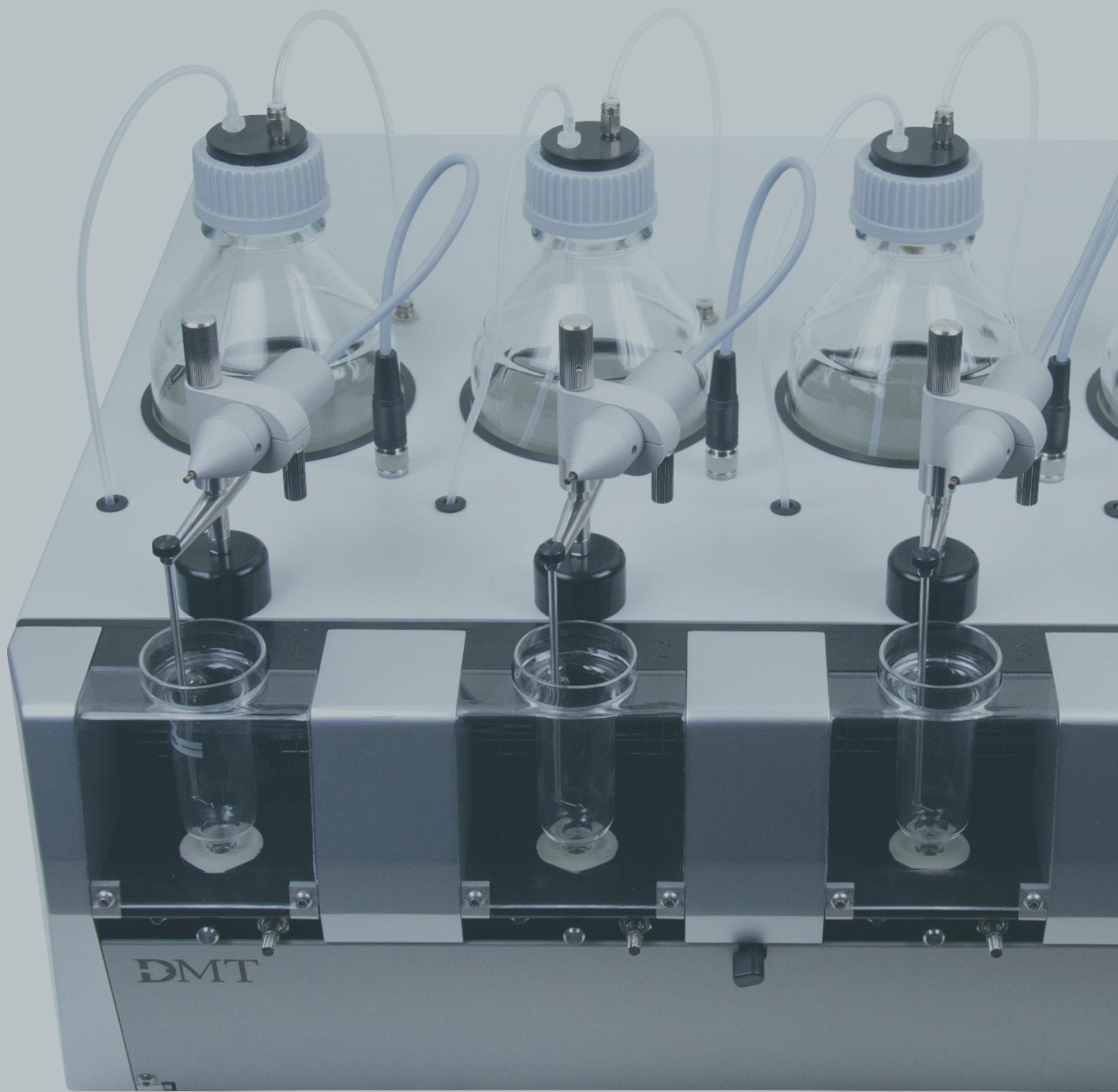


TRADEMARKS

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INTRODUCTION

The vertical Tissue organ bath system (TOBS) with its double walled glass chamber is a traditional experimental setup equipped for perfused tissues such as cardiac, skeletal and smooth muscle that has been used extensively for research purposes. The system can be used to investigate the physiological and pharmacological responses of perfused tissue such as smooth muscle (vascular and non-vascular) and other tissue preparations, through measurement of contractile forces, both isotonic and isometric whilst bathed in an appropriate physiological solution or buffer at 37°C. The main advantage of this study is the use of perfused tissue (in-vitro) which can be subjected to pharmacological agents whilst excluding the influence of systemic processes (in-vivo) that occur in intact animals. The results of these organ bath systems are generally more consistent, reproducible and lend themselves for screening and the measurement of dose response or concentration response curves.

The primary application of the organ bath assay is in cardiovascular research, using isolated aorta rings, heart tissue (papillary muscle) or arteries. However, the organ bath system has its use in gastrointestinal and respiratory research as well. In studying gastro-intestinal effects, preparations of ileum and colon are often used, but gastric antral muscle and sphincter can also be studied. Respiratory effects can be studied in isolated tracheal rings, phrenic diaphragm preparations, pulmonary arterial smooth muscle and even lung parenchyma. Other smooth muscle preparations that have been used in the organ bath system are urinary bladder, penile (corpus cavernosum) muscle strips and prostate.

Because of these inherent qualities and the versatility in use, the basic organ bath design has seen little change over the last few decades. Nevertheless, there are some practical drawbacks, for e.g. occupying far too much bench space, using circulating water that results in temperature delays and gradients and is tough to clean. To remedy these limitations DMT has now redesigned the organ bath system for the new millennium using the latest developments in material, computer and tissue engineering. We have incorporated fast air heating in place of water heating, installed disposable or more economical reusable chambers, added semi-automated control of filling, emptying and gassing. All of this packaged in a single system that takes up just a quarter of the space of a traditional system and experimental time is far less tedious.

Moreover, two systems can be linked together (via USB connection) so 8 channels/chambers can easily be linked together. The convenient compact bench-top design of the DMT's organ bath system can be placed in any existing laboratory environment whilst gaining back valuable bench space.

SAFETY

DMT Tissue Organ Bath System - 750TOBS has been designed for use only in teaching and research applications. It is not intended for clinical or critical life-care use and should never be used for these purposes: nor for the prevention, diagnosis, curing, treatment, or alleviation of disease, injury, or handicap.

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

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

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

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

UNPACKING THE 750TOBS SYSTEM

Description: Tissue Organ Bath System 750TOBS

1 x 750TOBS system unit

4 x Chamber units (20 ml)

4 x Schott Duran bottles (1 litre)

4 x Triangle hooks, Mounting supports

1 x Tubing Kit

1 x Calibration Kit (TOBS) (including marked tissue bath (20 ml) and 5 gram weight)

1 x USB cable

1 x Power Supply

1 x Cable Air – 5m and clamps

2 x Fasteners – 7 mm

1 x Grease for needle valve/linear slides

1 x Manual on a USB stick

4 x Isometric Transducer

1 x 750TOBS Manual

1 x PC Acquisition Software

CHAPTER 1 - SYSTEM OVERVIEW

On unpacking the 750TOBS system:

1. The Schott Duran 1 litre glass bottles, power supply and data acquisition USB cable are packaged separately and need to be connected into the 750TOBS as a complete working instrument. Connect the power supply and place the 1 litre bottles in the 750TOBS unit.
2. The 750TOBS system is present as a single unit as depicted in *figure 1* and the rear panel and tissue chamber as depicted in *figure 2* respectively
3. The 750TOBS system has the Tissue chambers in place along with their respective mounted force transducer stands.

1.1 FRONT PANEL - 750TOBS

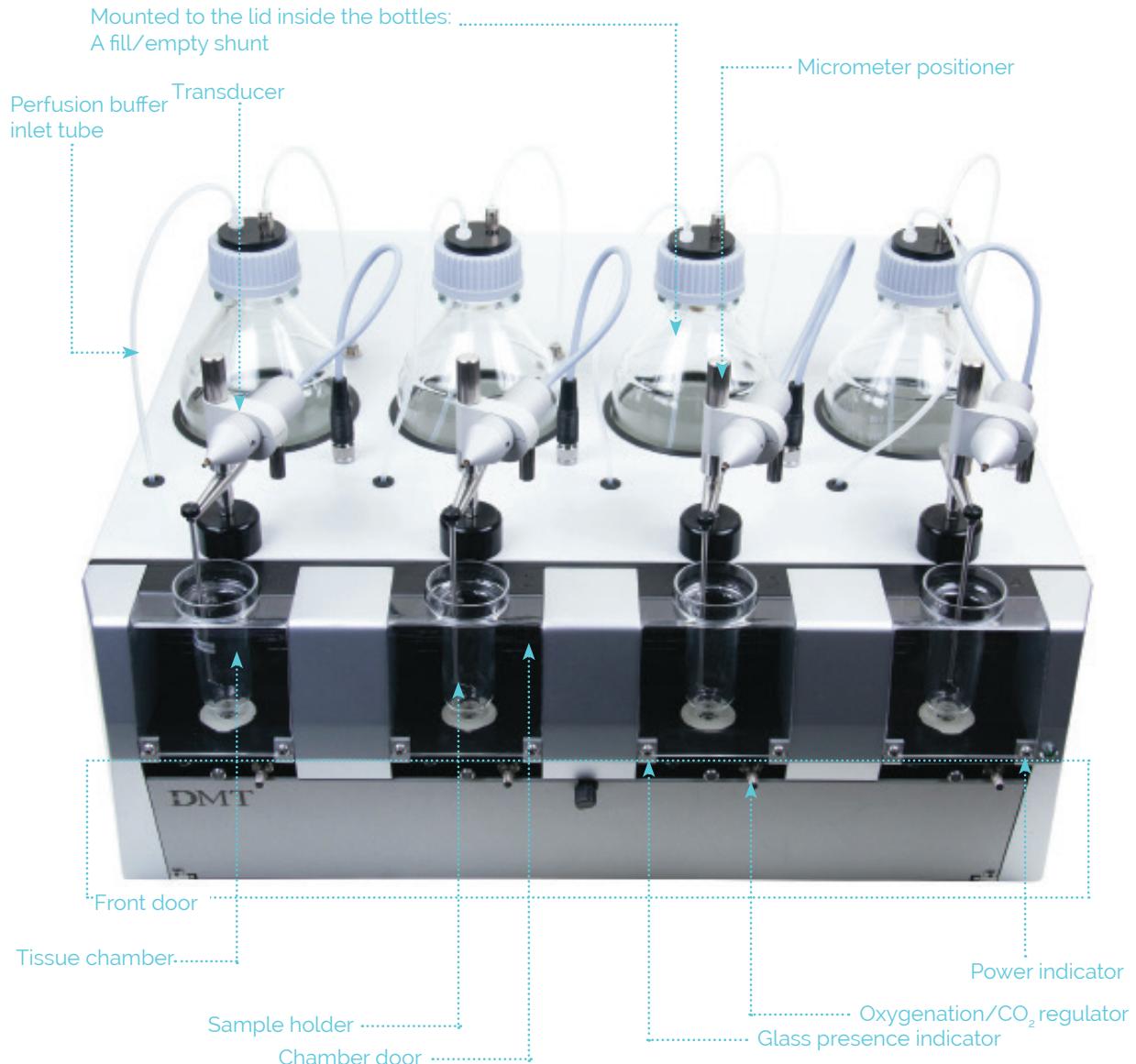


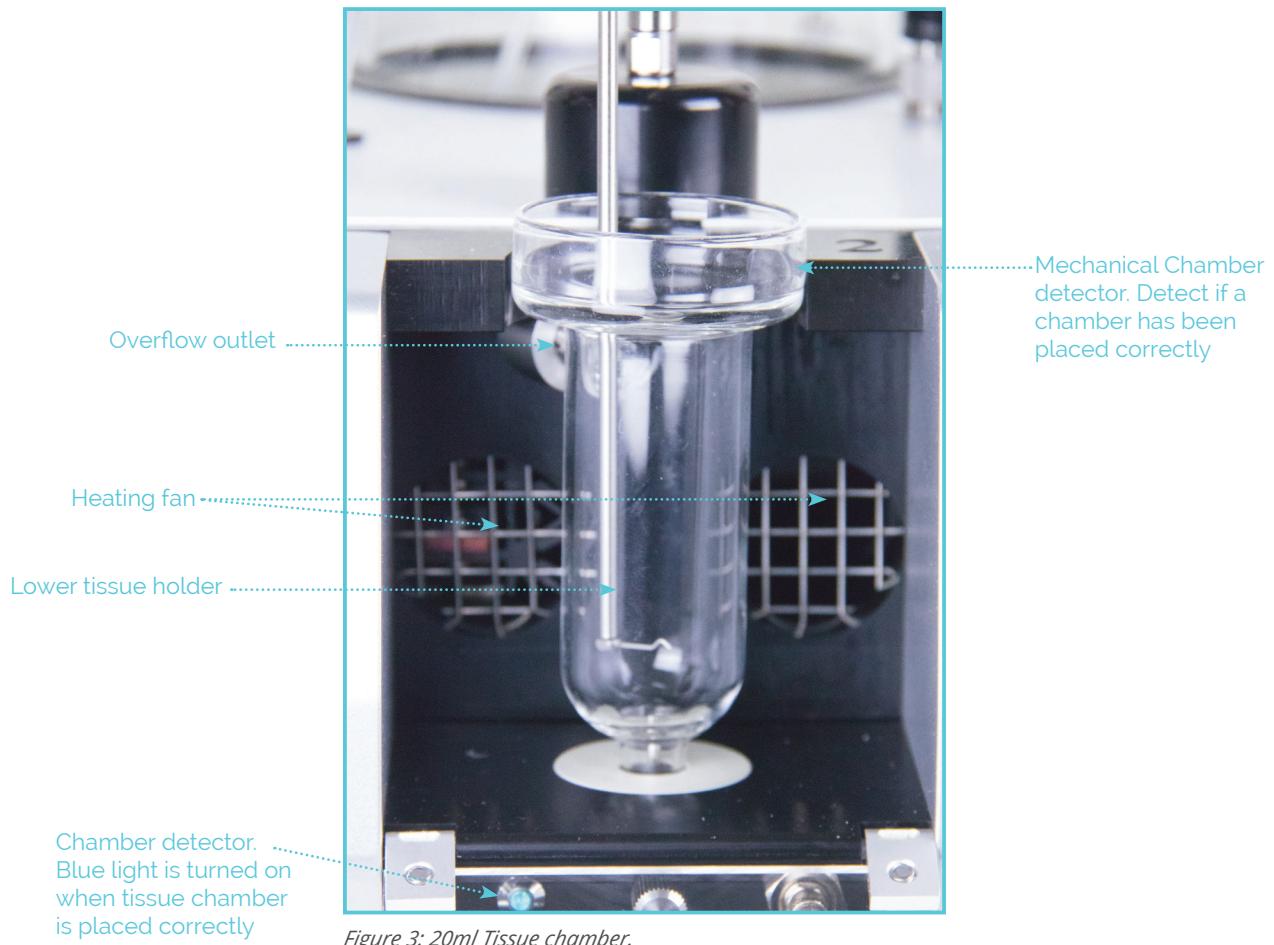
Figure 1: The 750TOBS system

1.2 REAR PANEL - 750TOBS



Figure 2: The rear panel of the 750TOBS system

1.3 CHAMBER - 750TOBS



NOTE: The Chamber Detector registers when the tissue chamber is in place. If the chamber is placed correctly the blue indicator lamp will be lit and the tissue organ bath will work.

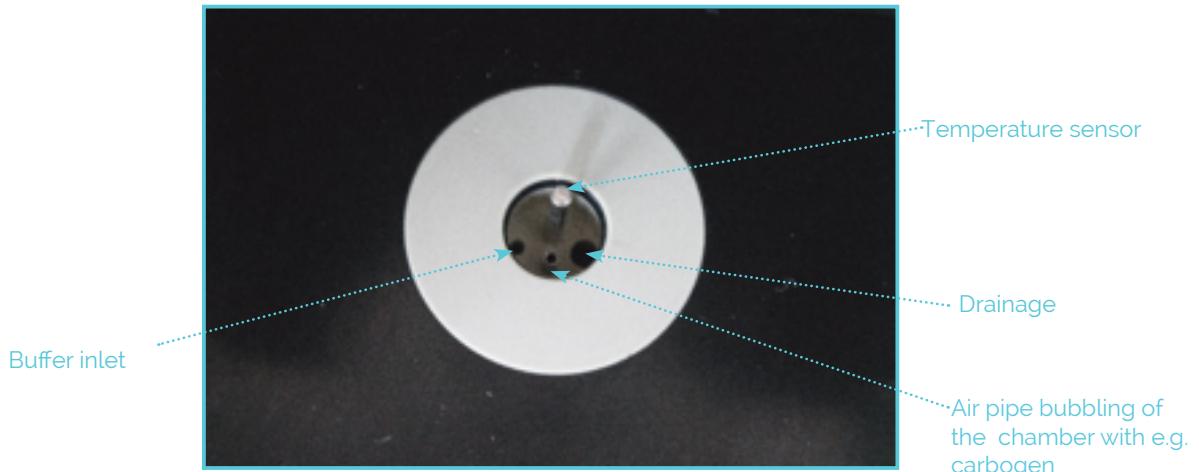


Figure 4: Picture of the Bottom of the chamber with the airpipe, Temperature Probe, Buffer inlet, and Drainage at the bottom.

To get access to the area with the tubing connections and valves open the front door of the 750TOBS system (see figure 5 and 6).



Figure 5: 750TOBS system – to open the front door turn the knob (red circle).



Figure 6: 750TOBS system – with open front door.

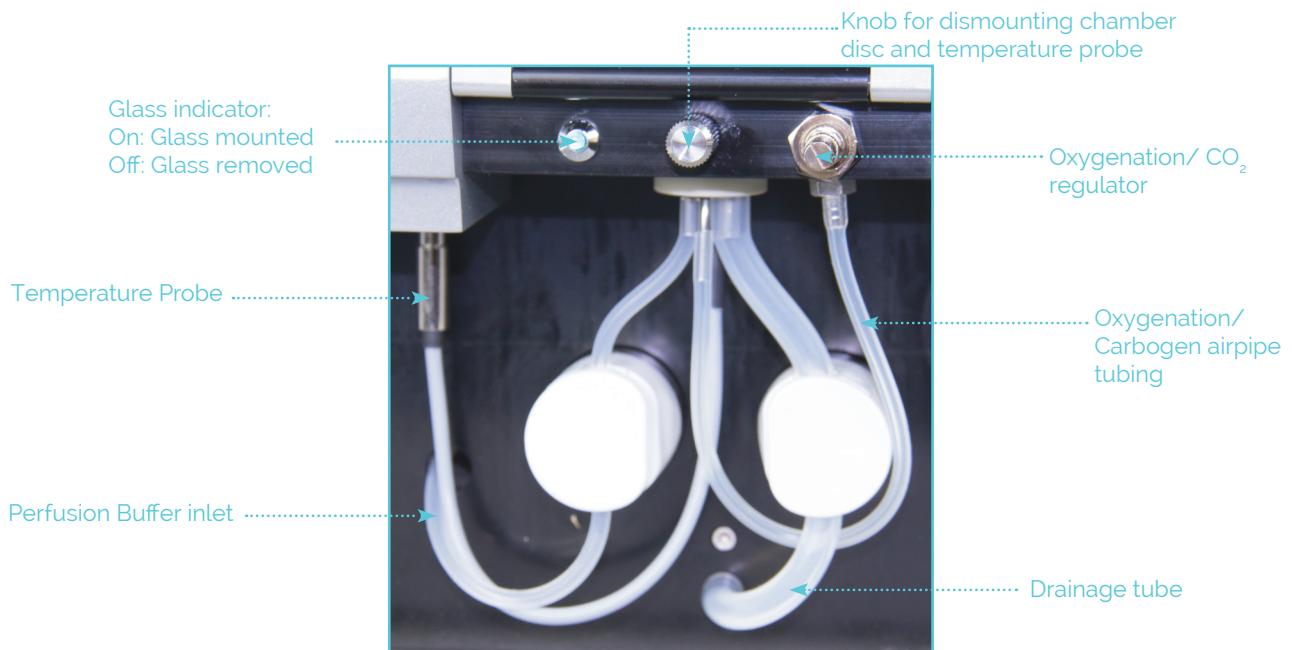


Figure 7: Tube temperature probe and oxygen regulator connection for each chamber.

NOTE: Regarding the oxygenation/CO₂ regulator: the regulators need to be greased AT LEAST twice a year. Also, make sure that the regulators are turned at regular intervals to prevent them from getting stuck. Put a small amount of the grease onto the needle valve spindle and move the pindle back and forth several times. Use ONLY the grease that came with the system.

CHAPTER 2 - SETTING UP

2.1 GETTING STARTED



Figure 8: Complete Tissue Organ Bath System 750TOBS

Step 1: Refer to the Complete Tissue Organ Bath System 750TOBS, (figure 8)

Users with 750TOBS and Labchart 8 Pro for Data acquisition

- Insert the Power cable to the Power connector point
- Connect the USB cable to the USB connection on the 750TOBS and the computer USB port
- Install the DMT Device Enabler on the computer (see Appendix A)
- Install Labchart 8 Pro on the computer (DO NOT use BCN cables for analog data recording)
- Install the 750TOBS Controller Software (see Appendix A)
- The vacuum pump with vacuum trap must be connected to the Drainage connection, marked 'VACUUM' for the waste buffer
- Connect the Oxygen/Carbogen gas supply to the inlet, marked 'OXYGEN' (min. 1bar pressure, max 10bar).

Users with 750TOBS and an analogue data acquisitions system (NOT FOR LABCHART 8 USERS)

Insert the Power cable to the Power connector point.

- Connect the BNC cables to the Analog recorder output ports, marked 'REC1, REC2, REC3 and REC4' and connect the BNC cables to the data acquisitions system
- Connect the USB cable to the USB connection on the 750TOBS and the computer USB port
- Install the 750TOBS Controller Software (see Appendix A)
- The vacuum pump with vacuum trap must be connected to the Drainage connection, marked 'VACUUM' for the waste buffer
- Connect the Oxygen/Carbogen gas supply to the inlet, marked 'OXYGEN' (min. 1bar pressure, max 10bar).

The isometric force transducer holder is already mounted on the 750TOBS assembled unit. If isotonic transducers have been ordered the transducers will be packed separately and have to be mounted on the 750TOBS. The isometric transducer is connected to the internal amplifiers, A/D-converters, the digital USB output (Labchart 8 Pro users) and the Analog output.

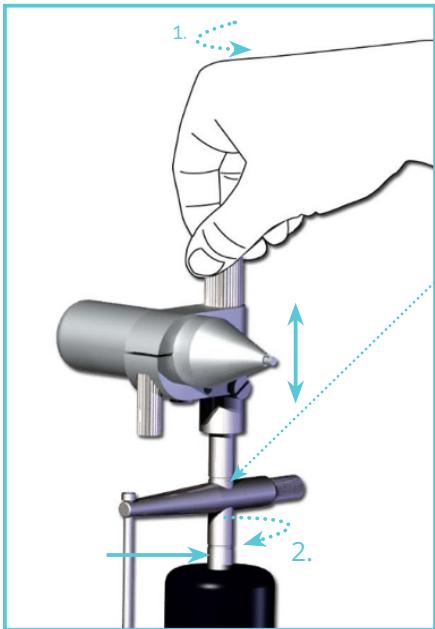


WARNING: Be very careful not to harm the isometric transducer during connection process. The transducer is very fragile and will break if more than 200g of force is applied to the transducer. There is NO warranty on the transducer.

Step 2: Switch power ON once the above connections are secure. The 750TOBS System is ready once you can hear two unlocking sounds.

2.2 POSITIONING/ALIGNMENT

ADJUSTMENT OF THE ISOMETRIC TRANSDUCER AND TISSUE HOLDER POSITION



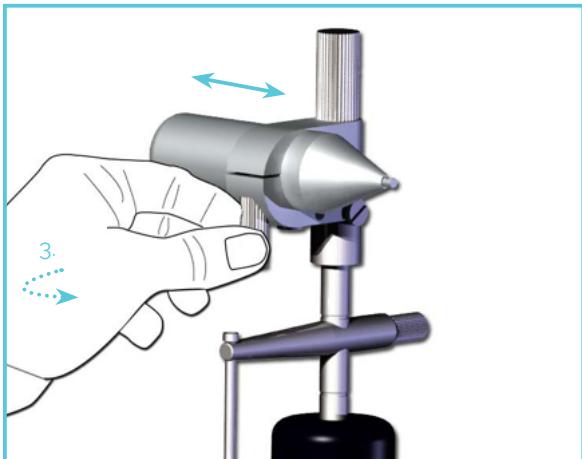
1. Vertical fine adjustment of the transducer:

Turn the micrometer screw clockwise to lower the transducer. Turn counter clockwise to lift the transducer. The marked line is the lowest position of the transducer. The micrometer positioner has a working range of 0 - 26 mm above the line.

NOTE: The micrometer positioner could have a small slag when shifting from clockwise to counterclockwise rotation and vice versa. It is easy to feel when the micrometer positioner starts to move the transducer.

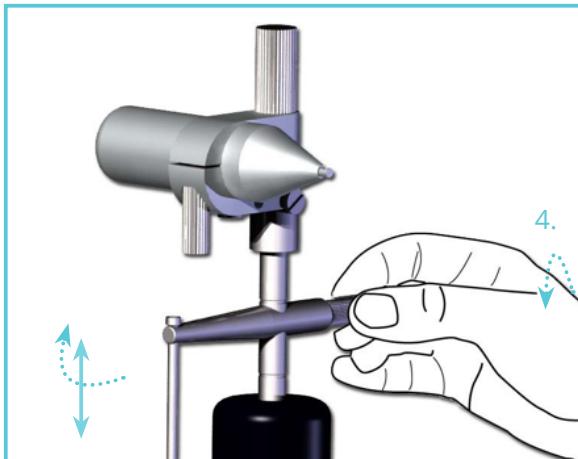
2. Final alignment of the transducer:

The transducer can be finally aligned in parallel to the tissue holder and secured by rotating the locking bolt.



3. Horizontal adjustment of the transducer:

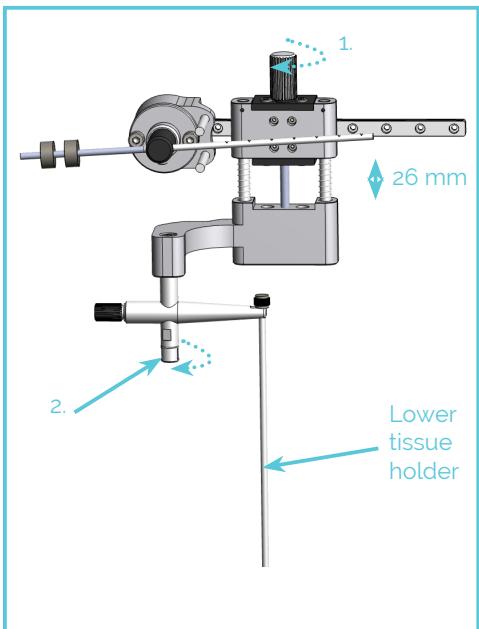
Turn the screw clockwise to free the transducer. The transducer can now be moved horizontal. Turn the screw counterclockwise to lock the transducer.



4. Course adjustment of the lower tissue holder:

Turn the screw counterclockwise to unlock the tissue holder. When unlocked, it is possible to move the holder in 4 orientations, up, down, left and right. Turn the screw clockwise to lock.

ADJUSTMENT OF THE ISOTONIC TRANSDUCER AND TISSUE HOLDER POSITION



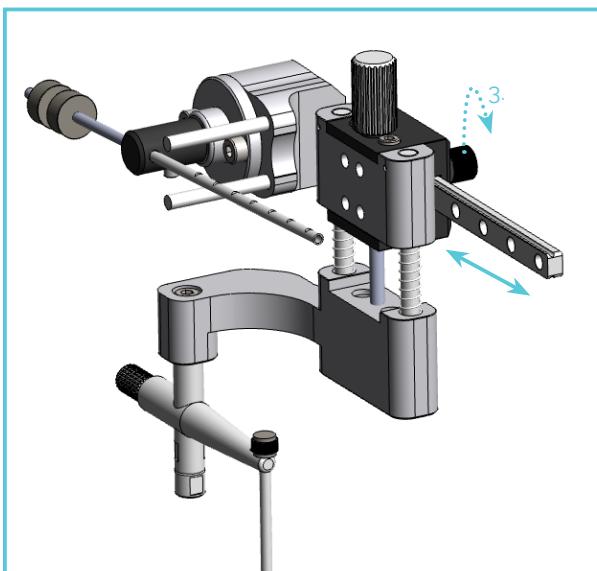
1. Vertical fine adjustment of the transducer:

Turn the micrometer screw clockwise to lower the transducer. Turn counter clockwise to lift the transducer. The micrometer positioner has a working range of 0 – 26 mm.

NOTE: The micrometer positioner could have a slag when shifting from clockwise to counterclockwise rotation and vice versa. It is easy to feel when the micrometer positioner starts to move the transducer.

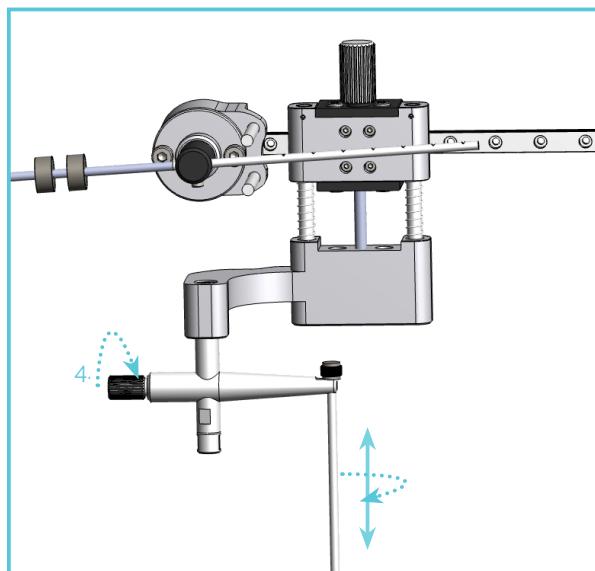
2. Final alignment of the transducer:

The transducer can be finally aligned in parallel to the tissue holder and secured by rotating the locking bolt.



3. Horizontal adjustment of the transducer:

Turn the screw clockwise to freely move the transducer horizontally. Turn the screw counterclockwise to lock the transducer position.

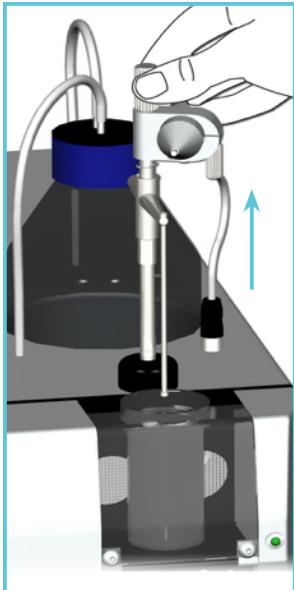


4. Course adjustment of the lower tissue holder:

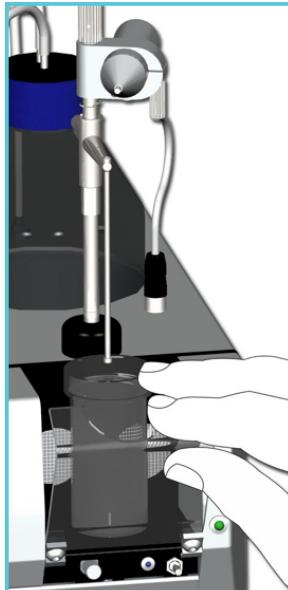
Turn the screw counterclockwise to unlock the tissue holder. When unlocked, it is possible to move the holder in 4 orientations, up, down, left and right. Turn the screw clockwise to lock.

2.3 TISSUE CHAMBER HANDLING PROCEDURE

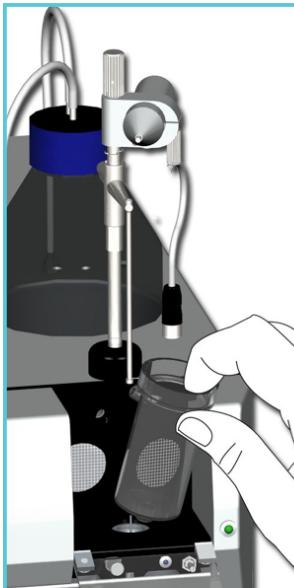
The following is how to place & remove the tissue chamber.



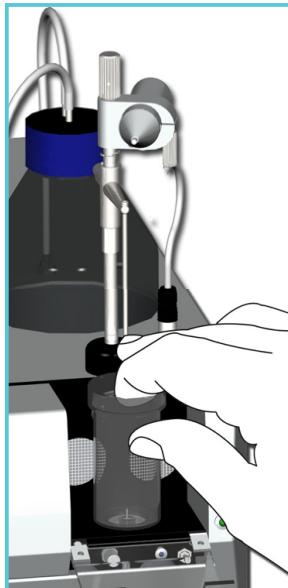
1. Move the transducer and mounting supports/holders upwards. Ensure the sample holder is free of the chamber.



2. Open the chamber door.



3. Removing the glass: Carefully pull the top of the chamber outwards pressing down on the bottom of the chamber a few millimeters until the chamber is free to move upwards. Then lift out the chamber.



4. Placing the glass: Place the pipe stub at the bottom of the chamber inside the chamber disc whilst aligning the pipe stub to the stub opening. Gently place the pipe stub situated on the inside of the heating compartment and secure in.

2.4 DIFFERENT TYPES OF TISSUE SAMPLE HOLDERS

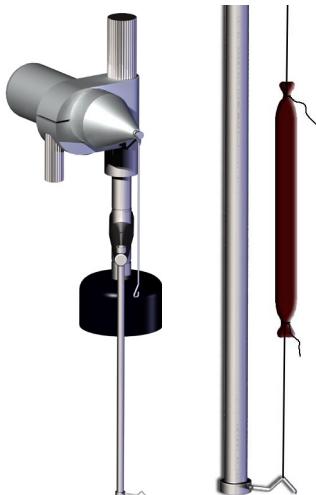


Figure 9A: Triangle hooks (standard)



Figure 9B: Closing clips (optional)



Figure 9C: Pins (optional)

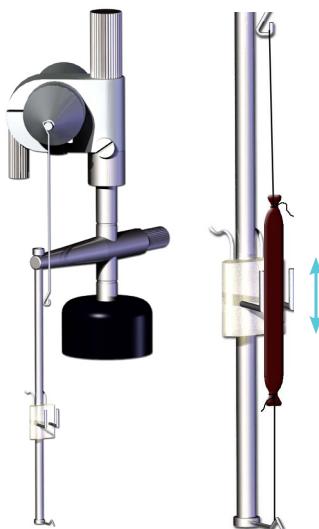


Figure 9D: Triangular hooks w/stimulation electrodes clips (optional)

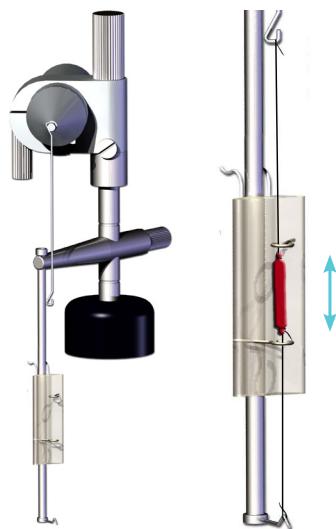


Figure 9E: Triangular hooks w/circular stimulation electrodes (optional)

NOTE: The above mounting supports (figure 9) are only examples of supports delivered by DMT. If other mounting supports are needed ask your DMT sales representative.

CHAPTER 3 - FUNCTIONS AND INTERFACE

3.1 THE 750TOBS USER INTERFACE

There are two ways to record the data from the four channels of the 750TOBS system.

First option: Digital Signal Output through the USB output connection. The USB cable is connected to the USB port on the 750TOBS and the USB port on the Computer. It is mandatory to have the Labchart 8 Pro data acquisition software and the DMT Device Enabler software installed on the computer to be able to record the digital signal output from all four chambers of the 750TOBS system (Appendix A).

Second option: Analog Signal Output through the BNC output connection (-2.5V to +2.5V). There are four connections corresponding to each of the 4 chamber systems. Any data acquisition system able to record a -2.5V to +2.5V output can be used for data acquisition from the 750TOBS system using the BNC connections.

3.1.1 CONTROLLER SOFTWARE INSTALLATION

The 750TOBS Controller software is needed to control the 750TOBS system, enter settings and do the transducer calibrations.

Install the TOBS 750 Control Software and DMT Device Enabler on the supplied USB memory stick on a computer running Windows 7 or newer (see Appendix A).

3.1.2 CONTROLLER SOFTWARE

Make sure the USB cable from the 750TOBS system is connected to the computer and the power of the 750TOBS system is ON. Start the TOBS 750 Control Software.

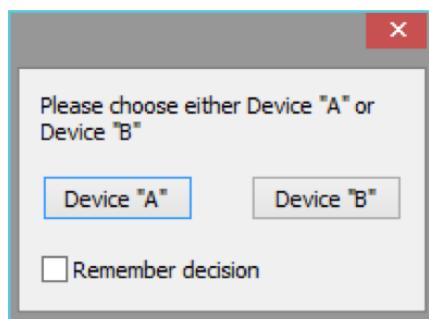


Figure 10: Select Device "A" for the first 750TOBS system

The 750TOBS Control software can control two 750TOBS systems per computer. If two 750TOBS systems has to be used then it is important to turn on the power of only one 750TOBS system and then start the TOBS 750 Control Software and select this as Device "A" (See figure 10). Then turn ON the power of the second 750TOBS system and start a second instance of the TOBS 750 Control Software. The second system will be named Device "B" (figure 11+12).

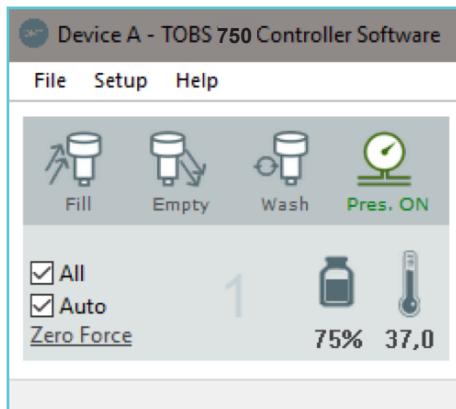


Figure 11: Two 750TOBS systems connected to the same computer

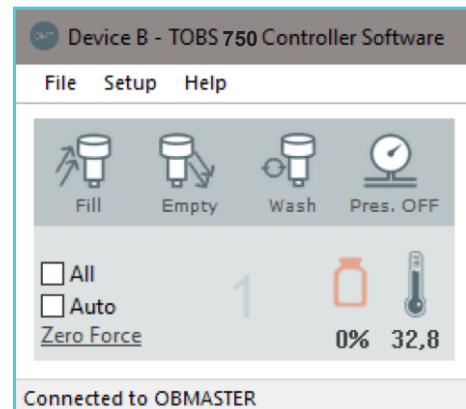
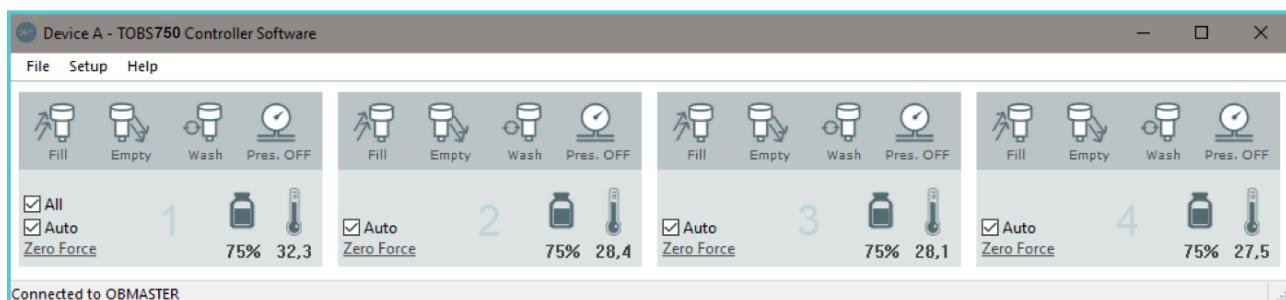


Figure 12: Two 750TOBS systems connected to the same computer

There are three primary menus:

- 3.1.2.1 File menu
- 3.1.2.2 Setup menu
- 3.1.2.3 Help menu

Each of these consist of their individual drop down sub-menus respectively.



Left-clicking on the main menu, provides the User their respective drop down sub- menus.

3.1.2.1 File menu:

This has the following options (figure 13):

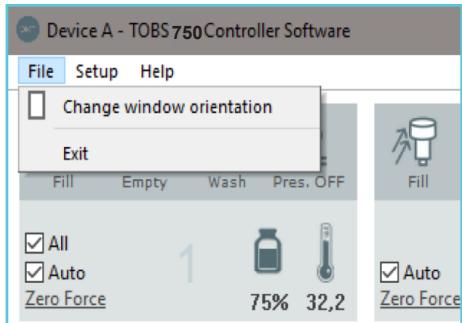


Figure 13

Change Window orientation: This changes the orientation layout of the User Interface to a vertical display as shown, from the default horizontal layout as shown in figure 14 and 15.



Figure 14: Horizontal orientation of the 750TOBS user interface



Figure 15: Horizontal orientation of the 750TOBS user interface moved to the top of the screen

Vertical orientation of the 750TOBS user interface as shown in figure 16 and 17.



Figure 16: Vertical orientation of the 750TOBS user interface

Vertical orientation of the 750TOBS user interface moved to the right side of the screen.

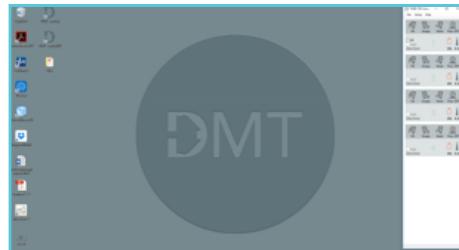


Figure 17: Vertical orientation of the 750TOBS user interface moved to the right side of the screen

3.1.2.2 Setup menu:

The Setup menu has the following sub-menu options (figure 18):

1. **Filling Settings** sub-menu
2. **Temp Settings** sub-menu
3. **Wash Settings** sub-menu
4. **Force Settings** sub-menu.

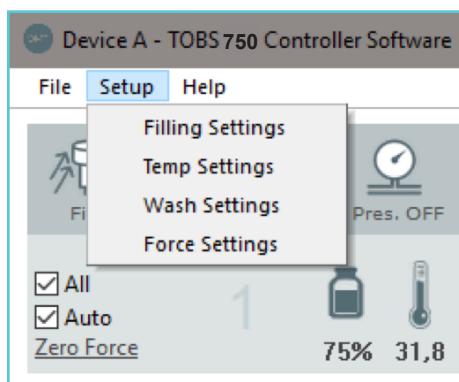


Figure 18: Setup menu

1. Filling Settings sub-menu:

This consists of;

- Fill Volume
- Buffer Start Volume
- Fill Calibration
- Empty Time
- Pressure

As shown in figure 19.

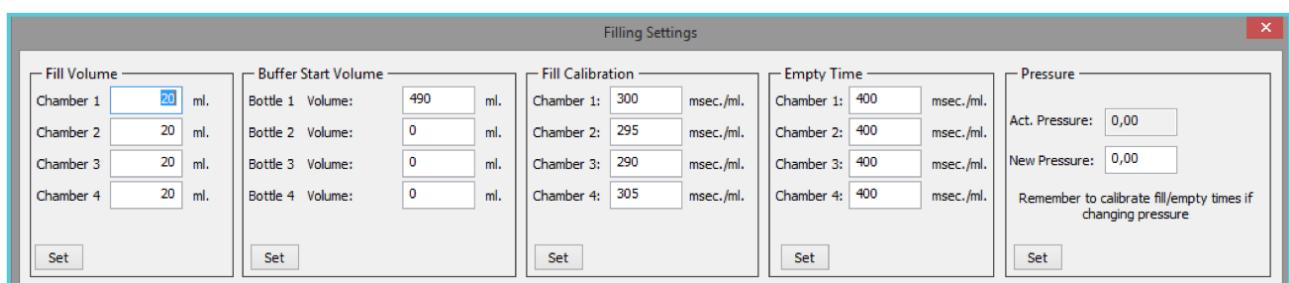


Figure 19: The Filling Setting menu

- **Fill Volume menu:** The default reading is 20 ml which is the Calibration Chamber volume. The Fill Volume is the volume filled into the chamber when pressing the Fill icon in the Main Menu (only when the Auto is selected as seen in figure 20).



Figure 20.

Set this volume to the volume needed for your experiment. If you want to have your mounted tissue in 15ml then enter 15ml into the Fill Volume and press Set to store the value (figure 21).

Next time the Fill icon is pressed in the Main Menu (with Auto selected) the given chamber will be filled with 15ml.

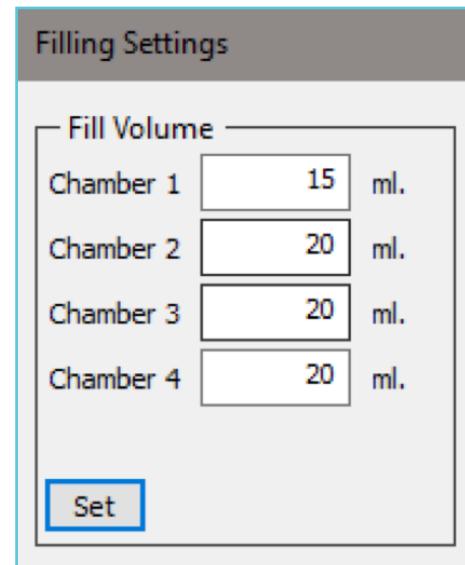


Figure 21: Changing the Fill volume

NOTE: Before using the Fill volume a Fill Calibration is needed. After the Fill Calibration the Fill volumes can be changed as wanted (see Fill Calibration).

- **Buffer Start Volume:** Before starting an experiment fill the bottle with a known volume of buffer. Enter the volume of buffer filled into each bottle and press Set. (The maximum volume of Buffer in each bottle is 800 ml).



WARNING: It is important to enter the right start volume of each bottle before starting an experiment. The 750TOBS uses the entered volume to issue a warning when the bottle is close to being empty.

- **Fill Calibration:** The Fill Calibration is needed for the Fill feature to work with the Auto selected (figure 22). This calibration is needed for being able to enter different fill volumes e.g. 15ml that will be filled into a given chamber when the Fill button is pressed.

Calibration of each chamber is performed with the marked tissue chamber marked with 20 ml as shown in figure 23.

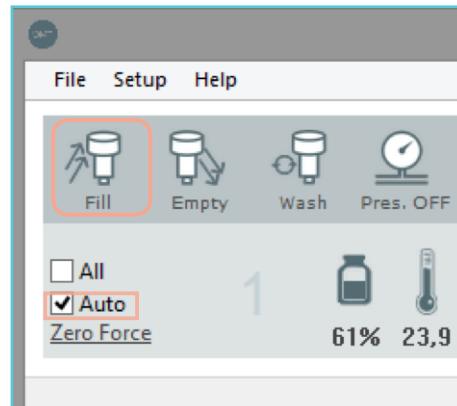


Figure 22: select Auto before starting the Fill Calibration.

Step 1: Place the 20ml Calibration chamber in the chamber position to calibrate.



Figure 23: 20ml Calibration chamber.

Step 2: Make sure the Buffer bottle is filled with buffer (max 800ml). Turn on pressure on the bottle by pressing on Pres. OFF icon (figure 24).



Figure 24.

which will change to Pres. ON in green color (figure 25).



Figure 25.

Step 3: Make sure that the 'Fill volume' which is in the Chamber Volume Settings sub-menu under 'Setup' is set to 20ml (figure 26).

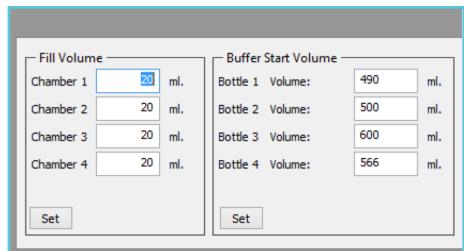


Figure 26

Step 4: Make sure the Auto has a tick mark and the All Do Not have a tick mark (figure 27).

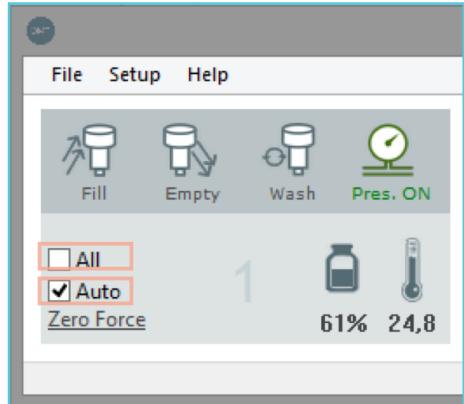


Figure 27

Step 5: Press the Fill icon on the given chamber (figure 28).

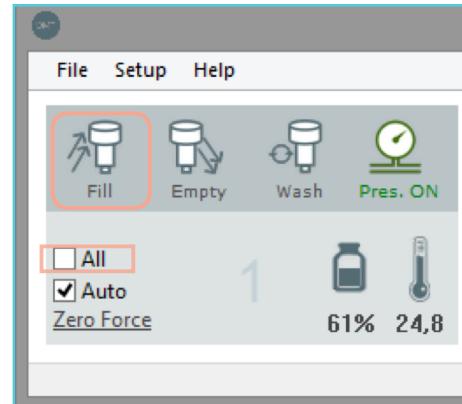


Figure 28.

The system will now fill the chamber for the given time shown in the Fill Calibration menu. In the example below it is 300msec/ml (figure 29) which means the system will fill the chamber for 20ml x 300msec/ml = 6000msec.

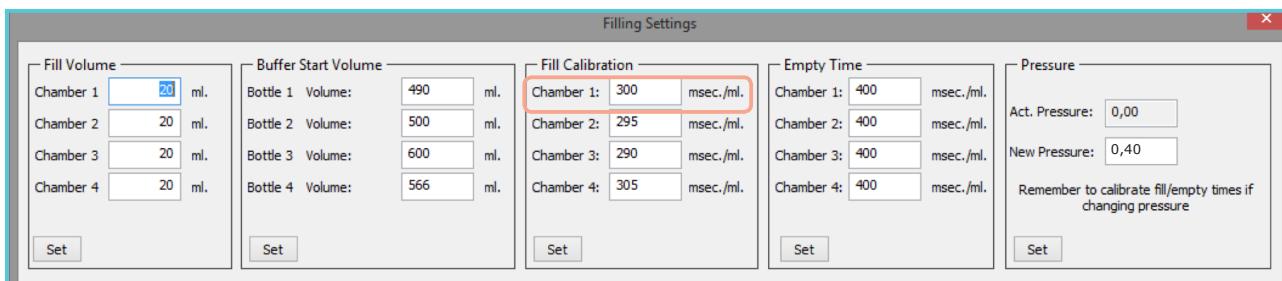


Figure 29: Fill calibration sub-menu.

Step 6: The 750TOBS will in the above example fill Chamber for 6.0sec and then stop. If the filling volume has not reached the 20ml line on the 20 ml Calibration glass (Lower meniscus level of the buffer) then the time in the Fill Calibration menu has to be increased e.g. to 306msec/ml. If the filling volume exceeded the 20ml line then lower the time in the Fill Calibration menu to e.g. 294msec/ml.

Step 7: After the 'Fill Calibration' has been set, empty the chamber and Press the 'Fill' icon to verify that the new Fill calibration time is correct.

Step 8: This procedure has to be done a number of times until the exact time taken to fill the 20 ml chamber has been attained.

Step 9: Calibrate the next chamber until all four chamber have been calibrated.

- **Empty Time:** is time regulated and this is activated during the calibration step for each chamber. Calibration of each chamber is performed with the marked 20ml Calibration chamber (figure 30).



Figure 30.

Step 1: Fill all chambers with 20ml buffer by placing a tick-mark in the Auto and All (figure 31). Enter 20ml the in Fill Volume and press the Fill button. All chambers are now filled with 20ml buffer (Can only be performed after the Fill Calibration have been made).

Step 2: Start the vacuum pump and make sure that the vacuum trap is connected to Vacuum port on the rear side of the 750TOBS system.

Step 3: Make sure that both the Auto and All has a tick mark as depicted in figure 31.

Step 4: Press the Empty icon (figure 32).

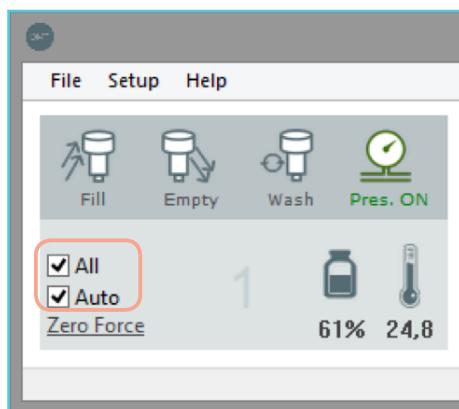


Figure 31

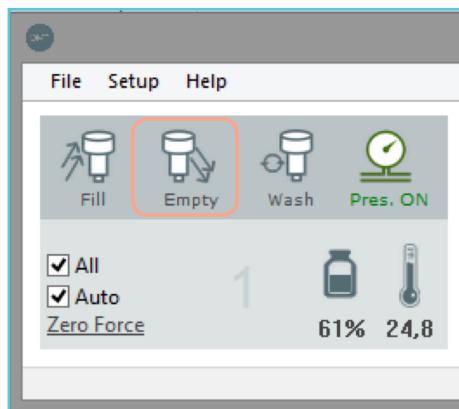


Figure 32

The system will now Empty the chambers for the given time shown in the Fill/calibration sub menu (figure 33). In the example below it is 400msec/ml which means the system will empty the chamber for $20\text{ml} \times 400\text{msec/ml} = 8000\text{msec}$.

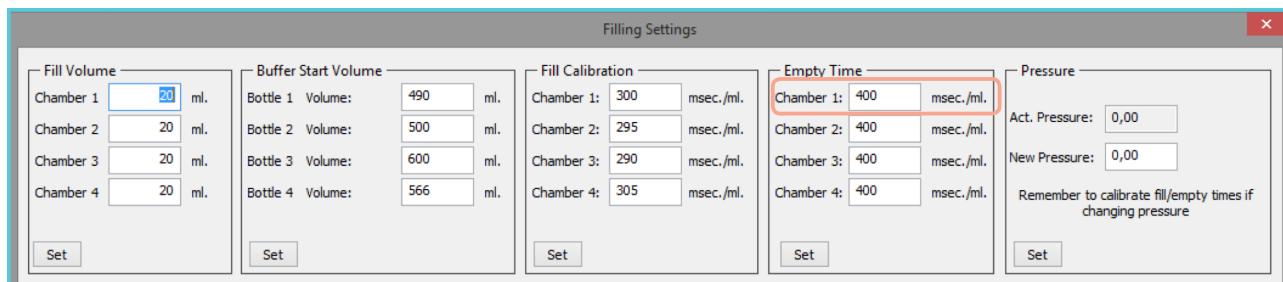


Figure 33: The Empty Time sub-menu.

Step 5: The system will in the above example empty the Chambers for 8.0sec and then stop. If the chambers has not been emptied completely according to the set 'Empty time' rate, then increase the time in the 'Empty Time' sub-menu e.g. to 410msec/ml. If the chamber is empty before the set 'Empty Time' reaches completion, then lower the time rate in the 'Empty Time' sub menu to e.g. 390msec/ml.

Step 6: After Calibration of the 'Empty Time' has been set, fill the chamber with 20ml buffer and press the Empty icon again to verify that the new 'Empty Time' is correct.

Step 7: This procedure has to be done a number of times until the exact time taken to Empty the 20 ml chamber has been attained.

The above method will ensure that all chambers are empty in the Auto mode when the Empty button is pressed with tick-marks in All (figure 31).

- **Pressure:** The Pressure is set at a default of 0.400 bar (figure 34). One can set or reset the target pressure anywhere between 0.01 and 0.999 Bar, by clicking on 'Set' (figure 35).

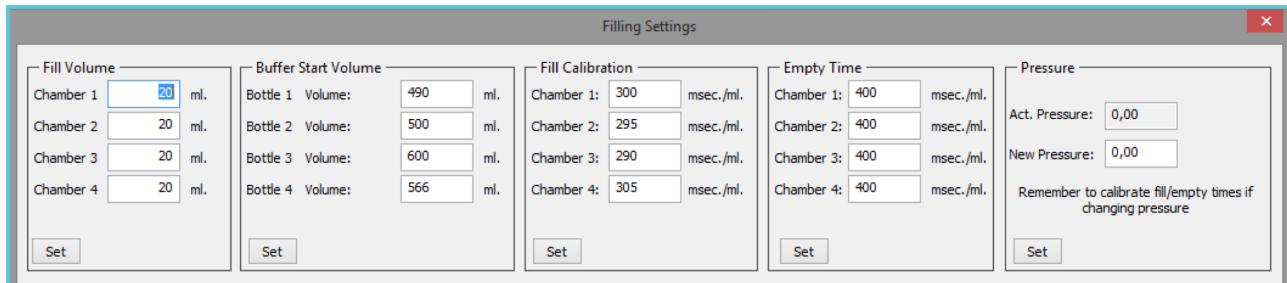


Figure 34: The Pressure sub-menu.

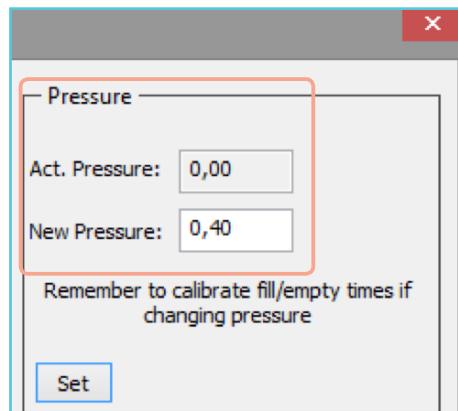


Figure 35.



WARNING: Fill Calibration needs to be redone for every new change in Pressure settings. Act. Pressure indicates the true pressure in the reservoir bottles. New Pressure: This is the Target Pressure or the new set pressure.

2. Temperature Settings sub-menu: Consists of;

- Heating Plate Temp
- Chamber Temp
- Temp Difference

Shown in figure 37. The Temperature settings menu is found in the Setup menu (see figure 36).

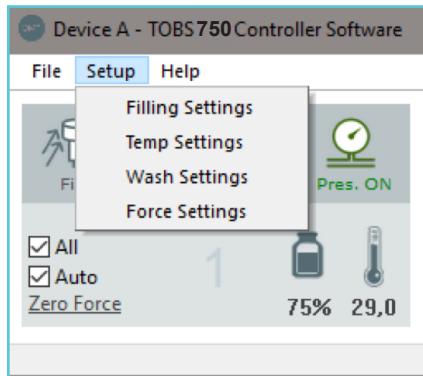


Figure 36: Setup menu

Heating Plate Temp.			Chamber Temp.			Temp. Difference		
Actual:	40,8	°C	Actual:	25,3	37,0	Chamber 1:	0,0	
Set:	41,5	°C	Set:	26,8	37,0	Chamber 2:	0,0	
Diff:	0,5	°C	Set:	26,1	37,0	Chamber 3:	0,0	
Set			Set			Chamber 4:	0,0	Set

Figure 37: The Temp settings menu

- **Heating Plate Temperature:** This is the temperature of the plate on which the buffer bottles are placed in the 750TOBS system. This temperature is chosen to be higher than the bath temperature to avoid gas formation and to compensate for cooling during chamber refill. Set temp is updated when clicking 'Set'. The difference due to heat loss can be compensated for, by entering the difference between the Actual temperature you measure in the buffer bottles. Place the buffer bottle with pre-heated buffer in the 750TOBS system. Let the 750TOBS heat the bottle for 30min or more at the given temperature e.g. 37°C. Measure the buffer temperature in the buffer bottle with a thermometer. If the thermometer is reading 36.5°C then set the Diff to 0.5°C. The Actual temperature is the temperature of the heating plate and is only used to verify that the heating plate is heating up.

NOTE: The heating plate temperature must be within 20- 50 °C. Entering a temperature below or above this range results in the following error message as shown in figure 38.

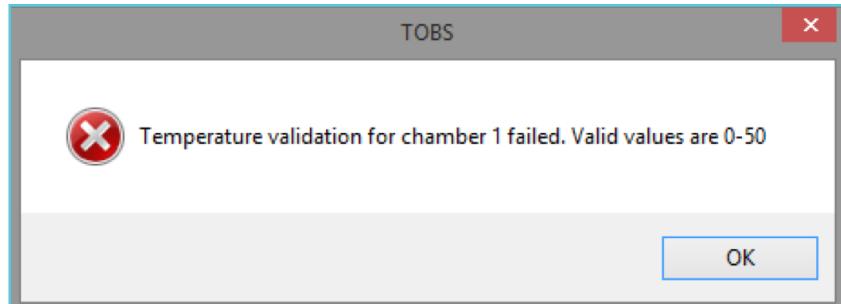


Figure 38.

- **Chamber Temp:** The Chamber Temp. is the target temperature for your experiment. The Actual temperature reading, will vary from the Set temperature due to the ambient temperature difference in the lab. The Chamber temperature Set value is the appropriate temperature for the experiment e.g. 37°C.

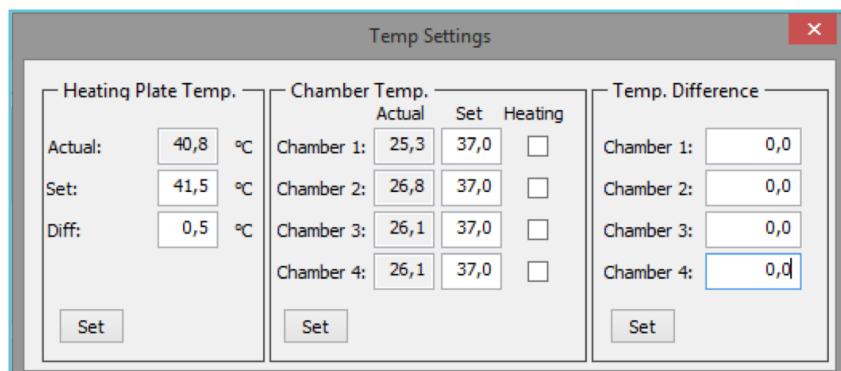


Figure 39.

To start the heating of a chamber, mark the box Heating in the Chamber Temp sub-menu for the given chamber (figure 39). Fill the chambers with heated buffer and wait 20min or until the Actual temperature is not changing anymore. If there is a difference between the Set and Actual temperature due to heat loss then the Temp. Difference must be used.

- **Temp Difference:** The difference due to heat loss can be compensated for, by entering the difference between the Actual temperature (also shown in main menu) and the Set temperature. In the example shown in figure 40.

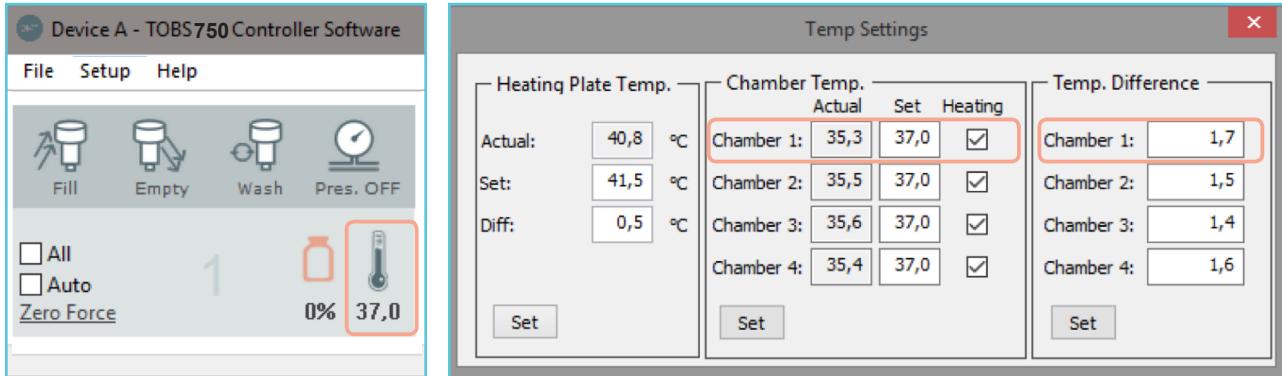


Figure 40: Temperature Difference setting to compensate for heat loss.

The Set temperature is 37°C and the Actual temperature is 35.3°C which is 1.7°C too low. To compensate for the 1.7°C heat loss in chamber 1 enter 1.7°C in the Temp. Difference. The same is done for all four chambers as can be seen on figure 40. Click on 'Set' to save the changes.

After 10-15 minutes verify that the actual temperature of the chambers are at the set temperature otherwise correct the Temp difference accordingly.

3. Wash Settings sub-menu: *The Wash function must only be used by users who cannot expose the experimental tissue to air!*

To prevent the tissue from being exposed to air for a short interval of time without nutrients, the wash protocol defined in the Wash Setting is washing steps where buffer is filled from the preheated reservoir without emptying the chamber first. The buffer will flow into the chamber from the inlet and run into the overflow automatically defined in the Wash menu setup. The Wash mode can be accessed in the Setup menu or by pressing the Wash icon in the main menu (figure 41) for each of the four chambers.

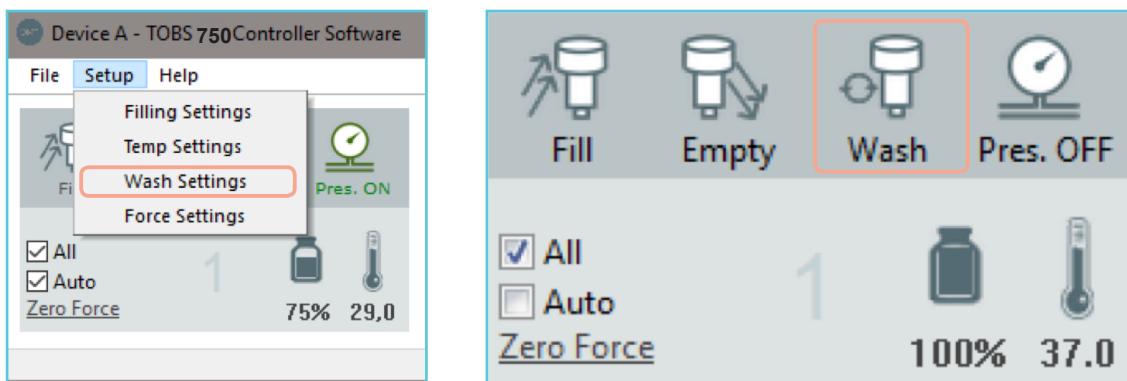


Figure 41: Wash protocol

- Wash:** From the Setup menu, the Wash menu allows define the wash protocol. The first step is to define how many seconds the 750TOBS should fill the chamber with buffer. The next step is to define the interval between fillings in whole minutes. The next step is to define the interval between fillings in whole minutes, including the wash time. The last step is to define how many times the filling should be repeated. In figure 42B. a wash protocol is made with a Wash time of 30sec, every 1 minute and repeated 2 times. This means that the 750TOBS will fill the given chamber for 30seconds and then wait 30 seconds (1minute minus 30sec) before it will fill the chamber again for 30 seconds. The wash protocol can be expanded with a second protocol as shown in figure 42C. Press Set to save the Washing protocol (figure 42C).

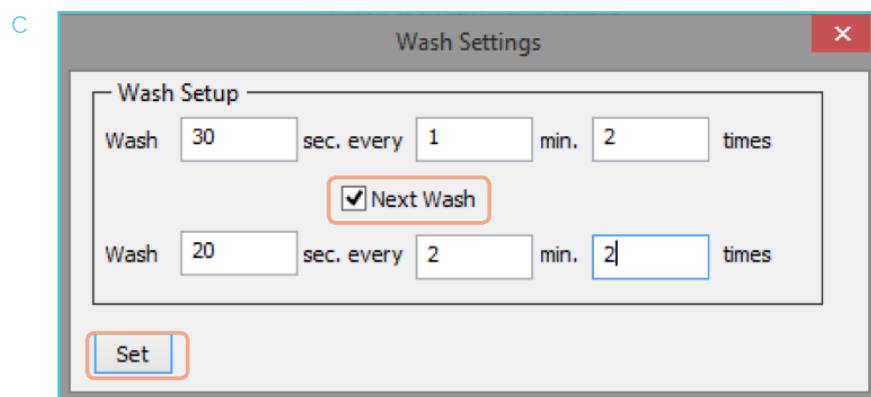
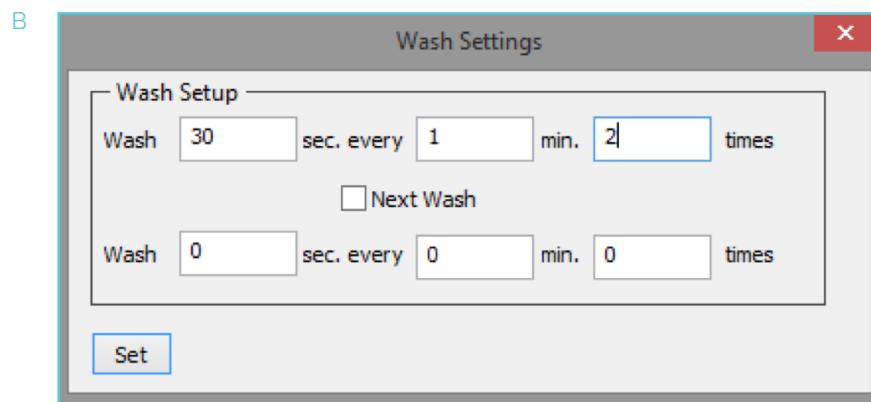
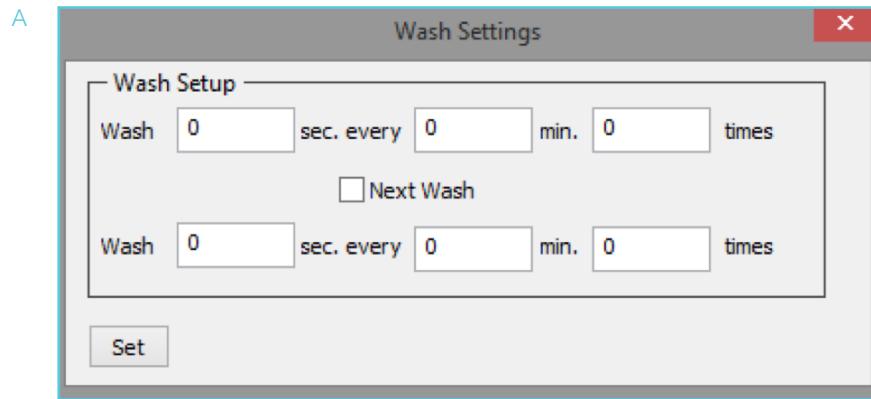


Figure 42A-C. Wash Setting menu to setup a washing protocol.

- **Wash - Main menu:** from the individual Chamber Panel, allows the user to define which chamber 1,2,3,4 or All should be subjected to the wash step (Figure 43). By selecting the Setup the Washing protocol can be defined as shown in figure 42A-C.

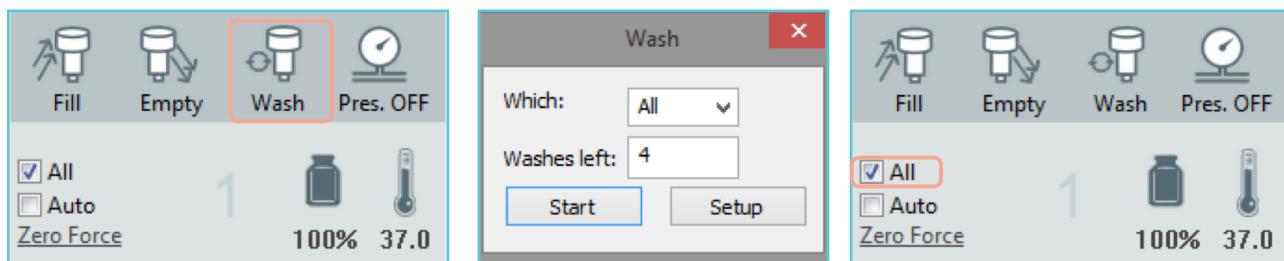


Figure 43.

Checking 'All' activates 'Wash' for all the 4 chambers simultaneously (figure 43).

With a setting of e.g. 260msec/ml in the Fill/Calibration menu a 10sec wash will result in 38.46ml buffer perfused through the chamber and into the overflow.

4. Force Settings sub-menu: Consists of:

- Force units
- Force transducer analog output
- Transducer menu.

as shown in figure 44.

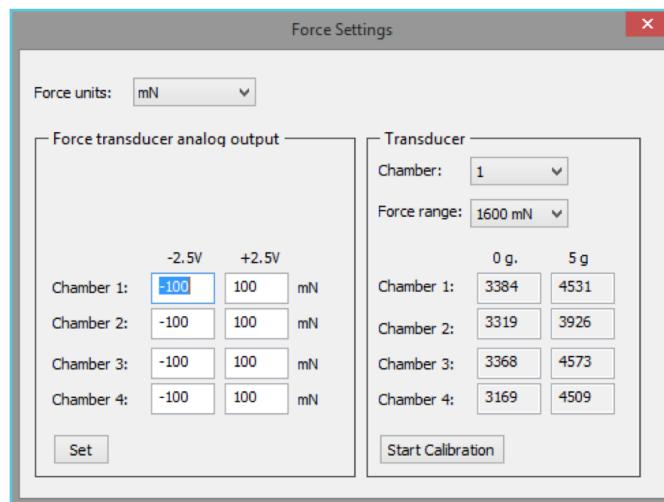


Figure 44: The Force Setting menu

- **Force Units:** The force recordings can be shown in mN or gram for users of digital output through the USB cable to Labchart as shown in figure 45.

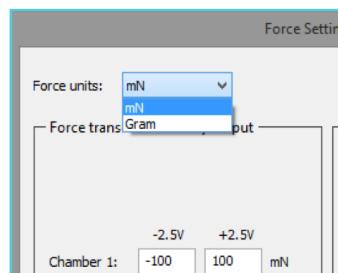


Figure 45: Selecting the force units for the digital output

NOTE: Every time the Force Units are changed Labchart must be restarted.

- Force transducer analog output:** The transducer analog output through the BNC cables can be set at -2.5volt which equal to e.g. -100mN and +2.5volt which is equal to e.g. to 100mN as shown in figure 46. If the 100mN is changed to -50mN and +50mN, respectively the resolution will be 2x better. By changing the settings in this menu the resolution of the analog output can be increase or lowered.

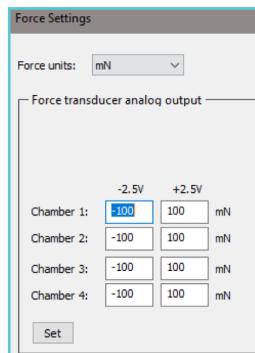


Figure 46: Force recording output for the analog Force output through the BNC plugs

NOTE: If the Transducer 'Force Range' is set to e.g. 400mN then the maximum force that can be measured is still 100mN unless the Force transducer analog output value is changed accordingly.

- Transducer menu:** The Force range can be set at max 200mN, 400mN, 800mN and 1600mN. Select the given chamber and the appropriate Force Range for your type of tissue (figure 47).

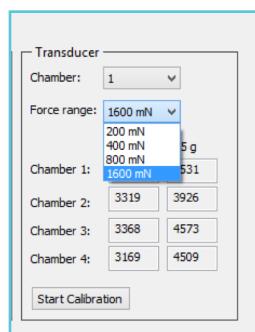


Figure 47: Selection of Force range for the isometric Force transducer

NOTE: It is very important that the Force transducer calibration is performed at 200mN force range. See Chapter 4 for how to do the transducer calibrations.

3.1.2.3 Help menu:

The Help menu (figure 48) has the following sub-menu options:

1. **About TOBS**
2. **Service Key**

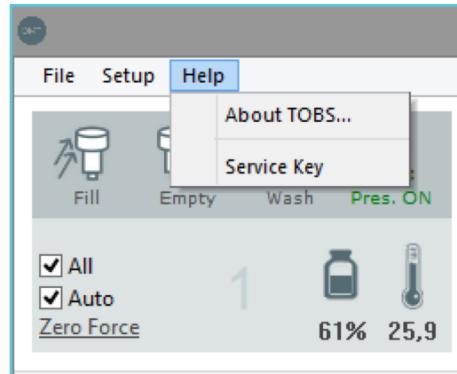


Figure 48: Help menu

1. **About TOBS:** Will list the TOBS Control Software version number as shown in figure 49 as well as other information relevant for DMT engineers.

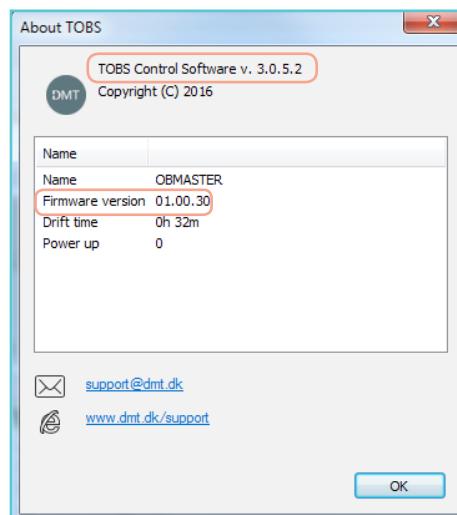


Figure 49: The About Tobs menu. Here the software version can be seen

2. **Service Key:** is only for the DMT engineers during a service (figure 50).

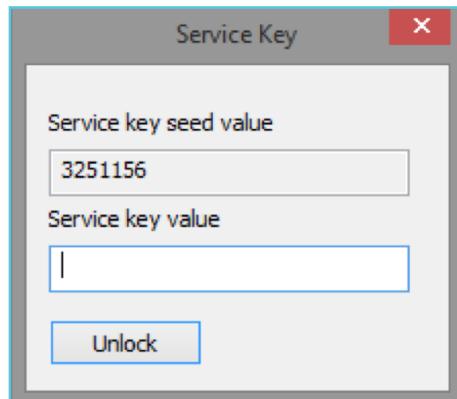


Figure 50.

3.1.3 TISSUE CHAMBER CONTROL INTERFACE

- Fill
- Empty
- Wash
- Pres. OFF
- Temperature Icon
- Buffer Bottle
- All
- Auto
- Zero Force

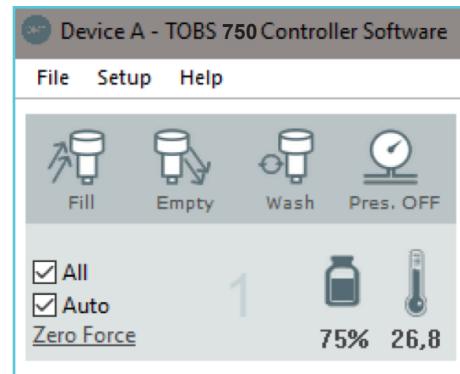
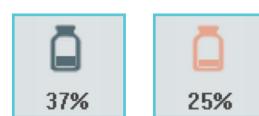


Figure 51: Help menu

- **Fill:** For filling buffer in that respective chamber. The icon lights up green when filling buffer and turns grey when finished. If Auto is not selected filling will continue until Fill is pressed again (manual filling)
- **Empty:** Activates the valve to drain the buffer from the chamber. The icon lights up green until the desired volume of buffer has been drained. If Auto is not selected emptying will continue until Empty is pressed again (manual emptying)
- **Wash:** Allows the user to view the number of Washes left for the respective chamber and allows the user to set a programmed number of washes/flushes in the Empty/Fill wash option.
- **Pres.OFF:** Pressure is activated/deactivated by clicking the 'Pres. OFF' button on the interface. If the pressure is ON, the icon lights up Green.
- **Temperature Icon:** This displays the Actual temperature of the buffer in the Chamber and is expressed in °C.
- **Buffer Bottle:** Displays the volume of Buffer present in the bottle in percent of 800ml (max volume). Here it is 37% of 800 ml which is 296 ml. The bottle icon will turn Orange when the residue volume is 25% or below as an alert to the user to refill the buffer bottle.



- **All:** If 'All' is checked, the activation of 'Fill', 'Empty', 'Wash', 'Pressure' and 'Zero', will be applied to all the chambers simultaneously (figure 52).

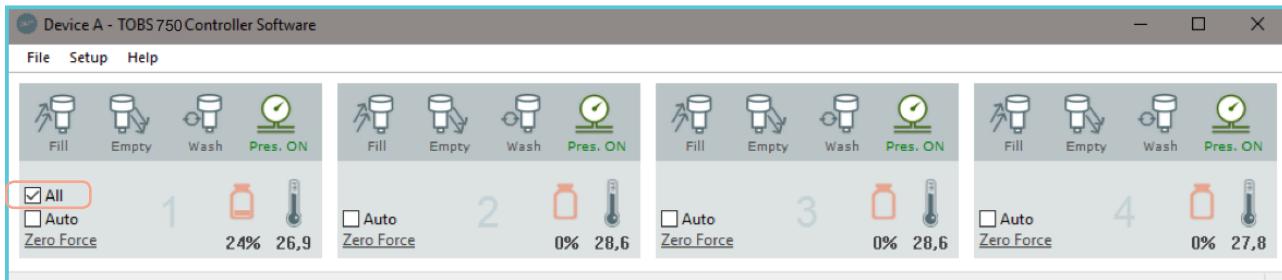


Figure 52: Selection of the All feature. This selection will make sure that e.g. pressing the Fill button will fill all four channels

- **Auto:** The Auto function is used together with the Fill and Empty functions/buttons. If Auto is checked as in figure 53.

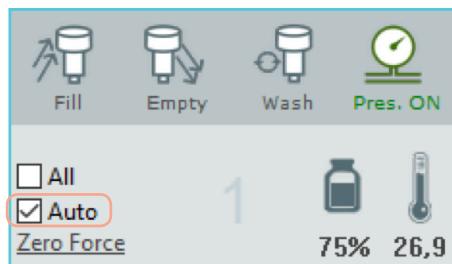


Figure 53: Selection of the Auto feature will make sure that the settings in the Filling setting will be used.

and the Fill button is pressed the chamber is filled up with the programmed amount of buffer in 'ml' that has been defined by the user in the 'Filling settings'. Here below in Figure 54, 20ml is entered,

Filling Settings			
Fill Volume Chamber 1: 20 ml. Chamber 2: 20 ml. Chamber 3: 20 ml. Chamber 4: 20 ml. <input type="button" value="Set"/>		Buffer Start Volume Bottle 1: Volume: 600 ml. Bottle 2: Volume: 600 ml. Bottle 3: Volume: 600 ml. Bottle 4: Volume: 600 ml. <input type="button" value="Set"/>	Fill Calibration Chamber 1: 300 msec./ml. Chamber 2: 295 msec./ml. Chamber 3: 290 msec./ml. Chamber 4: 305 msec./ml. <input type="button" value="Set"/>
			Empty Time Chamber 1: 400 msec./ml. Chamber 2: 400 msec./ml. Chamber 3: 400 msec./ml. Chamber 4: 400 msec./ml. <input type="button" value="Set"/>

Figure 54: The Fill Volume and Fill Calibration settings. Really important to make the Fill calibration before using the Fill Volume for your experiments.

If the Auto is not selected then the chamber is filled until the 'Fill' button is pressed again.

If Auto is selected and the 'Empty' button is pressed the time set in the 'Empty Time' settings as shown in figure 55, dictates the time at which the outflow from the chamber is activated. If the Auto is not selected then the chamber is emptied until the 'Empty' button is pressed again.

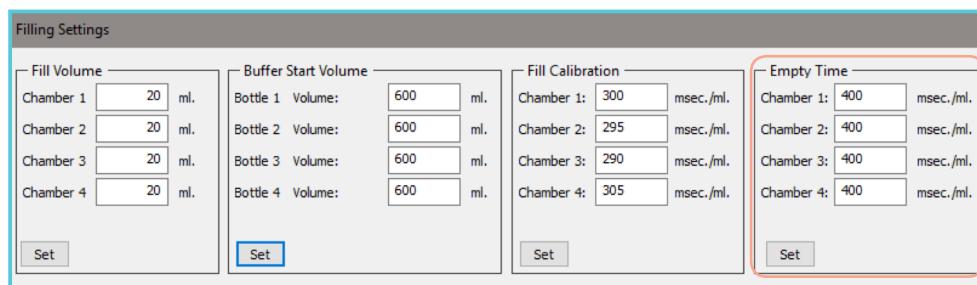


Figure 55: The Empty Time setting. Really important to set the Empty Time before start using the 750TOBS system for the experiments.

The values in the Fill Calibration and Empty time is found by performing the Flow calibration protocol described in 3.1.2.2 Filling Settings menu.

- **Zero Force:** To zero the force output:

To zero a specific channel force transducer remove the tick mark in All and then press Zero Force as shown in figure 56.

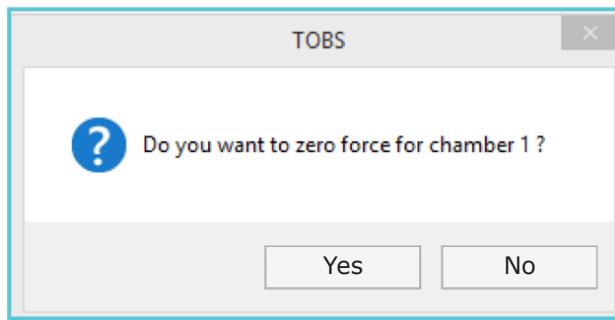


Figure 56.

Click on Yes to zero the force transducers or press No to cancel the zeroing.

If an isotonic transducer is used the procedure is the same to Zero.

There is a possibility to Zero all four transducers simultaneously by tick marking the All box and then clicking Zero Force as seen on the figure 57.

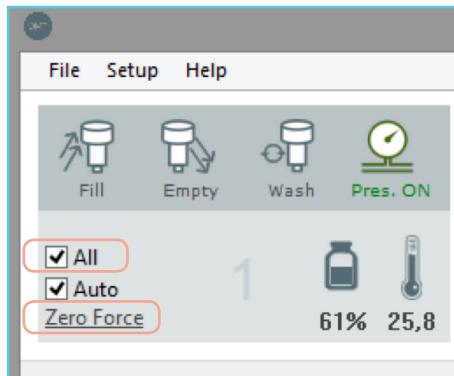


Figure 57: Zero of the force of all four channels simultaneously.

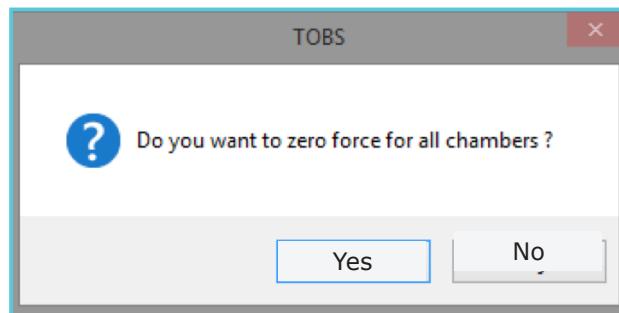


Figure 58.

Click on Yes to zero all four force transducers or press No to cancel the zeroing (figure 58).

CHAPTER 4 - TRANSDUCER CALIBRATION

Prior to shipment the 750TOBS has gone through 2 days of continuous testing, including a final transducer calibration. However, to ensure that the Tissue Organ Bath is working at its highest performance, DMT recommends that a new transducer calibration is performed before the first use of the 750TOBS system. As part of the general maintenance of the 750TOBS system, DMT also recommends that the bath is weight calibrated at least once every month, every time the system has been moved or has not been used over a long period.

4.1 ISOMETRIC TRANSDUCER:



Figure 59: Transducer with a 5 gram calibration weight.

4.1.1 WEIGHT CALIBRATION OF THE FORCE TRANSDUCER

The calibration is performed by going into the Setup menu and into the Force Setting sub-menu (figure 60).

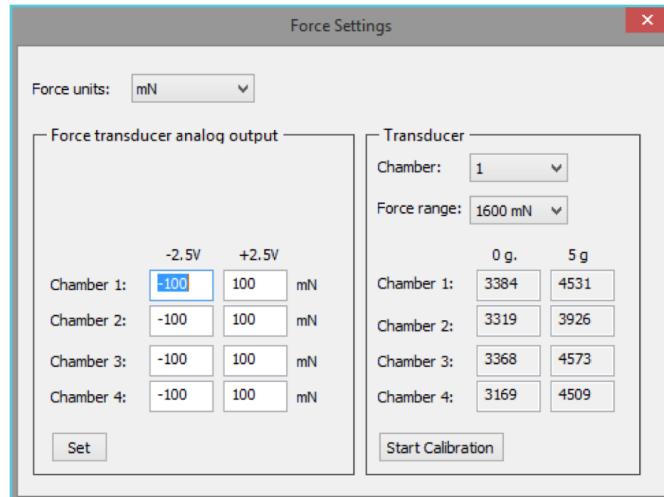


Figure 60: Force Settings menu.

The calibration is performed using a 5g weight (figure 59). Select the chamber to be weight calibrated (see figure 61).

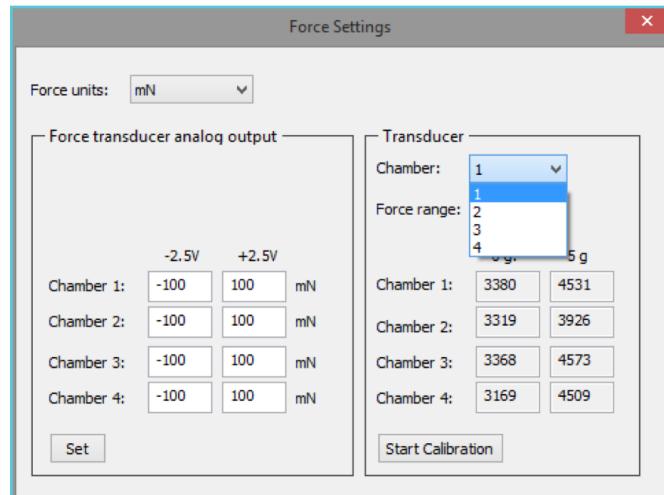


Figure 61: Select the chamber to be weight calibrated.

1. Make sure that nothing is attached to the transducer pin (remove mounting support from the transducer)
2. Select the Force Range to 200mN (figure 62)
3. Press Start Calibration

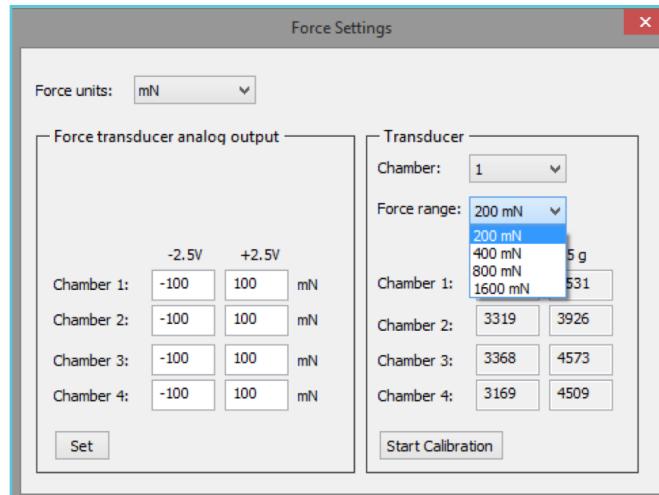


Figure 62: Select the 200mN Force Range before performing the weight calibration

4. Wait 10-15seconds and press next (figure 63)

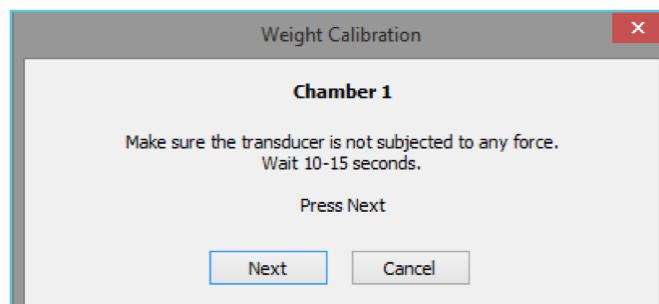


Figure 63.

5. Place the 5g weight on the transducer pin as shown in figure 59



Figure 64:

6. Wait until the force reading (Data acquisition system) is stable
7. Press OK (figure 64)
8. Repeat step 1-7 for chamber 2, 3 and 4.

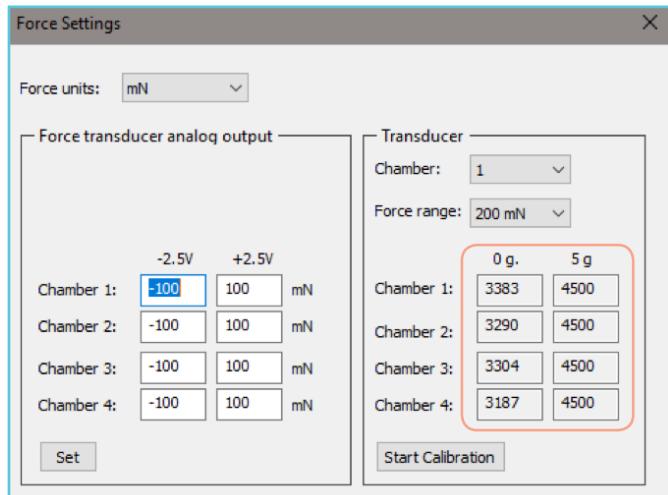


Figure 65: The 4-digit arbitrary numbers shown at 0g and 5g indicate the status of the isometric transducers. The numbers are important for your DMT sales representative.

After the calibration of the Force transducer an arbitrary value from the transducer will be shown automatically at Zero force and at 5gram weight (figure 65). These four-digit numbers has to be between 1000-5500. If the values are 0 or 6500 then the transducer is dead and beyond repair. If the value is 500-1000 or 5500-6000 then the transducer can be re-compensated at DMT and used again.

NOTE: If the arbitrary 4-digit value at Zero force is below 1000 or above 5500 then the transducer is damaged and needs to be either replaced or re-compensated. Please contact your DMT sales representative.

4.2 ISOTONIC TRANSDUCER CALIBRATION (OPTIONAL)

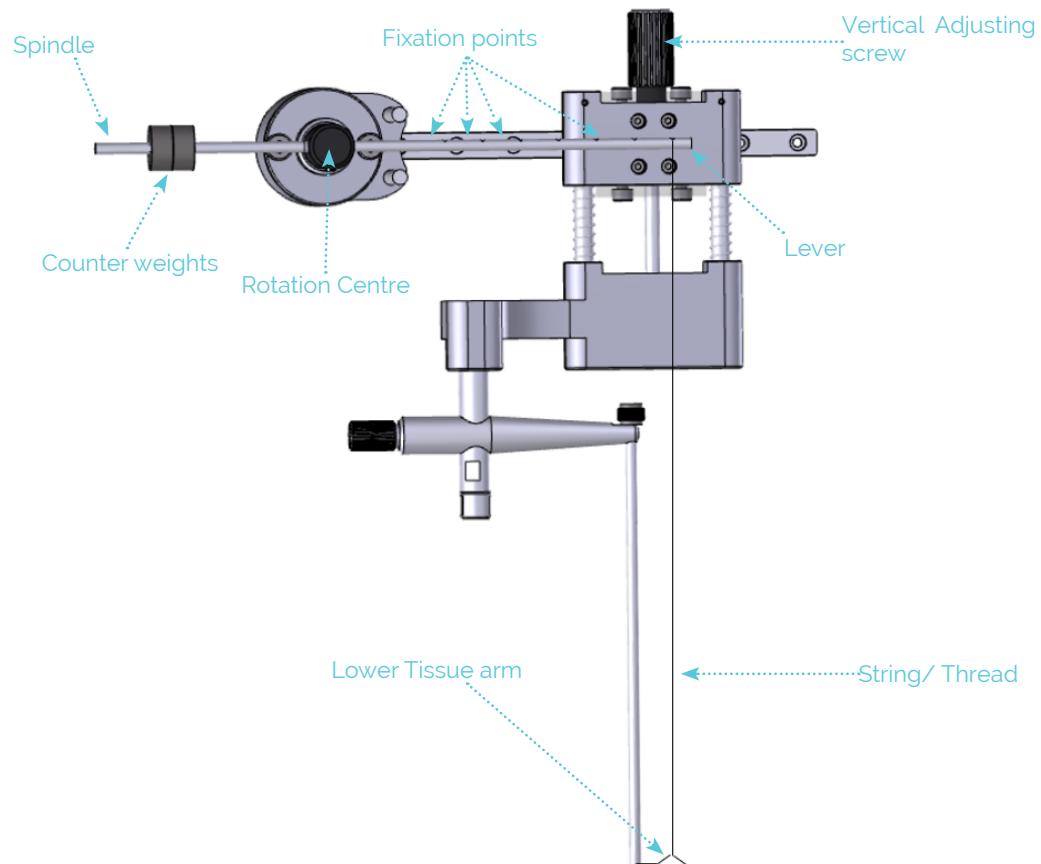


Figure 66: Schematic Diagram of the isotonic transducer. Lever length is 95mm with 7 fixation points at 10mm intervals. The first fixation point is 30mm from the rotation centre, the last point is 90mm from the rotation centre.

4.2.1 CALIBRATION OF THE ISOTONIC TRANSDUCER

This procedure is very essential to the start of every experiment. The calibration units of displacement can be displayed in meters, mm or μm depending on the choice of the user. The example shown in figure 66 for measuring the seventh fixation point which is 90 mm away from the rotation center. The procedure is same for the other fixation points.

Step 1: Turn ON the 750TOBS system. Start the TOBS 750 Control Software. Go into the Setup menu and into the Force Setting sub-menu of the TOBS 750 Control software

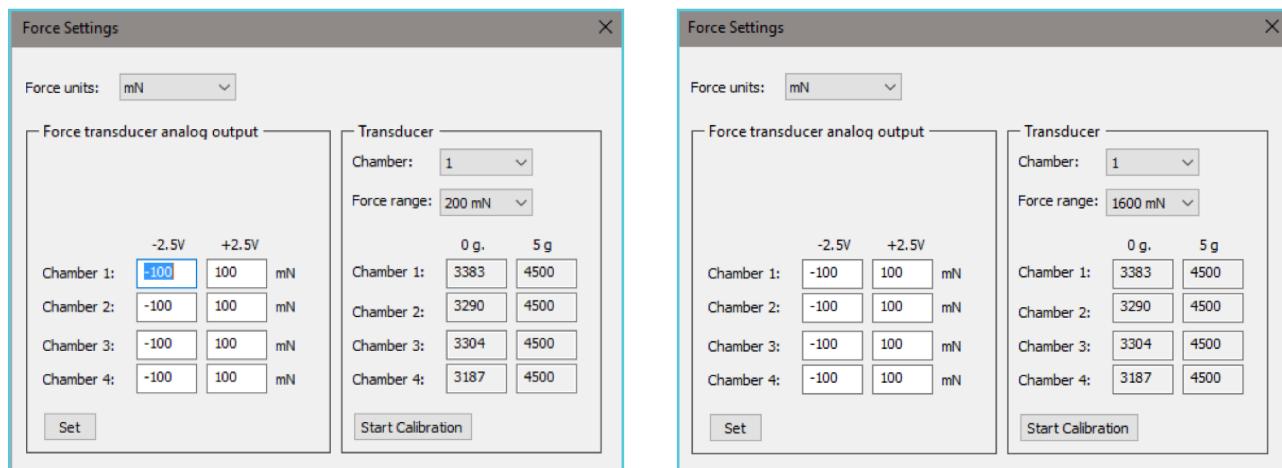


Figure 67: Before doing the Isotonic transducer calibration the Force Range has to be set at 1600mN for all four chambers.

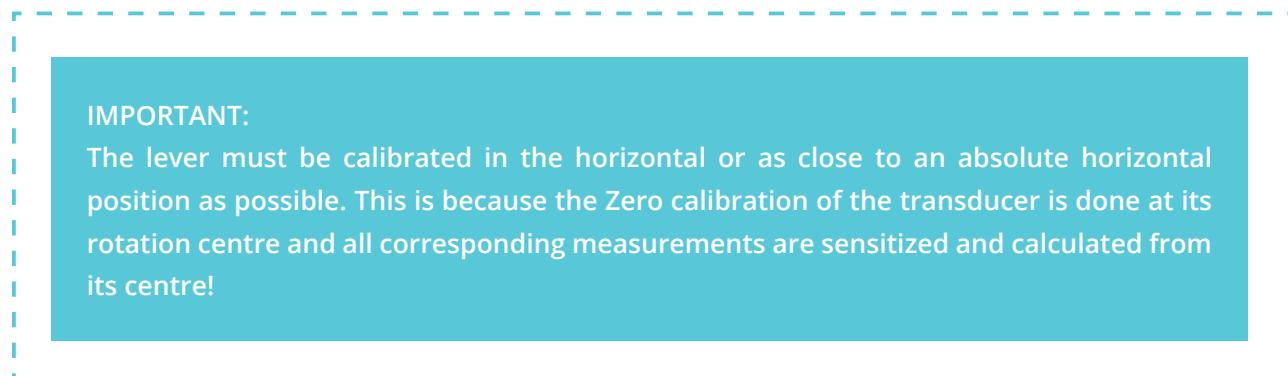
Set the Force range to 1600mN for all four chambers on the 750TOBS system (figure 67).

Start LabChart data acquisition (digital) or an appropriate analog Data acquisition software (BNC connections on the 750TOBS system).

Step 2: Fix and align the lever in the position as depicted in the figure 66 such that the lever orientation is horizontal in its position over the chamber. (This can be done by the fixation screw present just behind the 'Vertical adjusting screw' parallel to the lever.) The fixation point of the lever chosen should be directly above the center of the chamber below.

Step 3: Next, fix the lever to the lower tissue arm with a piece of string or thread. Adjust the counterweight provided on the other side of the rotation center such that the tension on the string is set to maximum until taut (figure 66).

Step 4: Take great care in adjusting the lever so that it is fixed in the horizontal position when seen at eye-level by the 'Vertical Adjusting screw' present above the lever as depicted in figure 66.



Step 5: Now, go into LabChart (figure 69), or a similar data acquisition system and start collecting data. Select the All and then click on the Zero force in the main control windows as shown in figure 68-69.

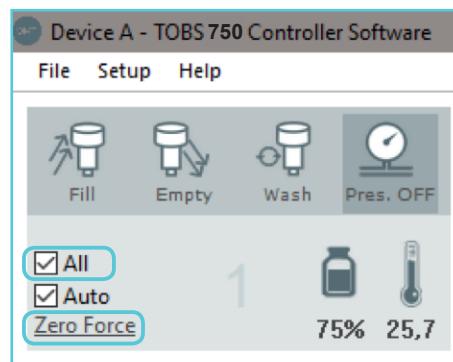


Figure 68: Select All and press Zero Force.

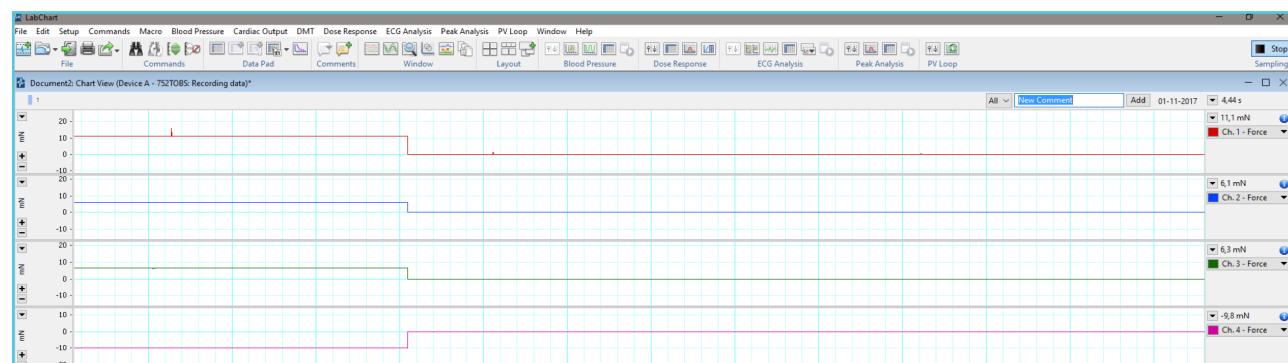


Figure 69: Labchart trace when Zero Force is pressed.

Step 6: Once the 'Force zero' trace is steady, turn the vertical adjusting screw exactly 3 turns in the clockwise (Contraction will result in negative values) or anticlockwise direction (Contraction will result in positive values). One turn of the screw is equal to 0.35 mm and therefore three turns corresponds to a 1.05 mm displacement. Use the small pinol screw for orientation when turning 3 rounds (figure 70).

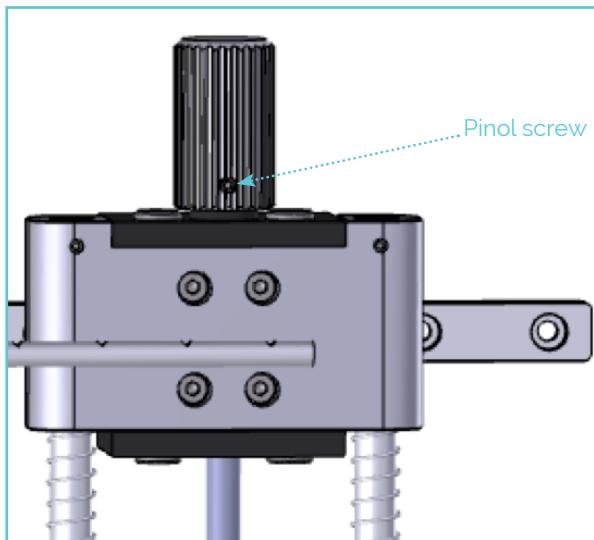


Figure 70: Pinol screw can be used for orientation when turning the positioner 3 turns



WARNING: The micrometer positioner could have a small slag when shifting from clockwise to counterclockwise rotation and vice versa. This has been intentionally manufactured to reorient the movement as desired by the user and reset the orientation whilst maintaining the calibrated instrument to the new orientation, it is not any defect of the micrometer positioner. It is easy to feel when the micrometer positioner starts to move the transducer.

NOTE: Only for DMT Device Enabler and LabChart Users: (Step 7 to 11)

Step 7: Stop the Trace, select the data trace from zero to the new trace reading after the 1.05mm displacement change, the region should appear grey (figure 71).



Figure 71: Labchart trace when Zero Force is pressed.

Step 8: Next, select 'Units Conversion' (figure 72).

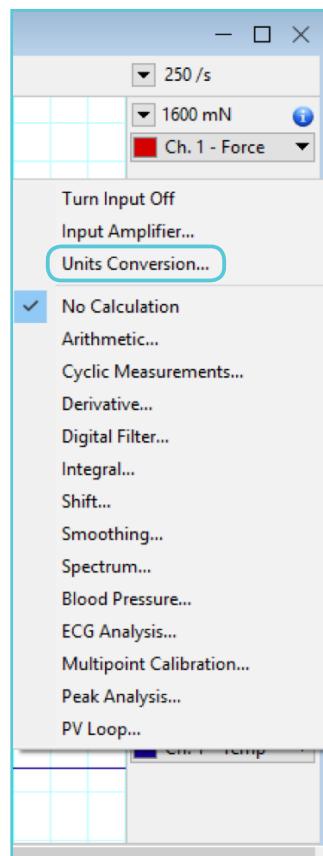


Figure 72.

Step 9: Next, select '2 Point Calibration', mark out the zero part of the trace and click the arrow for 'Point 1' (figure 73).

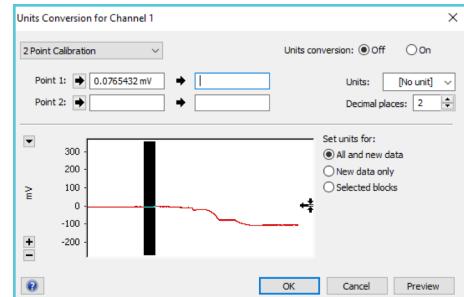


Figure 73.

Step 10: Mark out the new position of the trace that corresponds to 1.05 mm and click the arrow for 'Point 2' (figure 74).

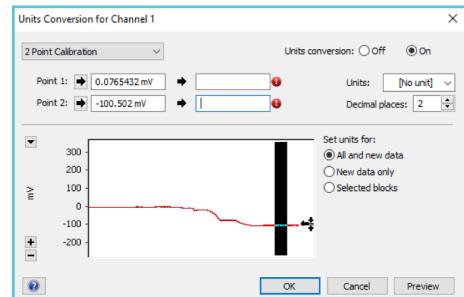


Figure 74.

Step 11: Next, in 'Units', select 'mm' or select 'Define unit' and enter units if your units of choice are not present in the default units drop down menu (figure 75).

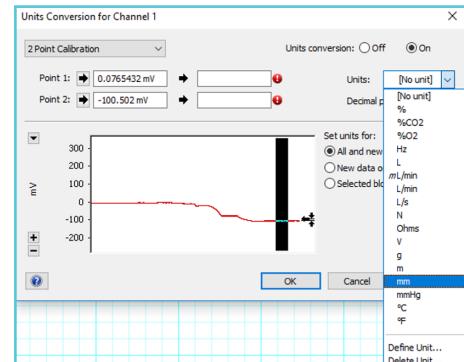


Figure 75.

Step 12: Now, Set the units for Point 1 and Point 2 as 0 and 1.05 respectively (if mm should be the unit) and click OK. The number of decimal places for the units can be edited according to preference. The transducer is calibrated and ready for the experiment (figure 76).

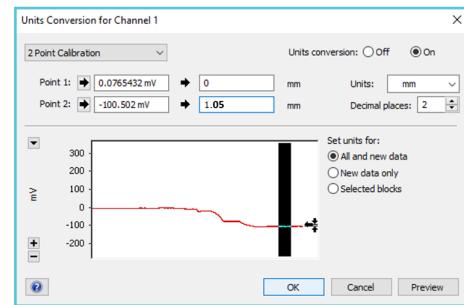


Figure 76.

IMPORTANT:

The fixation point at which the tissue is mounted for the experiment must be the same point at which the calibration was done. If a different fixation point is desired for the tissue, then the calibration at this position needs to be done.

NOTE: Users that do not use LabChart data Acquisition system have to follow the guidelines of the appropriate data acquisition system of how to calibrate the data acquisition system.

The Isotonic transducer is now ready for use. Calibration of all four chambers is performed accordingly.

CHAPTER 5 - EXPERIMENTAL SET-UP

Prior to beginning any experiment, the buffers must be pre-heated before use in the 750TOBS system either in a water bath or an external heating source to bring the temperature of the buffers to e.g. 37°C.

NOTE: The internal heating system for the buffer flasks is only for sustaining a selected temperature. It cannot be used for heating up the buffer from fridge or room temperature to 37°C.

Ensure that the Buffer bottles in the 750TOBS are filled with the appropriate buffer and enter the added buffer volume of each bottle in the Buffer Start Volume sub-menu (see figure 77).

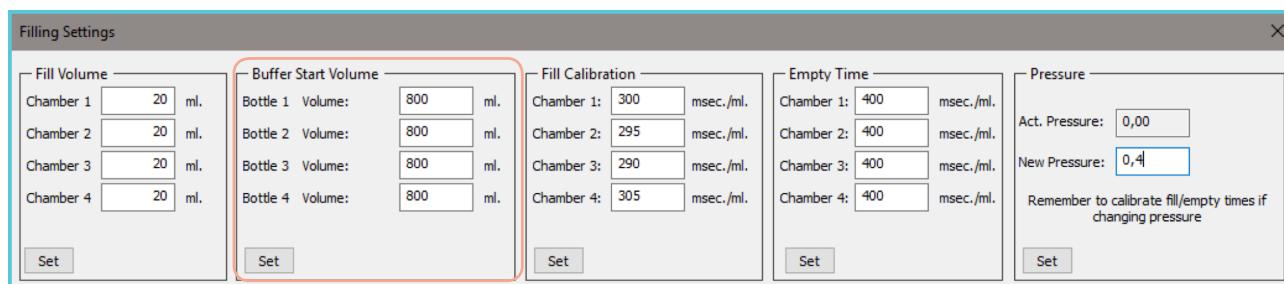


Figure 77: Setting the Buffer Start Volume before starting the experiments

Ensure the chamber is secured and chamber temperature has been set through the Temperature Setting sub-menu and make sure that the correct Temperature difference values has been entered to obtain the correct 'Actual temperature' (figure 78).

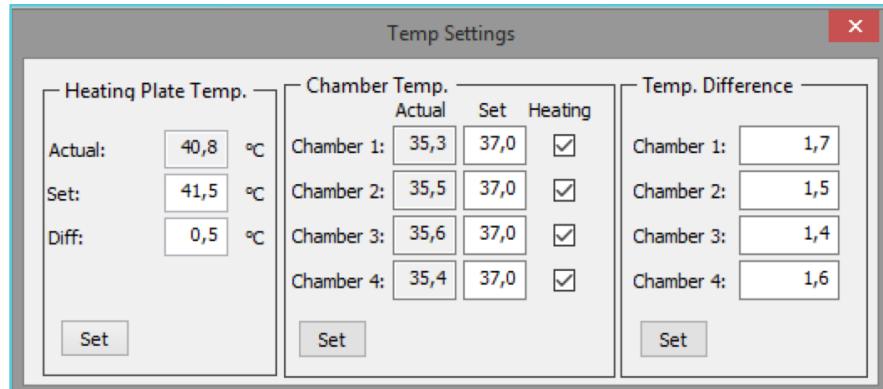


Figure 78: Setting the Temperature and Temperature Difference

On the rear panel, ensure that the vacuum trap is connected to the Vacuum port, and the USB cable to the computer with Labchart 8 Pro and the DMT device enabler installed, the oxygen/carbogen tubes are connected to their respective bottles, the carbogen pressure source (max 10bar) connect to the OXYGEN port.

Users of the analog output signal from the BNC connectors. Connect the BNC cables to the appropriate data acquisition system.

Turn on the Pressure by pressing the Pres. OFF icon and it will change to Pres. ON. (Figure 79)

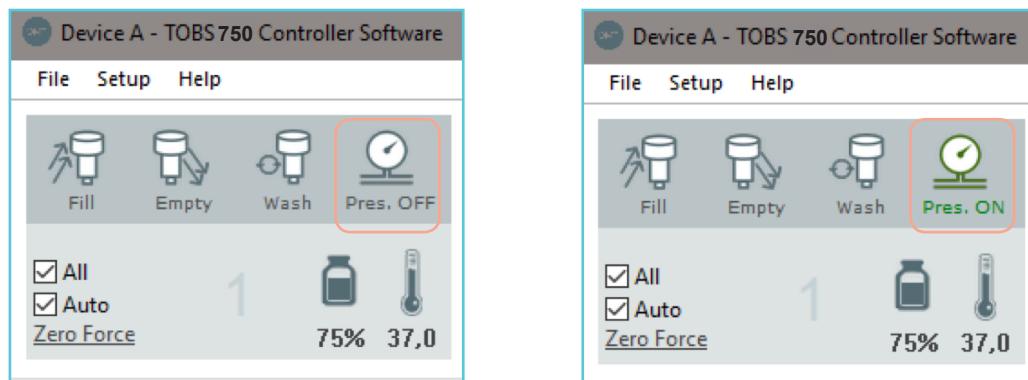


Figure 79: Set Pressure ON

Fill the chamber with the appropriate volume of buffer and make sure that the right actual temperature is reached before mounting your tissue. If using the Fill/Empty function of the 750TOBS system in the Auto mode then make sure the Fill Calibration and Empty Time have been performed (Section 3.1.2.2).

5.1 MOUNTING

DMT have a variety of different mounting supports for mounting of tissue strips and ring preparations. Please ask your DMT sales representative for guidance. Here below is a very short description of mounting a muscle strip. Secure one end of the muscle tissue and with e.g. a suture or thread on the hook of the lower tissue holder immersed in the chamber. The other end of the muscle tissue must be fastened and hung on the pin of the isometric transducer.

NOTE: The pin tip of the isometric transducer is extremely fragile, great care must be taken when mounting of the tissue on this, and must not be knocked or hit with any other sharp or blunt tool nor subjected to any rough use to avoid damage or breakage. More than 200g overload on the transducer will permanently damage the transducer.

Make sure that the mounted tissue has the correct pre-load after mounting. Please read the DMT Normalization Guide which contains a good description of how to identify the optimal pre-load tension for your type of tissue. The Normalization Guide can be found on the DMT home page or use the link below.

https://www.dmt.dk/uploads/6/5/6/8/65689239/dmt_normalization_guide.pdf

Now your experiments can be initiated.

CHAPTER 6 - MAINTENANCE

6.1 TISSUE ORGAN BATH MAINTENANCE

DMT recommends that the following sections are read carefully and that the instructions are followed always.

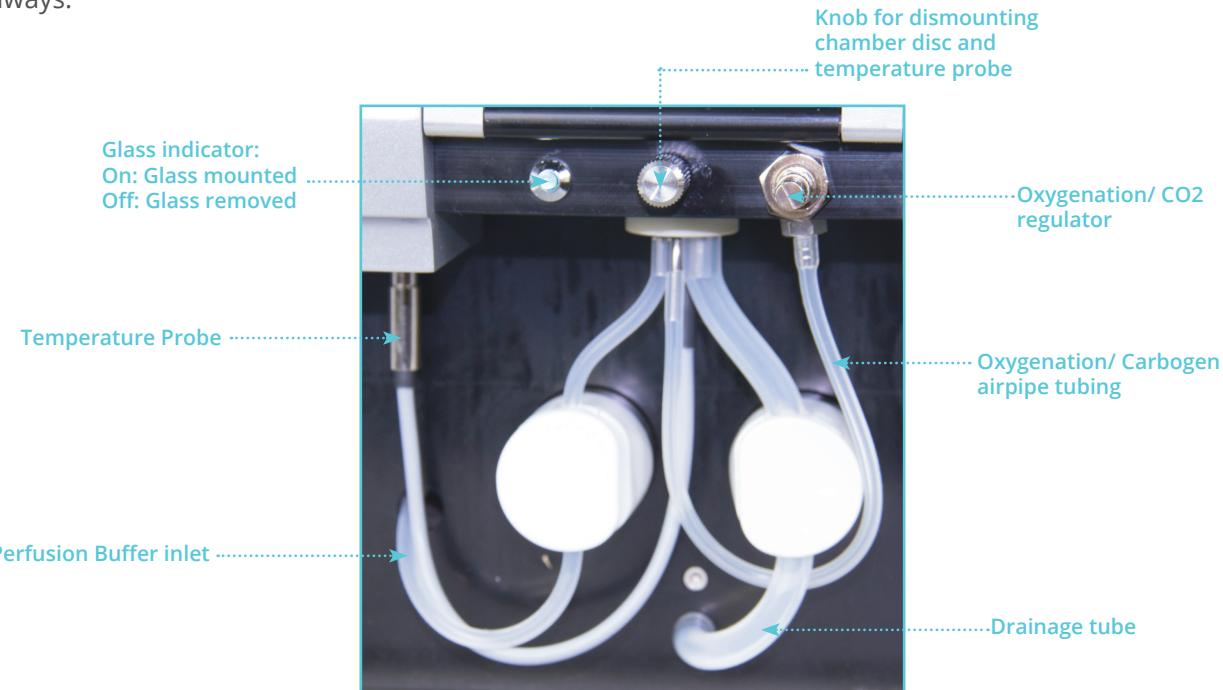


Figure 80: Tubes connected to the Chamber

The tubing can be replaced if unknown toxic chemicals, or if any kind of hydrophobic agents has been used.

The following tubes are changeable:

- Perfusion Buffer inlet
 - Oxygenation/Carbogen airpipe tubing
 - Drainage tube
-
- **The Perfusion Buffer inlet tubes** can be changed by unplugging the tubes from the bottle tops, then opening the chamber door and unplugging the tube from its port through. The new tube can then be pushed through from the top of the unit marked 1, 2, 3 or 4 and then plugged into its respective inlets as 1, 2, 3 and 4 as shown in the figure. Take great care in ensuring that the connections to buffer 1, 2, 3 and 4 are correctly secured as shown in figure 80.

- **The Oxygen Carbogen regulator** must be greased over time to ensure smooth and efficient tuning of the gaseous supply.
- **The Temperature probe and chamber disc** can be dismantled and cleaned over time by turning the knob anticlockwise and pressing down on the disc for dismounting them.

After each experiment the following cleaning procedure is recommended:

1. Turn Pressure OFF
2. Use clean Schott Duran bottles
3. Fill with distilled water
4. Turn Pressure ON
5. Rinse the silicone tubing with double distilled water by activating the Fill and Empty Control routine several times.
6. Change the tubing after the use of unknown or toxic chemicals, or if hydrophobic reagents have been used.
7. Carefully dismount the tissue bath chamber and perform a normal glass wash.
8. If aggressive solution or drugs have been used it is possible to disconnect the temperature probe and remove the chamber disc. Both can be cleaned with distilled water, finally dry with a paper towel.

In exceptional cases, it may be necessary to demount the sample holders for cleaning to make sure that all surfaces are thoroughly cleaned.

IMPORTANT:

It is of great importance that the tubing used is the original tubing from DMT, the quality, diameter and length are significant to the chamber - filling mechanism.

NOTE: Regarding the four oxygenation regulators: The regulators need to be greased at least twice a year. Also, ensure that the regulators are turned at regular intervals to prevent them from sticking.

APPENDIX A

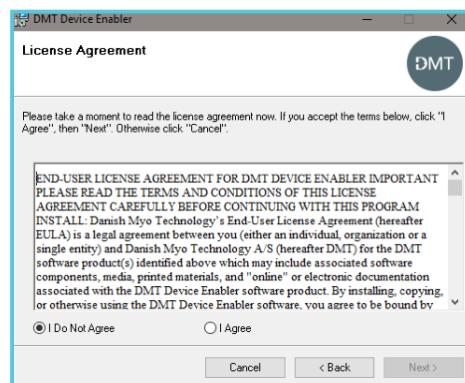
INSTALLATION OF THE DMT DEVICE ENABLER AND THE 750TOBS CONTROL SOFTWARE.

THE DMT DEVICE ENABLER SOFTWARE

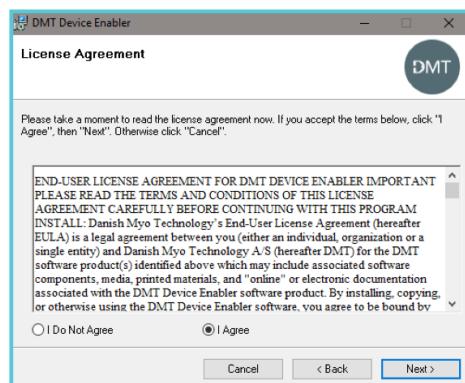
Step 1: Double click on the program file DMTDeviceEnabler.



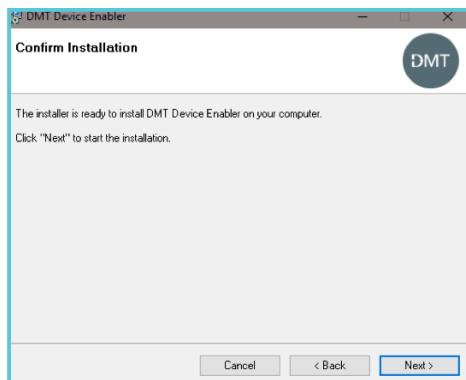
Step 2: Press Next.



Step 3: Select I Agree. Press Next.

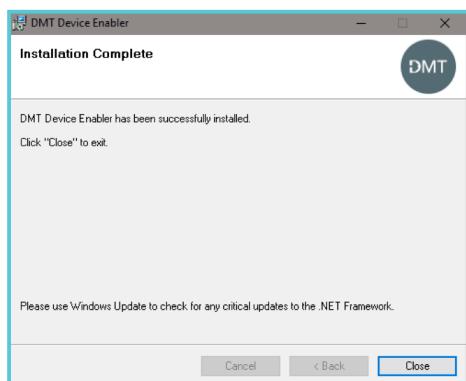


Step 4: Press Next. Wizard is complete. Click 'Install'



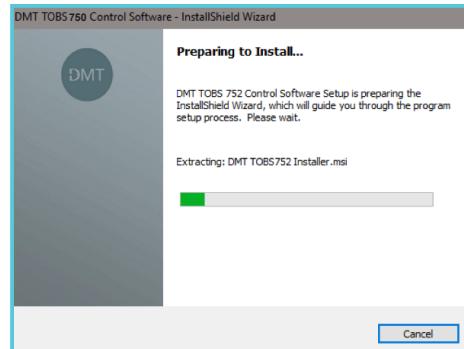
Step 5: Accept that the program is installed on the computer.

Press Close.

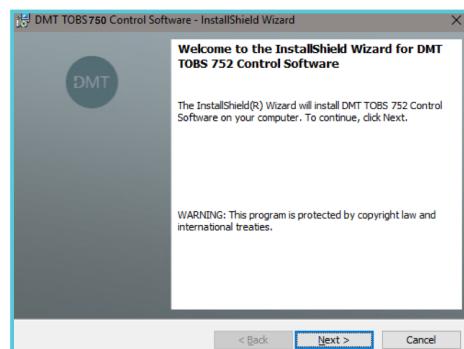


THE TOBS 750 CONTROL SOFTWARE

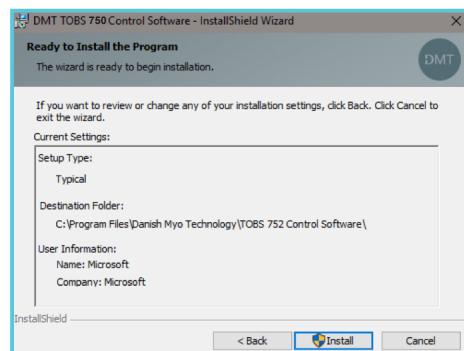
Step 1: Double click on the program file DMT TOBS 750 Installer



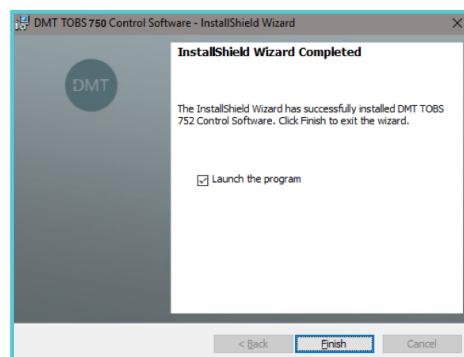
Step 2: Press Next.



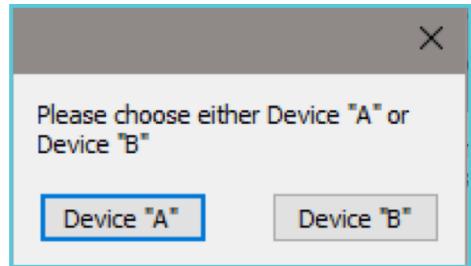
Step 3: Press Install.



Step 4: Press Finish to complete the installation of the TOBS 750 Control software.



Step 5: If there is only one 750TOBS System of 4 channels connected then select Device "A", if there are two 750TOBS Systems, then select either Device "A" or Device "B".



APPENDIX B

DATA ACQUISITION USING THE DIGITAL OUTPUT AND LABCHART DATA ACQUISITION SOFTWARE.

1. Make sure the DMT device enabler and the TOBS 750 Control software is installed on the computer (Appendix A).
2. Install Labchart 8 Pro on the computer
3. Connect the 750TOBS system with the computer using the USB cable.
4. Turn on the power of the 750TOBS system
5. Start the TOBS 750 Control Software. Make sure the Control software is connected to the 750TOBS system e.g. by pressing the Pressure Off button and see if the system responds.
6. Start Labchart 8 Pro. Labchart will now search for a 750TOBS system (figure 81A) and if two 750TOBS system is connected to the same computer then Labchart will find both system (figure 81B).

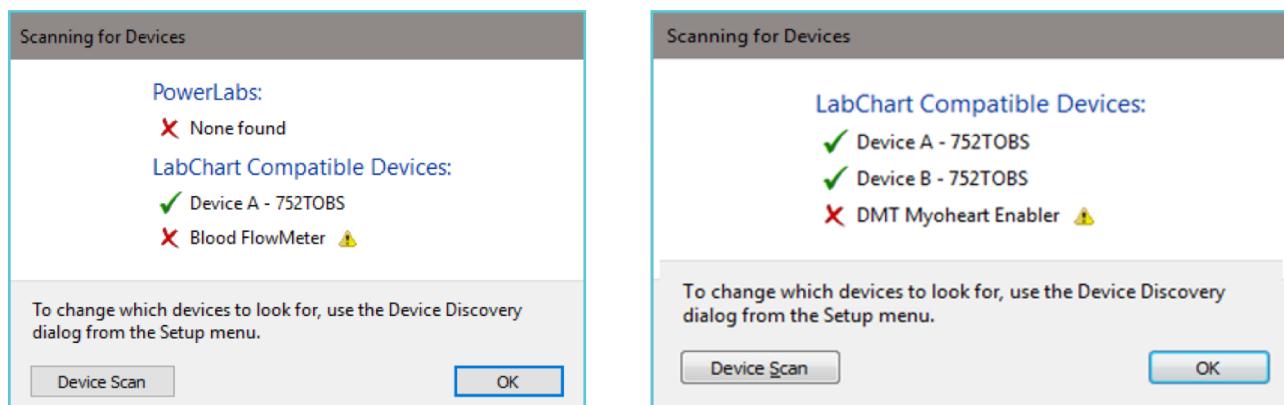


Figure 81A-81B. Labchart will find the 750TOBS systems connected to the computer.

7. Press OK. If Labchart does not find the 750TOBS system then check the USB connection between the computer and the 750TOBS system. Make sure the power is ON. Check if the TOBS 750 Control software is started. Then restart Labchart.
8. The Labchart Welcome center will turn up. Close it (figure 82).

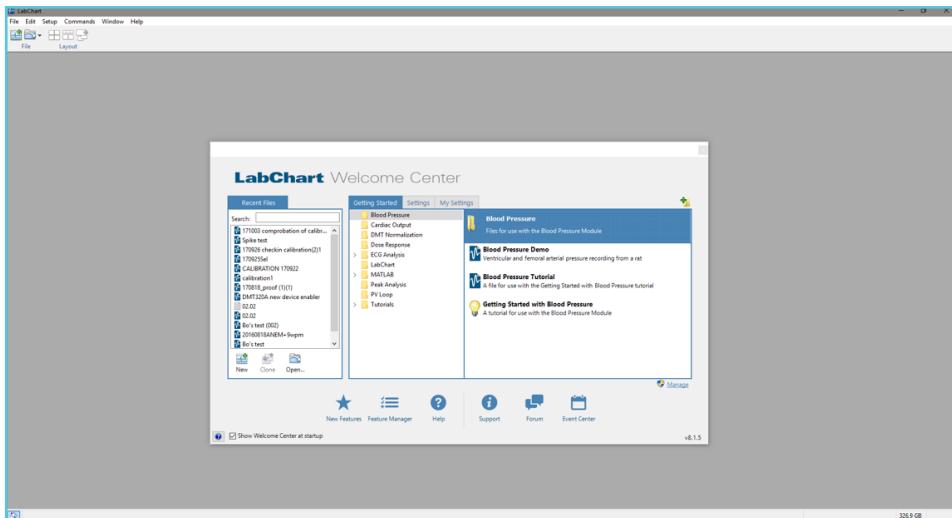


Figure 82.

9. Labchart will now show the force of the four chambers in channel 1-4 and also the temperature in channel 5-8 (figure 83).

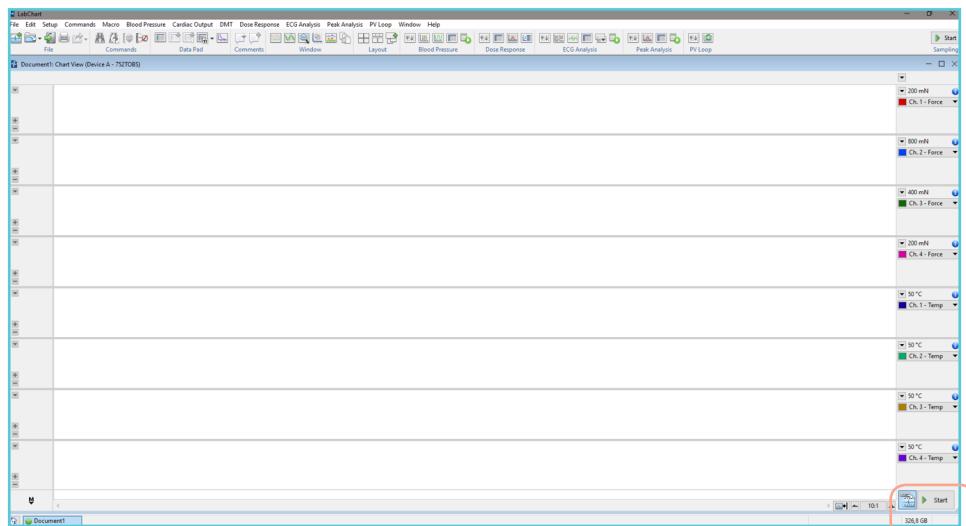


Figure 83: Labchart showing the force and temperature of the four 750TOBS chambers. Press start to start the trace recordings (red box).

10. The Labchart data acquisition system is now ready for recording the force and temperature from each of the four channels by pressing the Start button in Labchart (figure 83, 84).

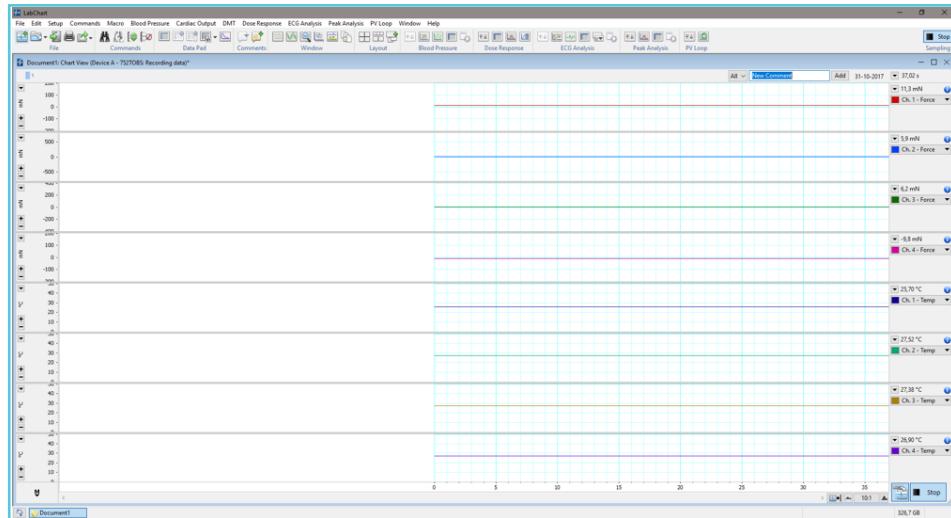


Figure 84

11. If two 750TOBS are connected to the computer go into the Setup menu and select the Devices and Channels menu (figure 85).

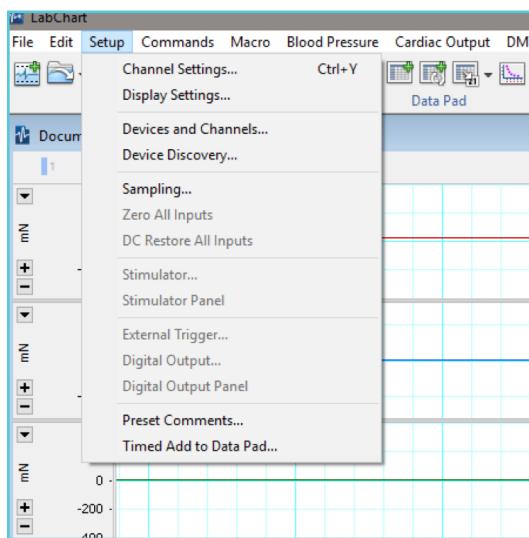


Figure 85: Select the Devices and Channels in the Setup menu

12. Select Device B – 750TOBS as shown in figure 86.

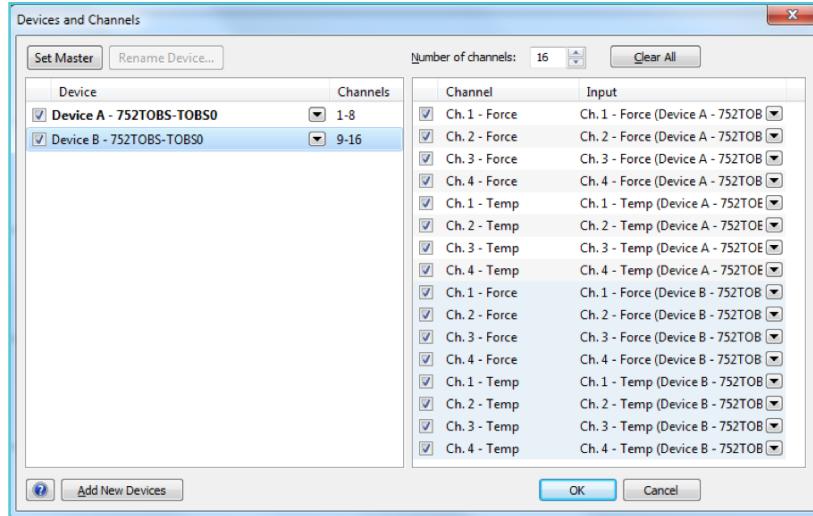


Figure 86: Select Device B – 750TOBS to add the 8 channels of Device B – 750TOBS to Labchart.

13. Now all channels of Device A and B, 16 channels in total will be shown in Labchart (figure 87).

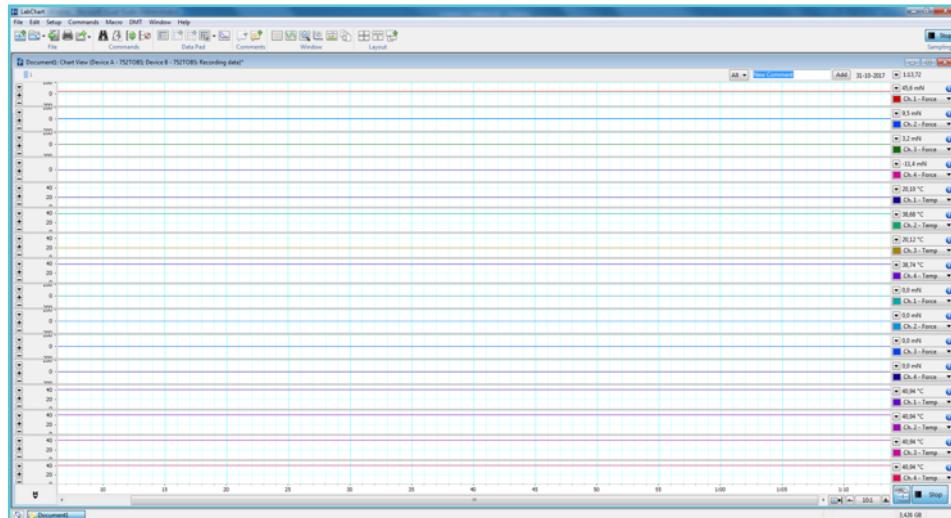


Figure 87: Labchart with two 750TOBS system connected to the same computer.

APPENDIX C

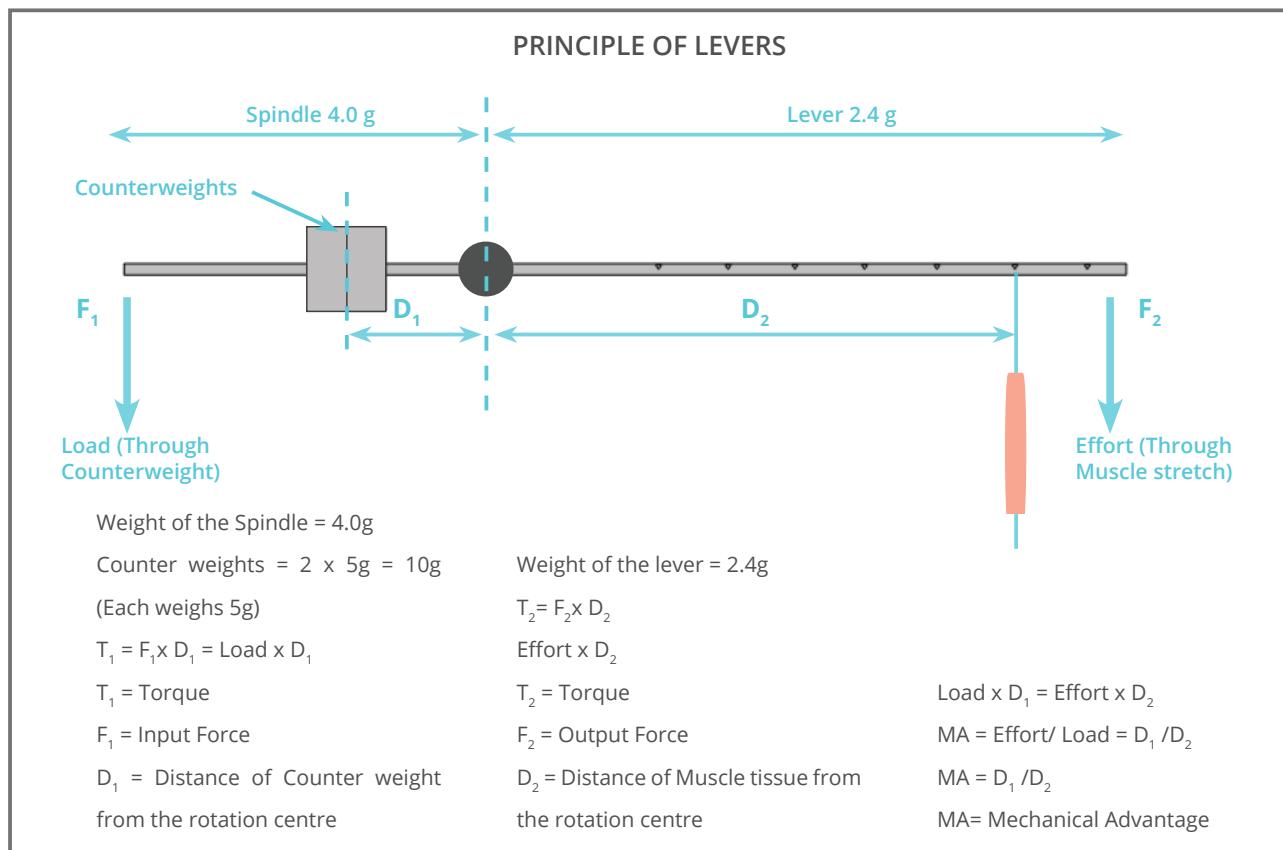
PRE LOAD FOR THE ISOTONIC TRANSDUCER

Secure one end of the muscle tissue to the lower tissue holder with a suture or thread and the other end to the same lever fixation point at which the calibration has been carried out.

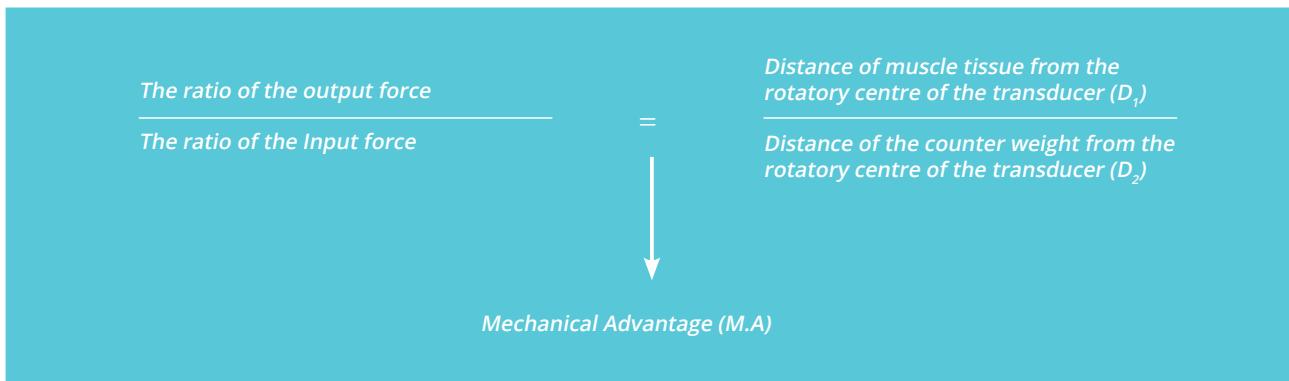
NOTE: It is very important to use exactly the same fixation point. If changing the fixation point, the transducer needs to be re-calibrated.

Begin the trace by clicking  in Labchart.

The muscle tissue can be preloaded with a desired weight or be mounted without any additional load before determining the maximum load at which the muscle contracts.



Most muscle contractions are based on the principle of the 3rd order lever. However, the load in this case is present between the rotatory center of the transducer and the muscle (effort) and therefore uses the principle of the 2nd order lever.



SETTING A PRE-LOAD ON THE SYSTEM:

Obtaining a specific pre-load on the system can be achieved in 2 ways:

1. **Using different known weights of desired pre-loads (5g here):** First adjust the position of lever for a desired mounting distance on the lever (D_2). If one desires to mount the muscle tissue in the hole on the lever that is 70 mm away from its rotation center, a known 5g weight must be hung at this point on the lever. As the counterweights, together are 10g on the spindle, adjusting this counterweight until balanced will give the user a pre-load of 5g on the system. (Refer to Figure above on the 'Principle of Levers')

Here below is the calculation of the D_1 distance to get a 5g pre-load when mounting a muscle strip on the lever 70mm away from the rotation center.

$$\text{Total weight on spindle} = (\text{Weight of Counterweights}) + (\text{Weight of the spindle})$$

$$14\text{ g} = 10\text{ g} + 4\text{ g}$$

$$\text{Total weight on lever} = (\text{Weight of appropriate pre-load}) + (\text{Weight of the lever})$$

$$7.4\text{ g} = 5\text{ g} + 2.4\text{ g}$$

The desired mounting distance of the muscle strip is 70 mm so place the Preload of 5g on the lever at,

$$D_2 = 70 \text{ mm}$$

$$\frac{\text{Mechanical advantage}}{\text{Load}} = \frac{\text{Effort}}{7.4 \text{ g}} \Rightarrow \frac{14 \text{ g}}{7.4 \text{ g}} = \frac{D_1}{70 \text{ mm}} \Rightarrow D_1 = 37 \text{ mm}$$

Setting a new pre-load: For any given muscle tissue, setting a desired pre-load on the muscle can be achieved as follows.

- Determine the position (D_1) of the counterweight on the spindle from its rotation center without changing the new pre-load position on the lever.

Eg: If a new pre-load of 7.5g is desired, the effort = 7.5g + 2.4g, the counterweight is constant at 10g + 4g on the spindle and must be positioned as follows.

$$\frac{\text{Mechanical advantage}}{\text{Load}} = \frac{\text{Effort}}{9.9 \text{ g}} \Rightarrow \frac{14 \text{ g}}{9.9 \text{ g}} = \frac{D_1}{70 \text{ mm}} \Rightarrow D_1 = 49.5 \text{ mm}$$

$D_1 = 49.5\text{mm}$ as calculated from the relation above.

In this way, the maximum or minimum pre-load and the distance at which the pre-load or load must be placed from the rotatory center of the transducer can be determined by adjusting the distance where the muscle tissue must be positioned on the opposite side of the lever.

2. Obtaining the correct pre-load by using the 5g weight applied with the system for calibration of the appropriate pre-load.

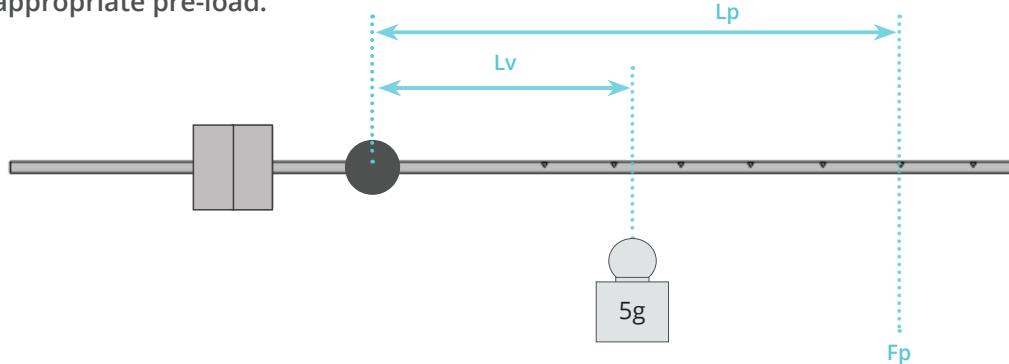


Figure 5.1 Using the 5g weight for setting the appropriate pre-load on a muscle strip.

The following formula should be used to find the correct distance (L_v) at which the 5g weight must be placed from the rotation centre to obtain a given pre-load

$$L_v = \frac{F_p * L_p}{5}$$

Where;

L_p : Distance of new desired Pre-load from the rotation center of the transducer

L_v : Distance of Pre-load of 5g weight from the rotation center of the transducer

F_p : The pre-load wanted to be applied to the mounted muscle at the given position of the lever.

Here below is an example of how to use a single fixed known pre-load weight (5g here) for obtaining different desired pre-loads and in this example pre-load of 2.75g is wanted:

First, choose a given hole on the lever where the muscle tissue must be mounted. In the above diagram, its demonstrated as the 6th hole, which is 80 mm from the rotation center of the transducer. So $L_p = 80$ mm and $F_p = 2.75\text{g}$.

$$L_v = \frac{2.75 * 80}{5} = 44 \text{ mm}$$

This results in a $L_v = 44\text{mm}$.

The 5g weight must now be hung on the lever 44 mm from the rotatory center. Next, adjust the counterweights on the spindle until balanced and the trace shows the value zero. Now the pre-load would be 2.75g when a muscle strip is mounted in the 6th hole of the lever.

APPENDIX D

FUSE REPLACEMENT

The main fuse of the 750TOBS is placed inside the power inlet on the 750TOBS . If the fuse blows it is easily changed using the following procedure.

IMPORTANT:

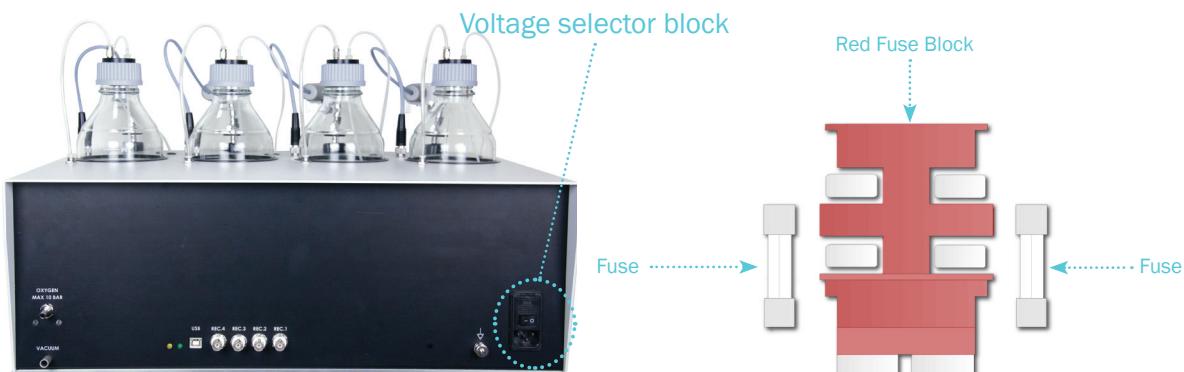
When a fuse blows, and needs to be changed, it is imperative that the replacement fuse is equal to the one blown.

DMT recommends that both fuses in the fuse block are changed at the same time, as it can be difficult to determine which fuse is blown.

To replace the fuses:

1. Open the voltage selector block using a small screwdriver.
2. Remove the red fuse block.
3. Remove the existing fuses.
4. Insert the new fuses.
5. Replace the fuse block and secure it back into the voltage selector block.

NOTE: Ensure that the correct voltage for your country is displayed.



APPENDIX E

HOW TO REPLACE AND CONNECT THE ISOMETRIC TRANSDUCER TO THE 750TOBS SYSTEM

If the force transducer is broken and has to be replaced remove the broken transducer by loosening the clamp/ring around the transducer house (figure A).



Figure A: The Isometric transducer must be connected as shown in the figure A by sliding it through the circular space and sliding it along the grooved space through its notch.

Replace the broken transducer with the new transducer. Tightening the transducer holder to secure the transducer (figure B).

Screw to loosen
or tightening the
transducer holder

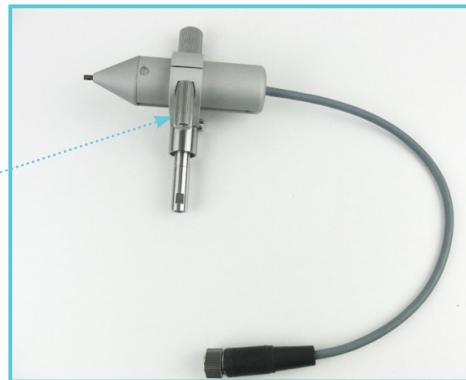


Figure B: The complete isometric transducer that is now ready to be mounted on the 750TOBS system.

Secure the Isometric transducer holder onto the 750TOBS system and secure the transducer pin in the center of the chamber (figure C).

Screw to loosen
or tightening the
transducer holder



Figure C: The complete isometric transducer that is now to be mounted on the 750TOBS system.

APPENDIX F - SPEC SHEET

CHAMBER:	
Chamber volume (min)	10, 20 (*1) or 50 ml
Chamber(s)	4
Chamber material	Glass
Mounting type	Pins, clips or hooks
Drain/fill	Semi-Automatic
Aeration	Yes
TEMPERATURE:	
Range	< 50.0 °C
Resolution	0.1 °C
Heating	Built-in air circulation
TRANSDUCER ISOMETRIC:	
Range	±200/±400/±800/±1600 mN
Resolution	0.1 mN
Force calibration	Yes
TRANSDUCER ISOTONIC:	
Angle Range	+/- 15° above and below horizontal axis. Movement of +/- 23.3mm above and below horizontal axis, at the last fixation point 90mm from the rotation center
Resolution	0.09°
Operating temperature	0 - 50°C
Breakaway Torque	max. 0.5g/cm
Lever length	95 mm with 7 fixation points at 10 mm intervals. The first point is 30 mm from the rotation center, the last point is 90mm from the rotation center
Counterweight	2 x 5g + 4g of the spindle. Total weight for the lever and spindle is 6.4g
Counterweight scale	0 - 5 g on lever at the last fixation point (90mm)
Lever weight	2.4 g
OUTPUT:	
Data communication	USB 2.0
Analogue output channels	4
Analogue output range	±2.5 V
RESERVOIR	
Amount	4 bottles
Volume	800 ml (x4)
Heating	Electronical - built - in
Temperature	< 40.0 °C
Temp. resolution	0.1 °C
Temp. stability	±1.0 °C
*1 20 ml chambers supplied as standard	

