MYOGRAPH SYSTEM - 630MA

USER GUIDE VOL. 3.0

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CONTENTS

CHAPTER 1 - WIRE MYOGRAPH OVERVIEW 3

CHAPTER 2 - THE MULTI WIRE MYOGRAPH UNIT 4
2.1 CHANGING AND ADJUSTING THE MOUNTING SUPPORTS 4
2.2 CALIBRATION OF THE FORCE TRANSDUCER 8

CHAPTER 3 - EXPERIMENTAL SET-UP 9
3.2 MOUNTING PROTOCOL FOR LARGER ARTERIES 15
3.3 NORMALIZATION 17
3.4 STANDARD START 24
3.5 ENDOTHELIUM FUNCTION 25
3.6 IN VITRO EXPERIMENT 1: NORADRENALINE CONTRACTILE RESPONSE 26
3.7 IN VITRO EXPERIMENT 2: ACETYLCHOLINE RELAXATION CURVE 28

CHAPTER 4 - CLEANING AND MAINTENANCE 30
4.1 CLEANING THE 630MA MYOGRAPH SYSTEM 30
4.2 MAINTENANCE OF THE FORCE TRANSDUCER 33
4.3 MAINTENANCE OF THE LINEAR SLIDES 37

APPENDIX 1 - BUFFERRECIPES 38
PHYSIOLOGICAL SALINE SOLUTION (PSS) 38
HIGH POTASSIUM PHYSIOLOGICAL SALINE SOLUTION (KPSS) 40

APPENDIX 2 - NORMALIZATION THEORY 41
MATHEMATICAL CALCULATIONS 41

APPENDIX 3 - READING AMILLIMETRE MICROMETER 43

CHAPTER 1 - WIRE MYOGRAPH OVERVIEW

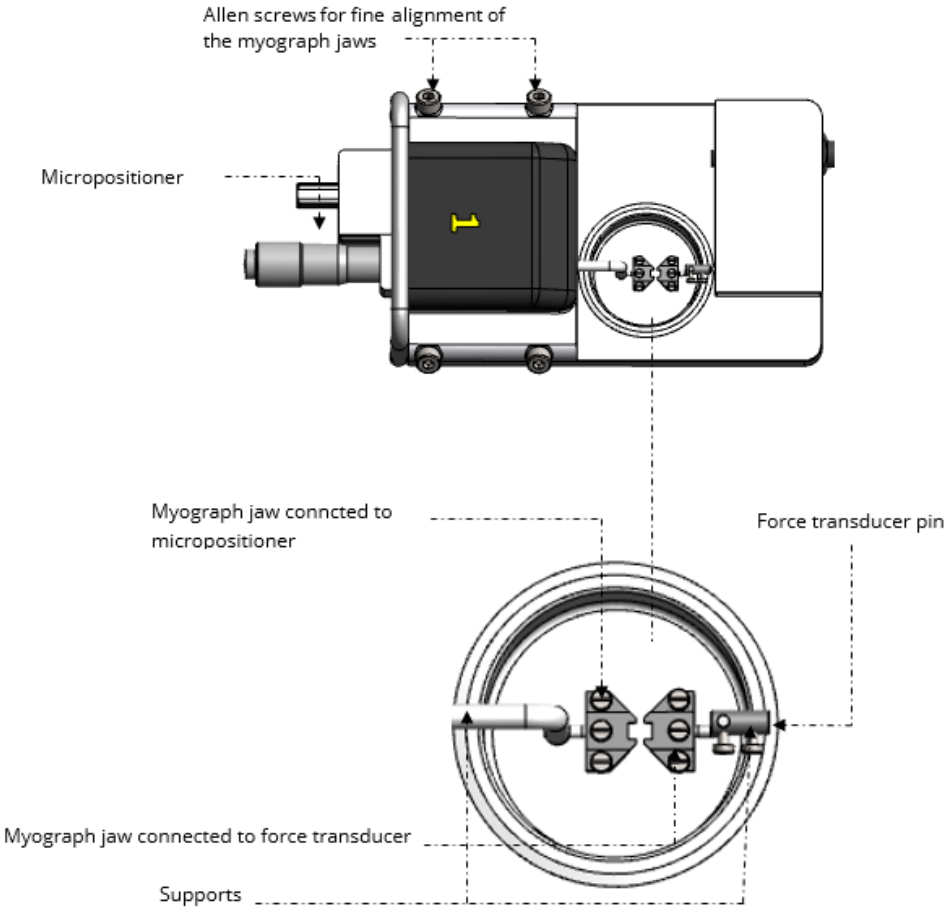


Figure 1.1 Close up of Multi Myograph unit and myograph jaws

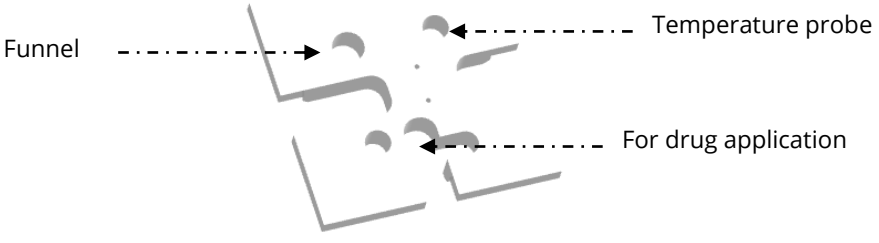


Figure 1.2 Chamber cover

CHAPTER 2 - THE MULTI WIRE MYOGRAPH UNIT

2.1 CHANGING AND ADJUSTING THE MOUNTING SUPPORTS

Each chamber can accommodate mounting supports for either small vessels (>30µm) or larger segments (>500µm). Because the mounting supports can be changed easily, experiments can be performed with different vessels of varying internal diameter. Continuous use and repeated greasing of the transducer pin holes will cause some misalignment of the mounting supports. The mounting supports, therefore, whether they are the jaws for wire or the pins, 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

2.1.1 CHANGING THE MOUNTING SUPPORTS (FIGURE 2.1)

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 transducer-side 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 fitting Allen key.
5. Pull the 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.

2.1.2 COARSE ADJUSTING THE JAWS FOR SMALL VESSELS (FIGURE 2.1)

1. Loosen screw A to move the micrometer-side jaw toward or away from the micrometer.
2. Loosen screw B to move micrometer-side jaw toward or away from the transducer and move the jaw sideways.
3. Loosen screw C to vertically align and rotate the transducer-side jaw. Screw C is the screw on the transducer- side support that is furthest away from the transducer.
4. Loosen screw D to move the jaw to the right or left on the transducer side.

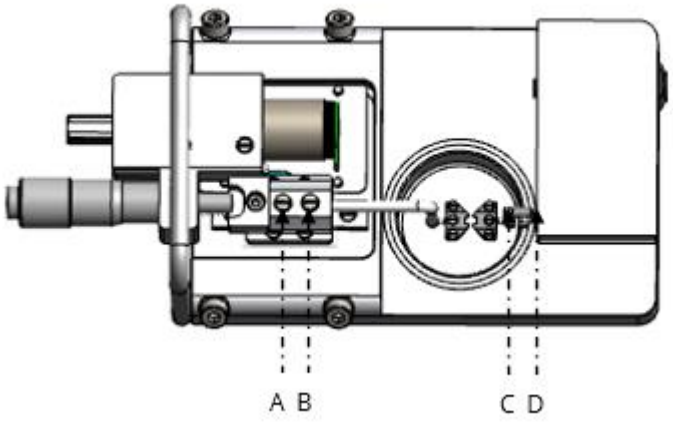
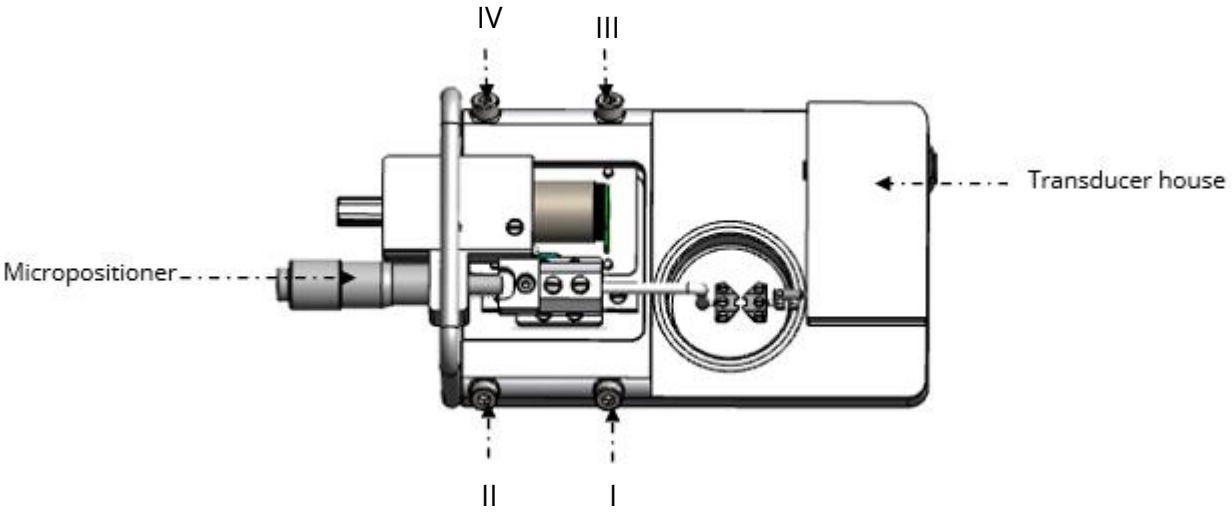
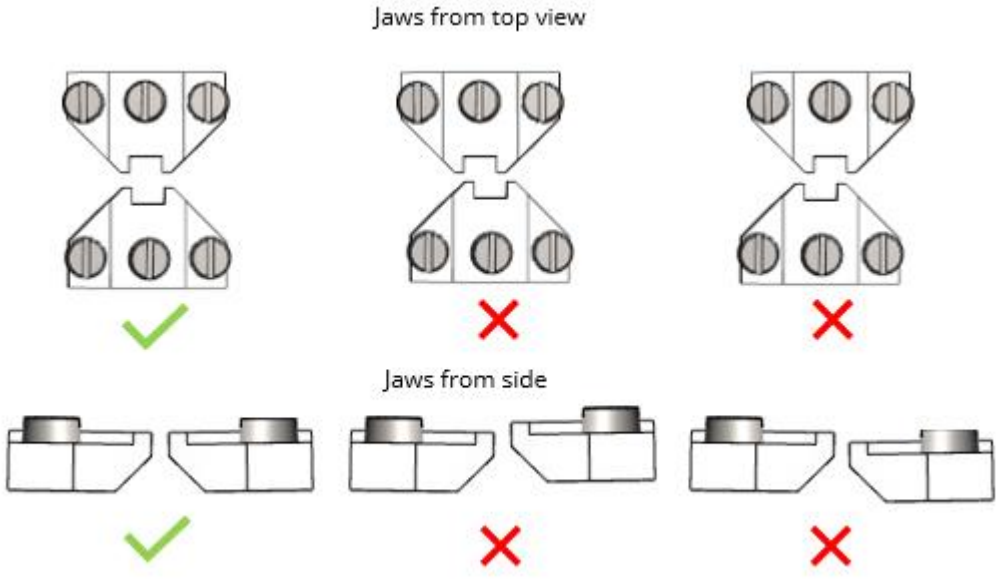


Figure 2.1 Illustration of the screws for changing supports and coarse adjustment of the jaws

2.1.3 FINE-ADJUSTING THE JAWS FOR SMALL VESSELS (FIGURE 2.2 AND FIGURE 2.3)

1. Tightening Screw “III” will move the micrometer side jaw downward and to the left.
2. Tightening both screws “III” and “I” will move the micrometer side jaw straight down
3. Tightening both screws “II” and “IV” will move the micrometer side jaw straight up.





2.1.4 FINE-ADJUSTING THE PINS FOR LARGER VESSELS (FIGURE 2.4 AND FIGURE 2.5)

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 and move the pins sideways.
3. Loosen screw C to align the micrometer-side pin vertically and rotate.
4. Loosen screws B and D to align the heights of the pins vertically.
5. Loosen screw E to move the pin left or right on the transducer side.

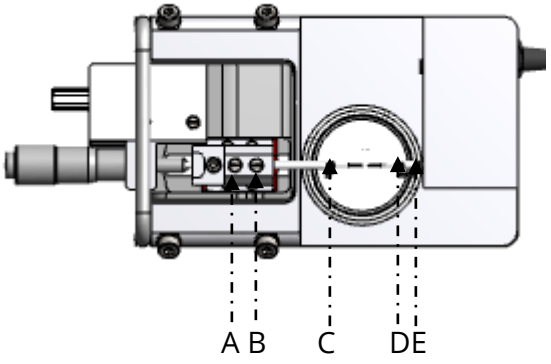


Figure 2.4 - Fine adjustments of the pins in the Myograph chamber

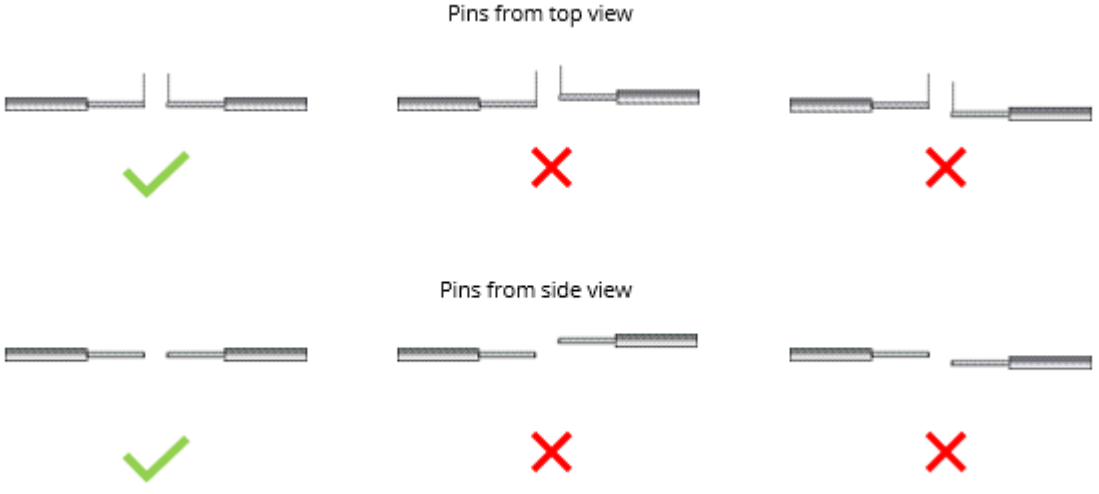


Figure 2.5 - Illustrations of properly aligned pins (depicted on the far left) and incorrectly aligned pins (depicted in the middle and far right).

2.2 CALIBRATION OF THE FORCE TRANSDUCER

As a part of the general maintenance of the Wire Myograph, DMT recommends that the Wire Myograph is force calibrated at least once a month. The Wire Myograph should also be force 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 Wire Myograph should also be calibrated if the system has been idle for longer than a month. A step-by-step procedure is explained in chapter 3 in Multi Myograph System User Manual.

CHAPTER 3 - EXPERIMENTAL SET-UP

This chapter contains experimental set-up for the Wire Myograph. For dissection of a vessel, please see Procedures for investigations of small vessels using a small vessel Myograph by M.J. Mulvany.

3.1 Mounting protocol for small arteries

The procedure involves attaching the mounting wires to jaws which are in turn mounted on the force transducer. This force transducer is capable of measuring with a sensitivity of about 0.01mN (1mg) but can be damaged if the applied force exceeds about 1N (100g). Therefore, care must be taken to avoid pressing the jaws too hard together. A movement of ~20µm after they have touched is sufficient to hold the wires clamped.

3.1.1 MOUNTING STEP ONE

- Cut lengths of 40µm wire ~2.2cm long. Mount one wire on left-hand jaw of the Wire Myograph as follows.
- Holding wire at far end, place center of wire between jaws and screw jaws together so that the wire is clamped (figure 3.1 A).

  NOTE
  DO NOT CLOSE THE JAWS TOO HARD AGAINST EACH OTHER.

- Bend the far end of the wire towards the left, and wrap it around under fixing screw, so the wire is wound clockwise: tightening the screw will then tighten the wire. This procedure should result in the wire being clamped between the jaws and with near end of wire pointing towards operator (figure 3.1 B-C).
- Fill the Wire Myograph chamber with PSS (at room temperature). See appendix 1 for example of buffer recipes.

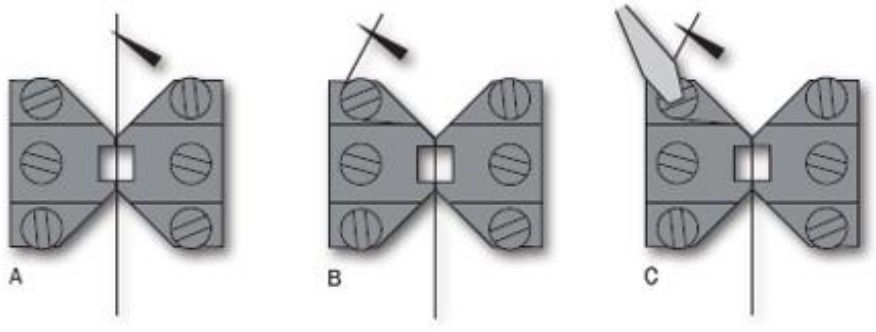


Figure 3.1 A, B and C Mounting step 1

3.1.2 MOUNTING STEP TWO

- Using forceps to hold the handle segment, transfer excised vessel from Petri dish to the Wire Myograph chamber. Hold the vessel as close to the proximal end as possible and try to mount the vessel onto the wire.
- If the lumen is shut, try one of the following possibilities:

  1. Use the wire to gently push the lumen open (blood streaming out is a good sign).

  2. Hold excised vessel about 3mm from the cut end with one set of forceps and use the other forceps to squeeze the blood remaining in lumen out through the cut end.

- Pull the proximal end of the excised vessel segment along the wire such that the vessel segment acts as its own feeder to be feed into the wire into the vessel (figure 3.2 A-C). Be careful not to stretch the vessel segment if the end of the wire catches the vessel wall.

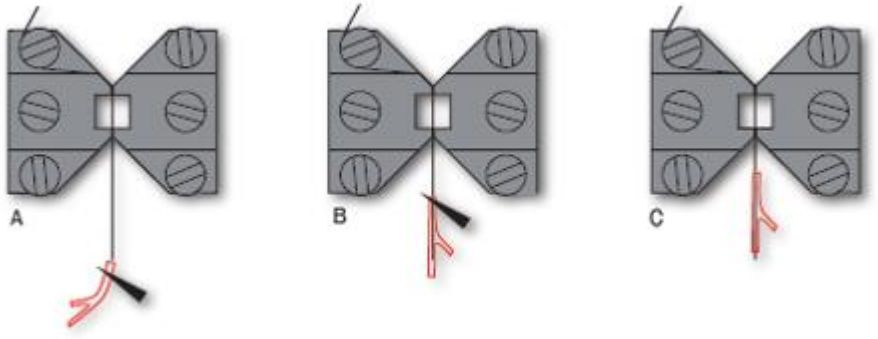


Figure 3.2 A, B and C *Mounting step 2*

3.1.3 MOUNTING STEP THREE

- Once the vessel segment is threaded onto the wire, catch the free end of the wire (nearest you) with the forceps and move the jaws apart.
- While controlling the movement of the wire with the forceps, use the other forceps to gently pull the vessel segment along the wire until the area of interest is situated in the gap between the jaws. The near end of the vessel segment shall lie about 0.1mm inside the jaw gap to insure no point of contact (figure 3.3 A).
- Still controlling the free wire end with the forceps, move the jaws together to clamp the wire and in one movement secure the wire under the near fixing screw on the left-hand jaw. Again, in a clockwise direction so that tightening the screw also tightens the wire (figure 3.3 B).

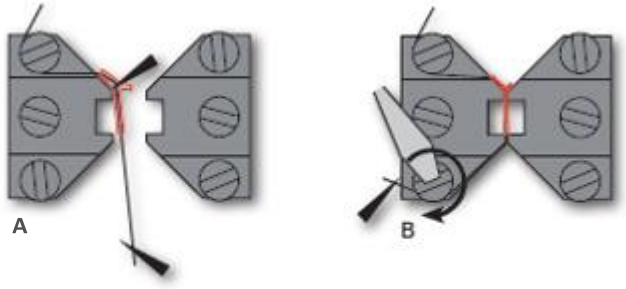


Figure 3.3 A and B *Mounting step 3*

3.1.4 MOUNTING STEP FOUR

- Using forceps, gently rub the vessel segment on the far side of the jaw to separate any excess vessel segment from the area of interest clamped in the gap between the jaws (figure 3.4 A). Make sure that the vessel segment is separated as close as possible to the jaws (figure 3.4 B). The excessive vessel segment is finally dissected free and removed from the wire (figure3.4 C).

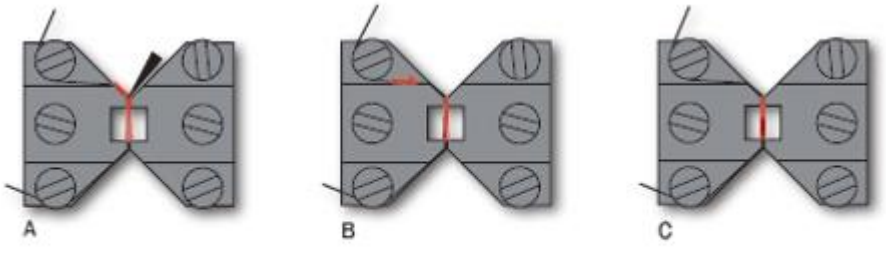


Figure 3.4 A, B and C Mounting step 4

3.1.5 MOUNTING STEP FIVE

- Move the jaws apart (figure 3.5 A). Take a second wire holding it about one third down from the far end using forceps. Align the wire parallel with the vessel segment such that the wire can be passed into the far end of the lumen. Gently feed the wire through the lumen of the vessel segment in one movement using the first mounted wire as a guide (figure 3.5 B-C). Hold the wire at a point at least 10mm from the vessel to prevent the vessel being stretched during the maneuver. Be careful not to touch the lumen of the vessel with the end of the wire and when pushing the wire end through the near end of the lumen. Once the wire has successfully passed through the lumen of the vessel segment, place the wire in a position, which ensures sufficient length for the wire to be secured both at the near and far fixing screws on the right-hand jaw.

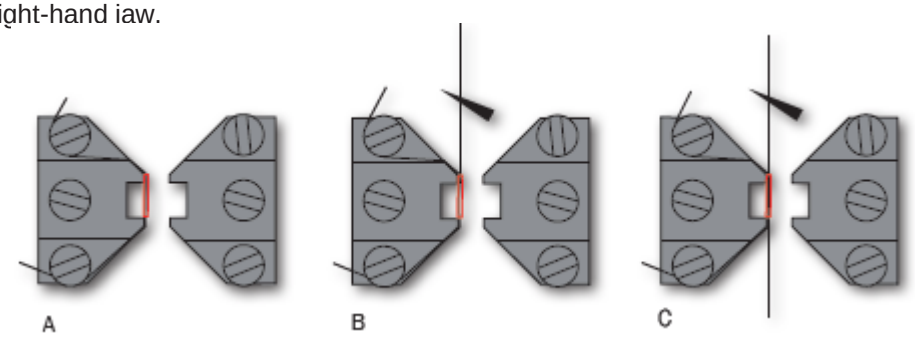


Figure 3.5 A, B and C Mounting step 5

3.1.6 MOUNTING STEP SIX

- Carefully move the jaws together while ensuring that the second mounted wire lies underneath the first one secured on the left-hand jaw (figure 3.6 A). The procedure clamps the second wire to prevent it from damaging the vessel segment when securing the wire to the right-hand jaw (connected to the transducer). Secure the near end of the wire in a clockwise direction under the far fixing screw on the right-hand jaw (figure 3.6 B).

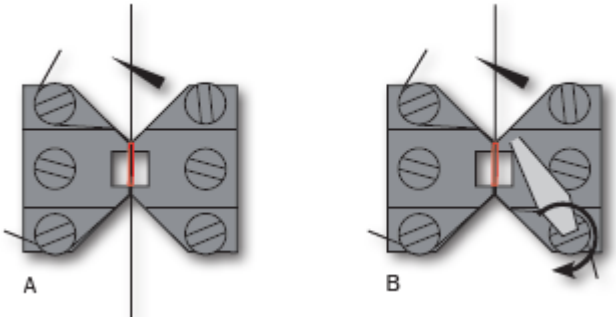


Figure 3.6A and B Mounting step 6

3.1.7 MOUNTING STEP SEVEN

- Secure the far end of the wire under the near fixing screw on the right-hand jaw. Again, the wire is passed clockwise around the screw stretching the wire as the screw is tightened (figure 3.7 A-B). Move the jaws apart to slightly stretch the vessel segment. Make sure that the vessel on the far side of the jaws does not extend beyond the jaws, as even a small extension will affect the normalization procedure. In case of excess of vessel on the far side of the jaws then move the jaws together again and remove excessive tissue using forceps as described in mounting step four. A better method for the skilled operator is to move the jaws slightly apart and use scissors to make a small slit in the vessel wall where the vessel is clamped. Move the jaws apart just enough to adjust the two wires so they are completely aligned and in the same height as shown in fig. 3.7C. If they are aligned correct the two wires will bump into each other when trying to close the gap between the two jaws. The mounted artery is now ready for the normalization described in the next section.

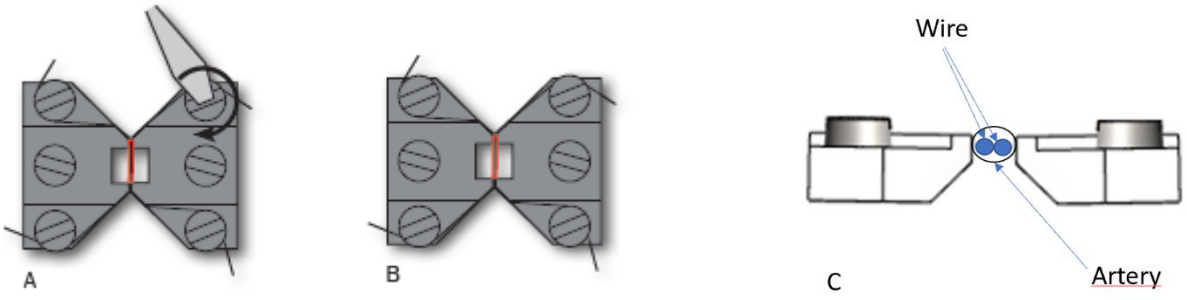
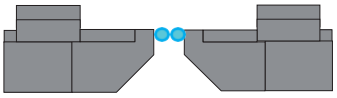


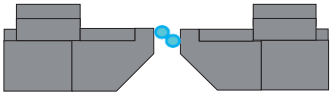
Figure 3.7 A and B Mounting step 7

3.1.8 MOUNTING STEP EIGHT

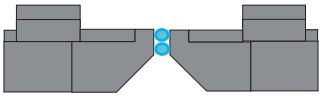
- Adjust the two wires in a way that the two wires will 'bump' into each other and not will go beneath or on top of each other as shown below by using the small screwdriver or forceps to move the wires on the two opposing Jaws a bit up or down.
- Use a dissection microscope at this step while moving the jaws together using the manual micropositioner until the two wires are as close to each other as possible but without touching.



Correct position of the two wires (Blue color) on the Jaws. This is a side view of the two wires on the two jaws.



Incorrect position of the two wires (Blue color). Here one wire is lower positioned than the other. Correct the wires before continuing. This is a side view of the two wires on the two jaws.



Incorrect position of the two wires (Blue color). Here one wire is completely above the other wire. Correct the wires as shown in first example before continuing. This is a side view of the two wires on the two.

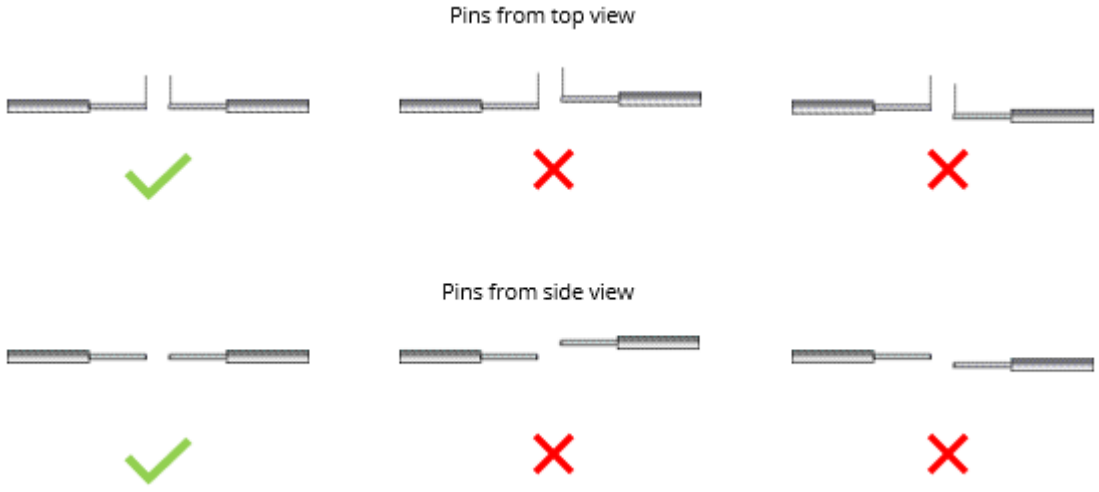
- The artery is now mounted correctly and can be moved onto the 630MA interface with the heat turned on. When the buffer in the chamber has reached 37^0C, replace the buffer in the chamber with fresh well aerated warm buffer. Wait 5 min. and start the Automated Normalization as described in Chapter 3.3.5.

3.2 MOUNTING PROTOCOL FOR LARGER ARTERIES

For mounting of larger arteries with an internal diameter larger than 500µm and up to 11mm replace the mounting Jaws with Pins (see chapter 2.1). With the 630MA system a box with 4 sets of 200µm pins is delivered. For large arteries pins with 250µm, 300µm or 400µm pins can be purchased from DMT.

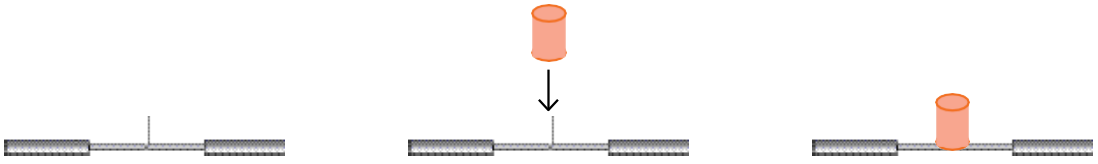
3.2.1 MOUNTING STEP ONE

- First make sure that the mounting pins are placed correctly across each other in the chambers as shown below.



3.2.2 MOUNTING STEP TWO

- Using the manual micropositioner move the two mounting pins as close together as possible without touching each other.
- Take the isolated artery/tissue section and slide it over the two pins as shown in the figures below.



The artery is now mounted correctly and the heat of the 630MA interface can be turned ON. When the buffer in the chamber has reached 37C, replace the buffer in the chamber with fresh well gassed warm buffer. Wait 5 min and start the Automated Normalization as described in Chapter 3.3.5.

NOTE: FOR LARGE ARTERIES IT COULD BE NECESSARY TO ADD A SMALL TENSION (1-2MN) TO THE MOUNTED ARTERY ON THE PINS TO AVOID THE ARTERY FALLING OF THE PINS DURING HEATING AND REPLACEMENT OF THE BUFFER. REMEMBER TO PLACE THE PINS AS CLOSE TO EACH OTHER AS POSSIBLE WITHOUT TOUCHING JUST BEFORE STARTING THE AUTOMATED NORMALIZATION.

3.3 NORMALIZATION

The importance of normalizing the preparation is
three-fold:

1. Experiments with elastic preparations like vessels can only have meaning if they are performed under conditions where the size is clearly defined.
2. Clearly defined conditions are required in pharmacological experiments as the sensitivity of preparations to agonists and antagonists is dependent on the amount of stretch.
3. The active response of a preparation is dependent on the extent of stretch, which makes it important to set the preparation to an internal circumference giving maximal response.

The aim of the normalization procedure is to stretch the segment to a so-called normalized internal circumference (IC_1): defined as a fraction of the internal circumference (IC_{100}) that a fully relaxed segment would have at a specified transmural pressure. For small rat arteries the target transmural pressure is typically 100mmHg = 13.3kPa.

3.3.1 PRINCIPLES OF THE NORMALIZATION PROCEDURE

In practice the normalization is performed by distending the segment stepwise and measuring sets of micrometer and force readings (figure 3.8). These data are converted into values of internal circumference (µm) and wall tension T (mN/mm) respectively.

Plotting wall tension against internal circumference reveals an exponential curve and by applying the isobar curve corresponding to 100mmHg, IC_{100} is calculated from the point of intersection using the Laplace relation (figure 3.9). IC_1 is calculated from IC_{100} by multiplying a factor, the Norm Factor, giving an internal circumference at which the active force production as well as the sensitivity to agonists of the segment is maximal. For rat mesenteric arteries the Norm Factor is 0.9 but both this factor as well as the transmural pressure has to be optimized for each particular segment. The normalized internal diameter is calculated by dividing IC_1 with π.

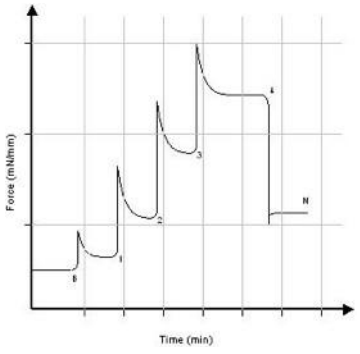


Figure 3.8 Illustration of the stepwise normalization procedure

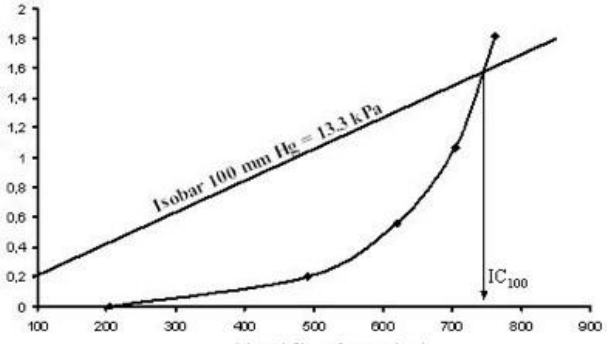


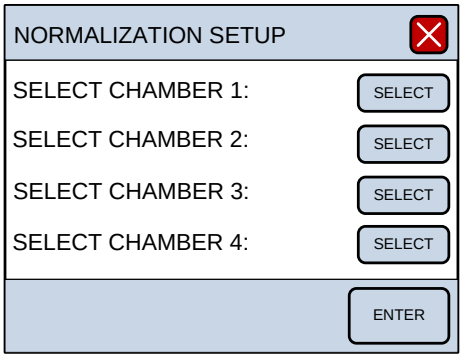
Figure 3.9 Illustration of the exponential curve fitting and determination of C_{100}

3.3.2 THE AUTOMATED NORMALIZATION PROCEDURE ON THE 630MA SYSTEM

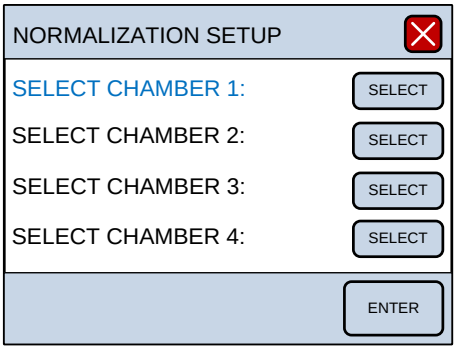
Make sure the wires/pins do not touch and are close together without touching.

1. Zero the force of the Chamber going to be normalized automatically.

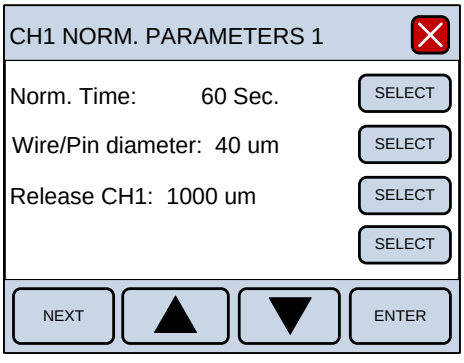
2. Go into the Norm. Set-up menu.



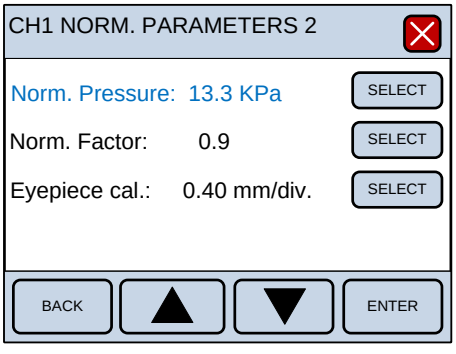
3. Select the appropriate chamber.



4. Enter the values for the Norm. Time, Wire/Pin diameter.
   DMT has the following wire and Pin products:
   Steel Wire: 40µm in diameter
   Tungsten wires: 25µm, 15µm and 10µm in diameter
   Pins:100µm, 200µm 250µm, 300µm and 400µm in diameter.



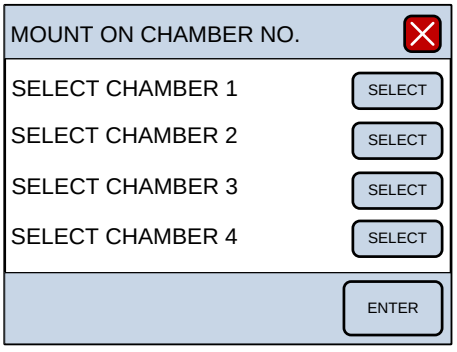
5. Press NEXT to go into the PARAMETERS 2 menu.



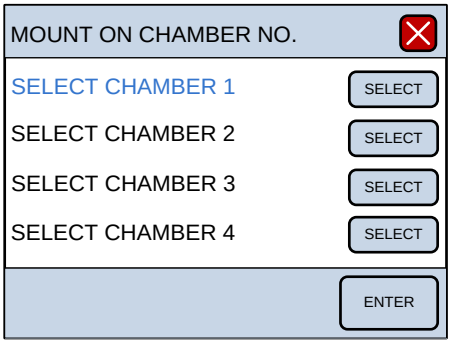
6. Enter the Norm. Pressure, Norm Factor and Eyepiece cal values. It is very important that the Norm Pressure and Norm factor is right for the type of artery mounted in the chamber. There can be a huge difference between artery type and species e.g. for rat mesenteric arteries the Norm Pressure is 13.3kPa and the Norm Factor is 0.9 but for mouse mesenteric arteries the Norm Pressure is 13.3kPa and the Norm Factor 1.1. To find the Norm factor for your specific artery and specie please read DMT Normalization Guide.

IMPORTANT: THE NORM FACTOR HAS TO BE FOUND FOR THE TYPE OF VESSEL AND SPECIES BEFORE USING THE AUTOMATED NORMALIZATION.

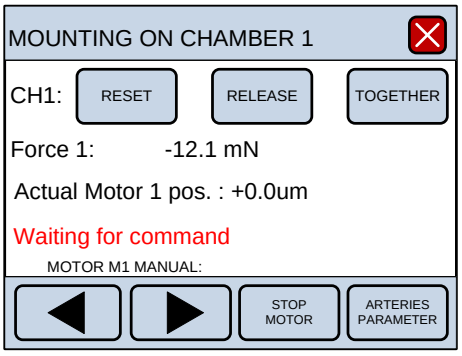
7. Go into the Mounting Artery



8. Select the appropriate Chamber

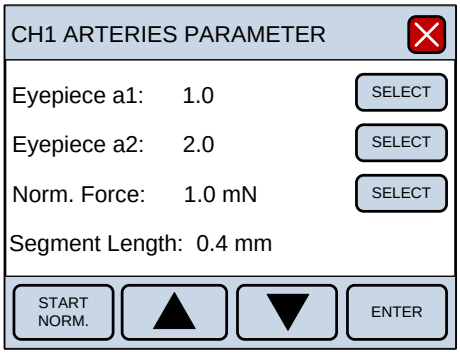


9. Press RESET to reset the motor positions to zero. Before pressing RESET the force should be zero or very close to zero but not negative. After RESET is pressed the Actual Motor position will be changed to zero.



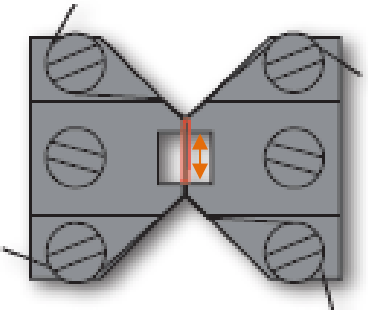
WARNING: DO NOT PRESS RELEASE AT THIS STAGE IT WILL DESTROY YOUR MOUNTED ARTERY.

10. Go into the ARTERIES PARAMETER menu

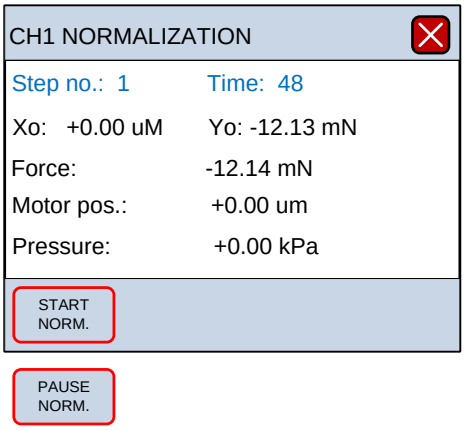


11. Enter the Eyepiece values of the artery mounted in the given chamber. When the a1 and a2 values have been entered the 630MA will calculate the artery Segment Length based on the a1, a2 and Eyepiece Cal values. Check if the segment length is approx. correct. The gap in the jaws are 2.00 mm (shown with red arrows in the figure to the right).

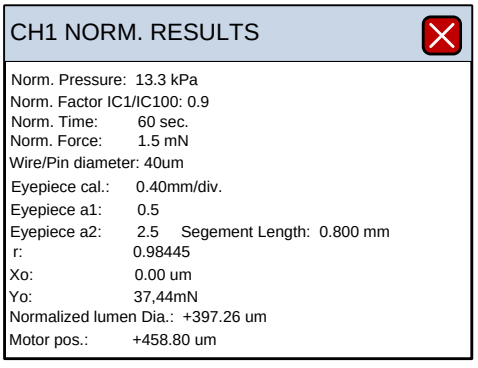
12. Enter the Norm Force. This value is the force that the vessel is stretched successively at the stretch steps 1, 2, 3.... The default is 1.0mN but this has to be lower for small arteries and higher for larger arteries for a successful normalization. If the normalization fails it can always be repeated with new values for the Norm Force.



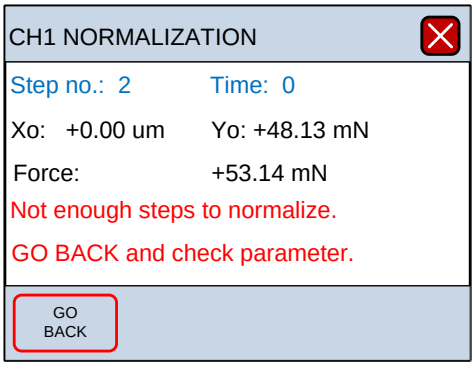
13. When all parameters are programmed, press Start Norm. to start the automated normalization of the given chamber.



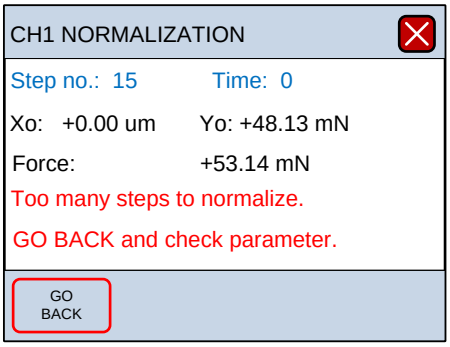
14. If the normalization is successful a parameter screen is shown, and the mounted artery is ready for use with the optimal pre-load tension.



15. If the normalization is NOT successful an error message is shown.



16. If the message “Not enough steps to normalize“ appears then go into the Norm. Force parameter in the Ch1 ARTERY PARAMETER menu and lower the Norm Force value. Also check if the Segment Length is in the right range.
17. If the message “Too many steps to normalize” appears then go into the Norm. Force parameter in the Ch1 ARTERY PARAMETER menu and Raise the Norm Force value. Also check if the right segment length has been entered.



18. While Chamber 1 is performing the normalization start mounting of the next artery segment on chamber 2.

NOTE: IT IS ONLY POSSIBLE TO PERFORM THE AUTOMATED NORMALIZATION ON ONE CHAMBER AT A TIME.

19. Therefore, the working process should be:

- Mount chamber 1 and start the automated normalization
- Mount chamber 2 and start the automated normalization when chamber 1 has finished
- Mount chamber 3 and start the automated normalization when chamber 2 has finished
- Mount chamber 4 and start the automated normalization when chamber 3 has finished

20. All chambers are now mounted, and the Standard start procedure can be started.

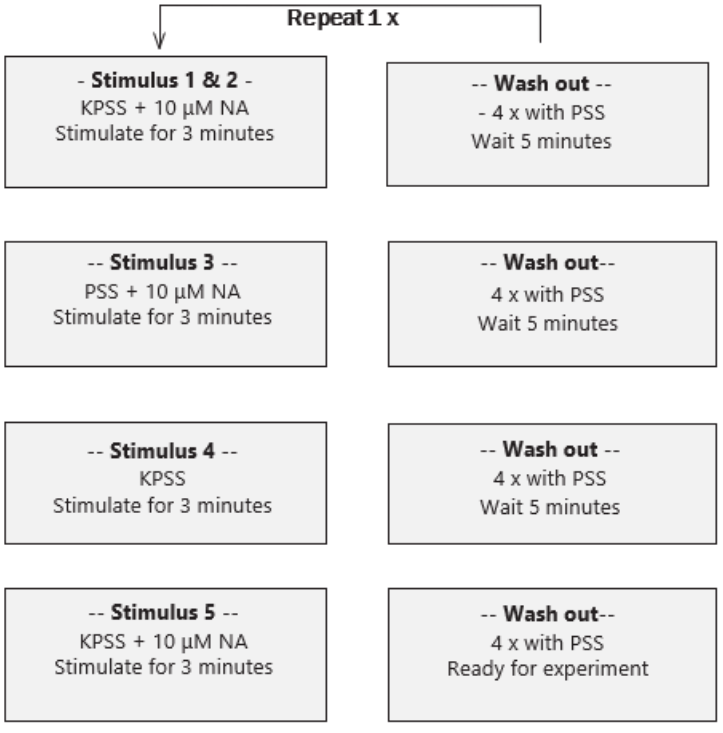
3.4 STANDARD START

The purpose of performing a standard start is to:

1. Re-activate the mechanical and functional properties of the vessel segment.
2. Check that responses to different types of stimuli are normal in appearance and thereby ensuring that the functionality of the vessel segment has not been damaged during the dissection or mounting procedures.
3. Ensure that the tension development gives an effective active pressure that is above the chosen accepted value (usually 13.3kPa = 100mmHg).

3.4.1 PRINCIPLES OF THE STANDARD PROCEDURE

The standard start procedure consists of a series of five stimuli and washout periods. The first two stimuli are performed using a mixture of KPSS and 10µM noradrenaline to give a maximum contractile response. The third stimulus is performed using a mixture of PSS and 10µM noradrenaline to give a maximum pure agonist mediated (α-adrenoceptor) contraction. The fourth stimulus is performed using KPSS to give a depolarizing contractile response (this stimulus also includes a component from neurally released noradrenaline). The final stimulus is performed using a mixture of PSS and 10µM noradrenaline. All solutions are preheated to 37°C and aerated with a mixture of 95% O_2 and 5% CO_2 before use. Instructions for making the necessary solutions are described in appendix 1.



3.5 ENDOTHELIUM FUNCTION

The reasons for checking endothelium function may include:

1. To check whether the relaxing function of the endothelium is intact. The procedure is performed to make sure that the endothelium is not damaged during the dissection or mounting procedure.
2. If an experiment requires removal of the endothelium this procedure is useful to check whether the endothelial cells were successfully removed.

The procedure can be performed after the vessel segment has been heated, equilibrated and normalized. Preferably the procedure should be done after performing a standard start to make sure that the vessel segment is viable.

The present procedure is for use with rat mesenteric arteries. Another procedure may be needed for other animal species and tissue or vessel types.

3.5.1 PRINCIPLES OF CHECKING ENDOTHELIUM FUNCTION

Stimulating a vessel segment with acetylcholine causes a release of nitric oxide (NO, also known as EDRF) from the endothelium cells and subsequent relaxation of the vascular smooth muscle cells. If the endothelium is undamaged by the dissection and mounting procedures, then a substantial relaxation will occur. With complete removal or damaged endothelium, a partial relaxation or no relaxation to acetylcholine is observed.

It is important to note that the amount of NO or EDRF in a vessel is often dependent upon its size. In certain vessels, endothelium-derived hyperpolarizing factor (EDHF) can contribute more or less than EDRF, and in other vessels the same stimulation with ACh can promote release of endothelium-derived contracting factor (EDCF). Therefore, it is important to check the existing literature in order to determine the expected response in your particular vessel with the given concentration of agonist.

3.6 IN VITRO EXPERIMENT 1: NORADRENALINE CONTRACTILE RESPONSE

The purpose of the present protocol is to determine the sensitivity of rat mesenteric small arteries to the vasoconstrictor noradrenaline/norepinephrine with a cumulative concentration-response curve.

3.6.1 BACKGROUND

Noradrenaline (norepinephrine) causes contraction of mesenteric small arteries through activation of α-adrenoceptors whereas noradrenaline activation of β-adrenoceptors causes vasodilatation. As the purpose is to determine the contraction sensitivity to noradrenaline, the vasodilatory effect of noradrenaline is eliminated throughout the experiment by the constant presence of the β-adrenoceptor antagonist, propranolol.

Rat mesenteric arteries are densely innervated by sympathetic nerves, which have a highly efficient re-uptake mechanism that removes noradrenaline from the neuromuscular junction. The re-uptake mechanism will create a concentration gradient between the solution around the vessel segment and the receptors on the smooth muscle. To correctly determine the sensitivity to noradrenaline it is necessary to eliminate this concentration gradient by performing the experiment in the presence of cocaine to block the noradrenaline re-uptake.

To determine the sensitivity to noradrenaline the vessel segment is exposed to increasing concentrations of noradrenaline. Each concentration is applied until a steady response has been reached and then the next concentration is applied. When the vessel segment is fully contracted or does not respond more upon increasing the noradrenaline concentration, the experiment is ended.

3.6.2 PROTOCOL

Prepare the following stock solutions:

Noradrenaline:	10^{-4}M, 10^{-3}M, 10^{-2}M
Propranolol:	10^{-3} M
Cocaine:	10^{-3} M (optional)

1. Mount and normalize the vessels as described in the former chapters.
2. Perform a standard start as described in chapter 3.4.
3. Incubate the vessel segment in 1µM propranolol (add 5 µL of 10^{-3} M to 5 mL PSS in chamber) and 3µM cocaine (add 15 µL of 10^{-3} M to 5mL PSS in chamber) for at least 10 minutes.
4. Add increasing concentrations of noradrenaline into the bath (use the table below as a guideline). Wait for a stable contractile response or a standard time such as 2 minutes between each application.

[NA] in chamber (µM)*	Volume of stock solution to add to chamber
0.1	5µL of 10^{-4} M
0.3	1µL of 10^{-3} M
0.5	1µL of 10^{-3} M
1	2.5µL of 10^{-3} M
1.3	1.5µL of 10^{-3} M
1.5	1µL of 10^{-3} M
3	7.5µL of 10^{-3} M
5	1µL of 10^{-2} M
10	2.5µL of 10^{-2} M

**In calculating the [NA] in the Wire Myograph chamber, the applied volume of noradrenaline is ignored.*

3.7 IN VITRO EXPERIMENT 2: ACETYLCHOLINE RELAXATION CURVE

The purpose of the present protocol is to determine the sensitivity of the endothelium dependent vasodilator acetylcholine in noradrenaline pre-contracted rat mesenteric small arteries.

3.7.1 BACKGROUND

Acetylcholine causes relaxation of rat mesenteric small arteries by activating of muscarinic M3 receptors at the endothelial cell layer leading to release of endothelium-derived relaxing factors.

Rat mesenteric arteries do not show spontaneous tone in the wire myograph, which is why it is necessary to first induce a contraction to be able to observe the relaxation to acetylcholine. In this protocol the contraction is induced by noradrenaline. The required concentration of noradrenaline needs to be optimized since a too low concentration makes it impossible to evaluate the relaxation. On the other hand, it may be difficult to relax super maximally contracted arteries, which may lead to an underestimation of the sensitivity to acetylcholine. Therefore, it is recommended to apply a concentration of noradrenaline inducing 60-70% of maximal contraction response. In practice this concentration is found by performing a noradrenaline concentration-response curve as described in the previous section.

The vessel segment is exposed to the noradrenaline concentration and when the response has stabilized, increasing concentrations of acetylcholine are added to relax the vessel. Each concentration is applied until a steady response has been reached and then the next concentration is applied. When the vessel segment is either fully relaxed or does not relax more upon increasing the acetylcholine concentration, the experiment is ended.

3.7.2 PROTOCOL

Prepare the following stock solutions:

Acetylcholine 10^{-4}M, 10^{-3}M, 10^{-2}M
Noradrenaline 10^{-2}M

1. Mount and normalize the vessels as described in the former chapters.
2. Perform a standard start and check the vessel segment for endothelium function, as described in chapter 3.4 and 3.5.
3. Add noradrenaline to obtain a response around 60% of maximum (determined from the previous noradrenaline concentration-response curve). When the contractile response is stable, add increasing concentrations of acetylcholine to the chamber, using the table below as a guideline. Wait for a stable contractile response or a standard time such as two minutes between each application.

[ACh] in chamber (µM)*	Volume of stock solution to add to chamber
0.1	5µL of 10^{-4} M
0.3	1µL of 10^{-3} M
0.5	1µL of 10^{-3} M
1	2.5µL of 10^{-3} M
1.3	1.5µL of 10^{-3} M
1.5	1µL of 10^{-3} M
3	7.5µL of 10^{-3} M
5	1µL of 10^{-2} M
10	2.5µL of 10^{-2} M

**In calculating the [ACh] in the Wire Myograph chamber, the applied volume of ACh is ignored.*

CHAPTER 4 - CLEANING AND MAINTENANCE

4.1 CLEANING THE 630MA MYOGRAPH SYSTEM

NOTE:

- *DMT STRONGLY RECOMMENDS THAT THE WIRE MYOGRAPH AND SURROUNDING AREAS ARE CLEANED AFTER EACH EXPERIMENT.*
- *IF ANY ACETIC ACID IS SPILLED OUTSIDE THE STEEL CHAMBER REMOVE IT IMMEDIATELY WITH A NAPKIN AND CLEAN WITH DISTILLED WATER TO AVOID DAMAGE OF THE ALUMINUM BASE OVER THE YEARS.*

At the end of each experiment, use the following procedure to clean the Wire Myograph:

1. Fill the chamber close to the edge with an 8% acetic acid solution and allow it to work for one minute to dissolve calcium deposits and other salt build-up. Use a cotton-tipped applicator to mechanically clean all chamber surfaces with distilled water.
2. Remove the acetic acid and wash the 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 chamber and supports with 1M HCl for 1 minute. In exceptional cases, incubate the chamber and supports with no stronger than a 3M HNO_3 solution for about 1 minute.
5. Wash the 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 jaws and pins.

To prevent the tubing from becoming blocked with buffer salt deposits after an experiment, remove the chamber cover from the chamber and turn on the vacuum and press the vacuum valve for about 10 seconds by holding down the valve button(s) down. Turn off the vacuum and gas supply. Remove any water or buffer remaining in the chamber or on the tubing using absorbent paper.

IMPORTANT:

- *BE VERY CAREFUL USING HCL OR HNO3 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 MYOGRAPH 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.*
- *ANY ACIDS SPILLED OUTSIDE THE HIGH-GRADE STEEL CHAMBER ONTO THE MYOGRAPH HAS TO BE REMOVED IMMEDIATELY.*

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 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 chamber and supports several times with double distilled water.
10. Incubate the 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 chamber and supports with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.
12. Wash the chamber and supports several times with double distilled water.
13. Dry the surfaces using absorbent paper (i.e. Kim-Wipes) or cotton-tip applicators.

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

4.2 MAINTENANCE OF THE FORCE TRANSDUCER

The force transducer is the most delicate and fragile component of the Wire Myograph. Extreme care should 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 (see figure 4.1). Insufficient grease in this area will allow buffer and water to enter the transducer housing and causing damage to the force transducer.

IMPORTANT:

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

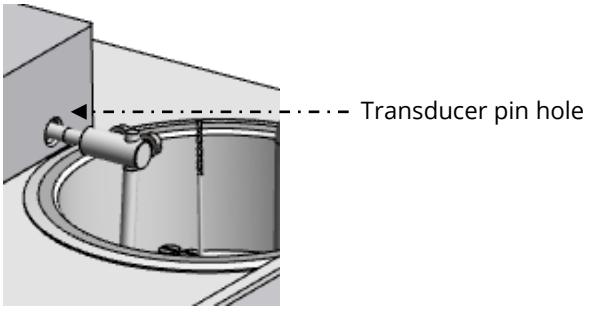


Figure 4.1 Close-up of transducer pin from outside.

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

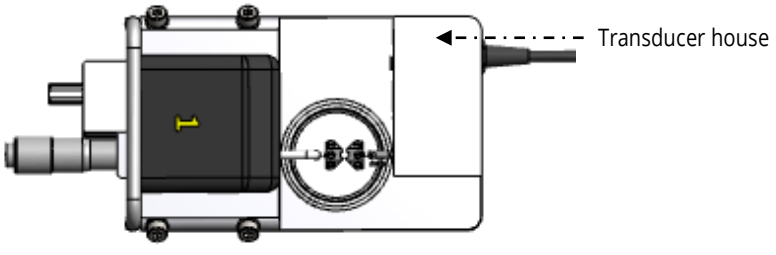
4.2.1 CHECKING THE FORCE TRANSDUCER

The force transducer is a strain gauge connected to a Wheatstone bridge. The force transducers for each Myograph are housed in a separate, protective compartment (see figure 4.2). While the protective cover offers some mechanical protection for the force transducers, they are still very vulnerable to applied forces exceeding 1600mN (160grams) or fluid running into the transducer compartment due to insufficient greasing of the transducer pinhole.

If the force readings on the Multi Interface appear unstable or noisy, then first check that the Myographs are connected properly to the Multi Interface and that the chambers are plugged all the way into the Multi Interface.
If the force reading(s) are still unstable or noisy, then perform a new calibration as described in chapter 3.5.1 in 630MA Myograph System User Manual. Electrical noise from the surroundings can be removed by grounding of the system using the ground connection on the back side of the 630MA system.
During the new calibration, monitor the relative force reading values in the Force Calibration sub-menu on the Multi Interface 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 1500 or greater than 4500, the force transducer is broken and needs to be recompensated at DMT. Contact your DMT sales representative.
- If the message “OFF” is displayed on the main page of the Multi Interface, even though the Wire Myograph is plugged in at the rear of the Multi 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 re-calibrated, 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.



4.2.2 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 or jaw from the transducer pin coming out of the transducer house.
2. Disconnect the Wire Myograph from the 630MA Interface.
3. Turn the Wire Myograph upside down and remove the transducer housing by loosening the two screws (A+B) and the other eight screws indicated with arrows as illustrated in figure 4.3.
4. Disconnect the transducer connector marked with the blue box in figure 4.4.
5. Lift carefully the broken transducer and discard it. (Figure 4.4)
6. The replacement transducer will be shipped with the new transducer inside a new transducer house.
7. Place a small amount of vacuum grease (clear or whitish grease) around the bottom of the transducer housing to seal when set back in place.
8. Drag the transducer connector through the chamber base and connect it again. Carefully realign the transducer housing with the new transducer on the Wire Myograph and reinsert the Allen screws through the bottom of the Wire Myograph.
9. Tighten the screws and place some vacuum grease around the transducer pin that protrudes from the transducer housing, see figure 4.1. 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.

IMPORTANT:
CALIBRATE THE NEW FORCE TRANSDUCER BEFORE PERFORMING A NEW EXPERIMENT.

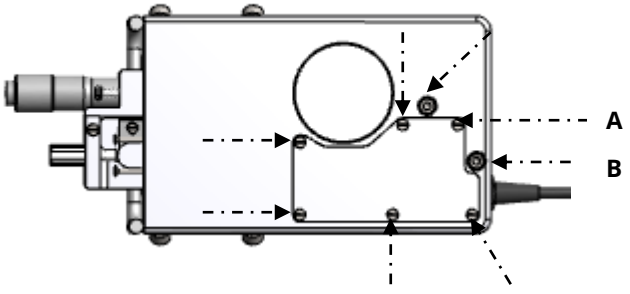


Figure 4.3 - The 8 screws that secure the transducer house to the chamber

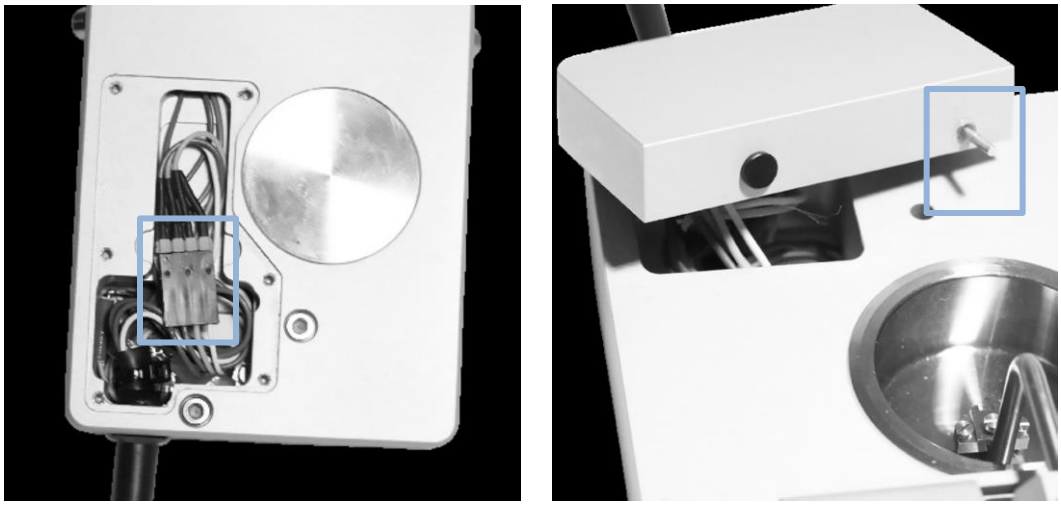


Figure 4.4 Inside the transducer housing and close-up of transducer pin.

4.3 MAINTENANCE OF THE 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 4.5 below).

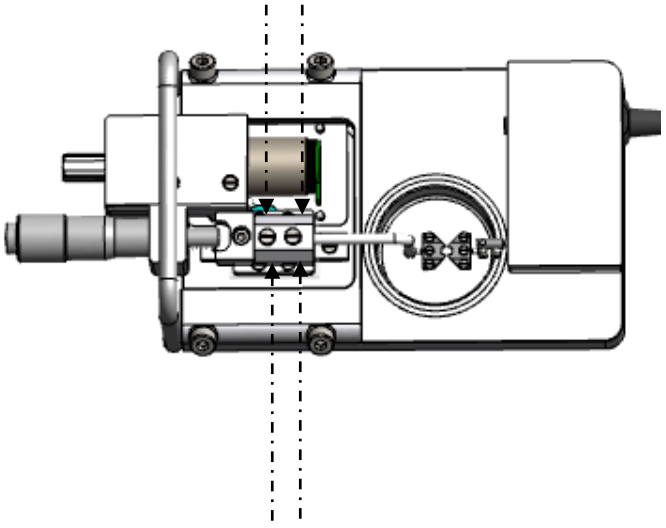


Figure 4.5 The areas where linear slide grease may be applied for smooth micropositioner movement

APPENDIX 1 - BUFFER RECIPES

PHYSIOLOGICAL SALINE SOLUTION (PSS)

1x PSS:

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	130	3.799	7.598	15.20	30.39
KCl	(74.557)	4.7	0.175	0.35	0.70	1.40
KH_2PO_4	(136.09)	1.18	0.08	0.16	0.32	0.64
$MgSO_4 7H_2O$	(246.498)	1.17	0.145	0.29	0.58	1.16
$NaHCO_3$	(84.01)	24.9	1.05	2.10	4.18	8.37
Glucose	(180.16)	5.5	0.545	1.09	2.18	4.36
EDTA	(380)	0.026	0.005	0.01	0.02	0.04
$CaCl_2$	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of $CaCl_2$ (110.99) in double-distilled H_2O. Filter-sterilize the calcium solution through a 0.22 µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals except the $CaCl_2$ in approximately 80% of the desired final volume of double distilled H_2O while being constantly stirred. For example, if 1 liter of PSS is to be made, then dissolve all the chemicals in 800mL of double distilled H_2O.
3. Add the appropriate volume of 1.0M $CaCl_2$ for the total volume of PSS being made (for example, 1.6mL of 1.0M $CaCl_2$ for 1 liter of buffer). Continue to stir the PSS while the $CaCl_2$ is being added.
4. Bring the solution up to the final volume with double-distilled H_2O. Continue to stir the solution until the EDTA is fully dissolved. This takes about 15 minutes at room temperature.
5. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.

25x Concentrated PSS (Stock solution):

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	3250	94.98	189.96	379.92	759.84
KCl	(74.557)	117.5	4.375	8.75	17.5	35.0
KH_2PO_4	(136.09)	29.5	2.0	4.0	8.0	16.0
$MgSO_4 7H_2O$	(246.498)	29.25	3.625	7.25	14.5	29.0
EDTA	(380)	0.65	0.125	0.25	0.50	1.0
$CaCl_2$	(110.99)	40	20mL	40mL	80mL	160mL

1. Make a 1.0M solution of $CaCl_2$ (110.99) in double-distilled H_2O. Filter-sterilize the calcium solution through a 0.22 µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals in approximately 80% of the desired final volume of double distilled H_2O while being constantly stirred. For example, if 1 liter of PSS is to be made, then dissolve all the chemicals in 800mL of double distilled H_2O.
3. Bring the solution up to the final volume with double-distilled H2O. Continue to stir the solution until the EDTA is fully dissolved. This takes about 15 minutes at room temperature.
4. Store cold until use.

Before use:

5. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.
6. Add:
   1.09 g/L Glucose
   2.10 g/L $NaHCO_3$
7. Add the appropriate volume of 1.0M $CaCl_2$ for the total volume of PSS being made (for example, 1.6mL of 1.0M $CaCl_2$ for 1 liter of buffer). Continue to stir the PSS while the $CaCl_2$ is being added.
8. Bring the solution up to the final volume with double-distilled H_2O. Aerate the solution with carbogen (95%O_2 + 5%CO_2) for at least 20 minutes. If necessary, wait further for the pH of the buffer to reach pH 7.4.

HIGH POTASSIUM PHYSIOLOGICAL SALINE SOLUTION (KPSS)

1x 60mM KPSS:

Chemical	Mol.Wt	mM	g/0.5L	g/L	g/2L	g/4L
NaCl	(58.45)	74.7	2.18	4.36	8.72	17.44
KCl	(74.557)	60	2.24	4.48	8.96	17.92
KH_2PO_4	(136.09)	1.18	0.08	0.16	0.32	0.64
$MgSO_4 7H_2O$	(246.498)	1.17	0.145	0.29	0.58	1.16
$NaHCO_3$	(84.01)	24.9	1.05	2.10	4.20	8.40
Glucose	(180.16)	5.5	0.5	1.00	2.00	4.00
EDTA	(380)	0.026	0.005	0.01	0.02	0.04
$CaCl_2$	(110.99)	1.6	0.8mL	1.6mL	3.2mL	6.4mL

1. Make a 1.0M solution of $CaCl_2$ (110.99) in double-distilled H_2O. Filter-sterilize the calcium solution through a 0.22 µm filter. The sterilized solution can be stored in the refrigerator for up to 3 months.
2. Dissolve all the chemicals except the $CaCl_2$ in approximately 80% of the desired final volume of double distilled H_2O while being constantly stirred. For example, if 1 litre of PSS is to be made, then dissolve all the chemicals in 800mL of double distilled H_2O.
3. Add the appropriate volume of 1.0M $CaCl_2$ for the total volume of PSS being made (for example, 1.6mL of 1.0M $CaCl_2$ for 1 litre of buffer). Continue to stir the PSS while the $CaCl_2$ is being added.
4. Bring the solution up to the final volume with double-distilled H_2O. Continue to stir the solution until the EDTA is fully dis- solved. This takes about 15 minutes at room temperature.
5. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.

APPENDIX 2 - NORMALIZATION THEORY

The importance of making a normalization before initiating an experiment with any tubular tissue segment is described in chapter 3.2. In this appendix the mathematical rationale and calculations underlying the normalization procedure are described in detail.

MATHEMATICAL CALCULATIONS

Let (Xi, Yi) be the pair of values representing the micrometer reading (see appendix 3) and force reading respectively characterizing each step in the normalization procedure. Y0 is the force reading at the start position of the normalization procedure where the wires are just separated, and the force reading is approximately zero. Then, given that tension on the vessel is equal to force divided by wall length, the wall tension at the i-th micrometer reading is calculated by:

$$T_i = \frac{(Y_i - Y_0)}{2\delta \bullet (a_1 - a_2)}$$

where δ is the microscope eyepiece reticule calibration factor in mm per division and a measuring the length of the mounted vessel segment.

The internal circumference of the mounted vessel at the i-th reading is calculated by:

$$IC_i = IC_0 + (2 \bullet (X_i - X_0))$$

where IC_0 is the internal circumference of the mounted vessel when the wires are just separated and is given by:

$$IC_0 = (2 + \pi) \bullet d$$

where d is the wire diameter. For 40 µm wires, IC_0 = 205.6 µm.

Using the Laplace relation, the effective pressure P_i is calculated for each pair of readings. The effective pressure is an estimate of the internal pressure, which is necessary to extend the vessel to the measured internal circumference.

$$P_i = \frac{T_i}{\left(\frac{IC_i}{2\pi}\right)}$$

The stepwise distension is continued until the calculated effective pressure exceeds the target transmural pressure. The target value needs to be optimized for the individual tissue preparation (optimal active force as determined by the length-tension relationship for that tissue). For rat mesenteric arteries the target transmural pressure is normally 100 mmHg (13.3 kPa):

$$T_{100\ mmHg} = 100\,mmHg \bullet \left(\frac{IC}{2\pi}\right)$$

An exponential curve is fitted to the internal circumference pressure data as illustrated in figure 3.9. Now the isobar corresponding to 100 mmHg is used to calculate the IC_{100} value from the point of interception between the function of the exponential curve and the function of the 100mmHg isobar.

The normalized internal circumference IC1 is calculated by multiplying the internal circumference corresponding to 100 mmHg, IC_{100}, by a factor k. The factor is for rat mesenteric arteries 0.9. Again, this value should be optimized for the particular tissue preparation being used by a length-tension curve.

$$IC_1 = k \bullet IC_{100}$$

The normalized internal (lumen) diameter is then calculated by:

$$d_1 = \frac{IC_1}{\pi}$$

The micrometer reading X1 at which the internal circumference of the normalized vessel is set to is calculated by:

$$X_1 = X_0 + \frac{(IC_1 - IC_0)}{2}$$

APPENDIX 3 - READING A MILLIMETRE MICROMETER

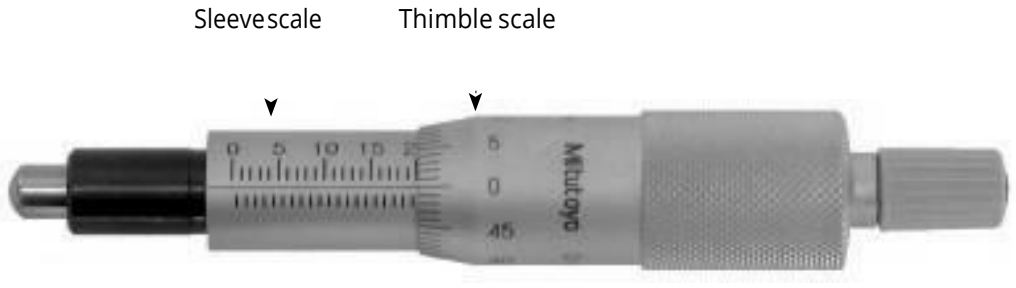


Figure A3.1 Overview of the micrometer parts (actual reading 20000 µm = 20 mm)

Sleeve scale

The micrometer sleeve scale has a total length of 25mm divided into 50 equal parts. Each part of a division above the horizontal line represents 1mm, where each 5th line is marked by a longer line and a number designating the length in mm. Each division below the horizontal line is placed between each 1mm mark (scale above the horizontal line) and represents 0.5mm.

Thimble scale

The thimble is divided into 50 equal parts, and one complete rotation of the thimble is indicated by the smallest division on the sleeve, which equals 0.5mm. Each division on the thimble scale is 10µm. If the thimble scale falls between two lines, then a number between 0 and 10 µm must be approximated.

Example 1

1. Note that the thimble has stopped at a point beyond “10” on the sleeve indicating 10000µm (10mm).
2. Note that there is no mark completely visible between the 10mm mark and the thimble.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

Figure A2.2 Example 1: reading = 10380µm

A.	Reading on sleeve:	10000µm
B.	No additional mark visible:	0µm
C.	Thimble reading:	380µm
	Total reading:	**10380µm**

Example 2

1. Note that the thimble has stopped at a point beyond “16” on the sleeve indicating 16000µm (16mm).
2. Note that this time a mark is visible between the 16mm mark and the thimble indication 500µm.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

Figure A2.3 Example 2: reading = 16780µm

A.	Reading on sleeve:	16000µm
B.	One additional mark visible:	500m
C.	Thimble reading:	280µm
	Total reading:	**16780µm**