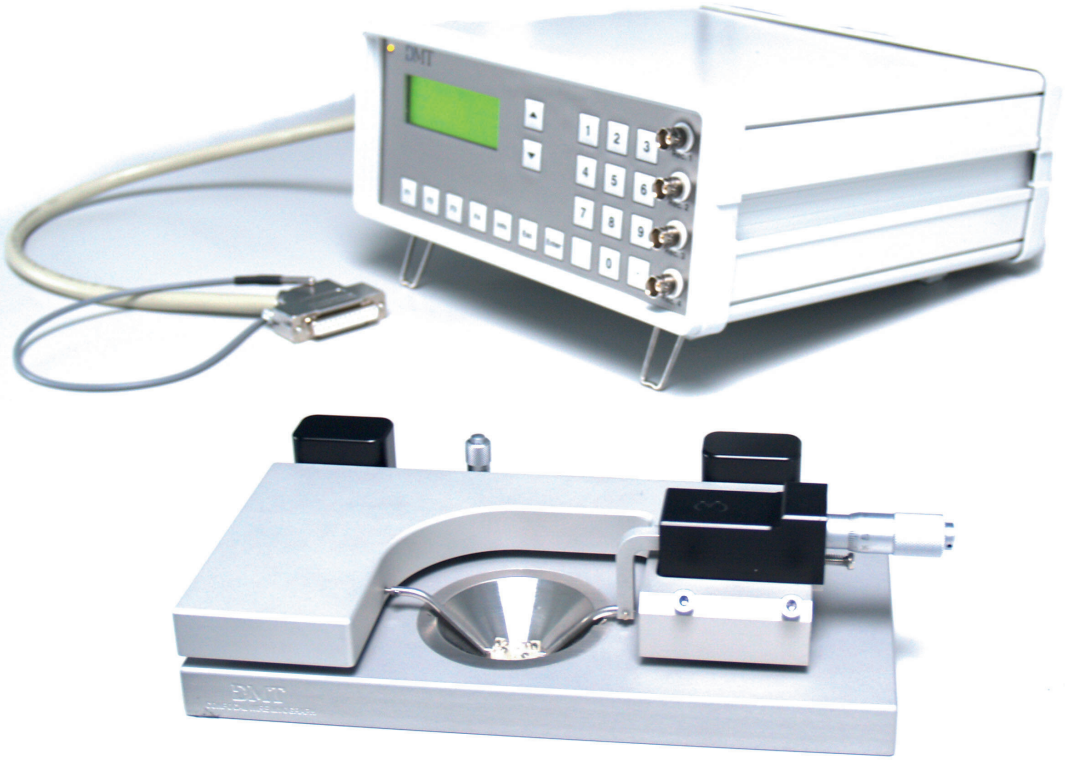
Confocal Wire Myograph System

Model 120CW



User Manual

Version 3.3

DMT

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Confocal Wire Myograph System - Model 120CW

User Manual

Trademarks

This documentation is provided with the DMT confocal wire myograph system – Model 120CW – v. 3.3

Document Number: 120CW – UG3.3A

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Introduction

The 120CW confocal wire myograph is specially designed to provide very close optical access to the mounted artery segment. High-resolution images of fluorescent dyes or markers within the living tissues such as those by laser scanning microscopy (LSM) become possible. Combining LSM technology with myography allows simultaneous measurement of isometric force and dynamic intracellular events (such as changes in intracellular Ca^{2+}or pH) occurring in living cells in the wall of isolated blood vessels.

The unique design of the myograph combines the precision and stability of our conventional wire myographs with the added feature of precise Z-axis movement with a micrometer. This optimizes the use with different LSM's and various high magnification and high numerical aperture objective lenses.

The conical bath design on the underside of the chamber allows the high numerical aperture lenses used on inverted microscopes and also direct immersion lenses used on standard upright microscopes to come directly in contact with the coverslip of the chamber window. Also, by using special mounting supports designed specifically for vertical positioning, an isolated blood vessel can be positioned directly above or on the chamber window. This permits use of lenses with working distances smaller than 250 µm on an inverted LSM. This may be advantageous for simultaneous electrophysiological measurements. The chambers are supplied with custom covers for inverted or upright microscope systems with connections for suction, gassing or measurement electrodes (pH, NO, O_2 tension). The myograph are supplied with jaws for inverted scopes but can be used with conventional jaws on an upright system. For experimental work requiring electrical field stimulation a set of plastic jaws with attached platinum electrodes is available.

During the experiment, the circumference of the vessel is kept constant, i.e. the vessel is examined under isometric conditions. Compounds are added directly to the chamber and the resulting contractile changes in vessel force/tension are measured. While on the LSM, data acquired from the myograph such as force and temperature can be recorded continuously, either through the serial interface output or with the Myo-Interface analog outputs connected to an external data acquisition system.

Safety

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

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

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.

EMC / EMI

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

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

Approvals

Complies with the EMC standards:

EMC 89/336/EEC: EN 61326-2-6:2005
EN 61000-3-2.

Certified with the safety standards:

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

Certificate of Conformity

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

Confocal Wire Myograph System
Model 120CW, version 3.3

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

EN 61010-1:2001 EN61010-1/Corr.1:2003 EN 61010-1/Corr.1:2003	Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements.
EN 61010-2-101:2003	Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
EN 61326-2-6:2005	Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment.
	With reference to regulations in the following directives: 2006/95/EC, 89/336/EEC

Contents

About this manual

This manual contains a complete list of procedures describing how to install, maintain and get started using the confocal wire myograph system – model 120CW – version 3.3.

Chapter 1 provides an overview of the construction and basic features of the Myo-Interface and the confocal wire myograph unit.

Chapter 2 describe step by step how to set-up a complete confocal wire myograph system, including connection to data acquisition.

Chapter 3 is a complete manual to the Myo-Interface. The chapter describes in detail the construction of the menu system and how to use all the features of the Confocal wire myograph system.

Chapter 4 contains procedures describing general as well as daily maintenance of the myograph unit; e.g. adjustment of supports, weight calibration of the force transducer and cleaning instructions.

Chapter 5 describes how to get started using the wire myograph system. This includes a complete dissection and mounting procedure for small mesenteric arteries, and a few basic pharmacological experiments.

Appendices contain additional information about normalization theory, ocular calibration, myograph service, shipping instructions, system specifications, equipment lists (accessories and spare parts), and fuse replacement.

Unpacking the myograph system

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

We recommend that you store the packing material for any possible future transport of the Confocal wire myograph system. In case of transport and the original packing material is unavailable, please contact DMT Sales Department for advice and packing instructions.

After unpacking your new Confocal wire myograph system, please use the following list to check that the system is complete:

1. **Myo-Interface unit:**
- Myograph connection cable with a temperature probe
- Power cord (The shape of the AC plug varies by country; be sure that the plug has the right shape for your location)
- Automatic voltage selector

2. **Confocal wire myograph unit:**
- Calibration kit (including bridge, balance and 2 g weight)
- Chamber cover
- Vacuum valve (manual)

3. **Accessories**
- 1 roll of 40 µm stainless steel wire
- 1 tube of high vacuum grease
- 1 tube of grease for linear slides
- 4 spare screws for mounting of jaws
- 10 extra myograph window glasses
- 2 Allen keys
- 1 small screwdriver
- 40 mm funnel
- Serial cable

4. **Manuals**
- User manual for “Confocal wire myograph system – model 120CW”
- “Procedures for investigation of small vessels using small vessel myograph”, by Professor M. J. Mulvany, Department of Pharmacology, Aarhus University, Denmark

1.1 Confocal wire myograph unit

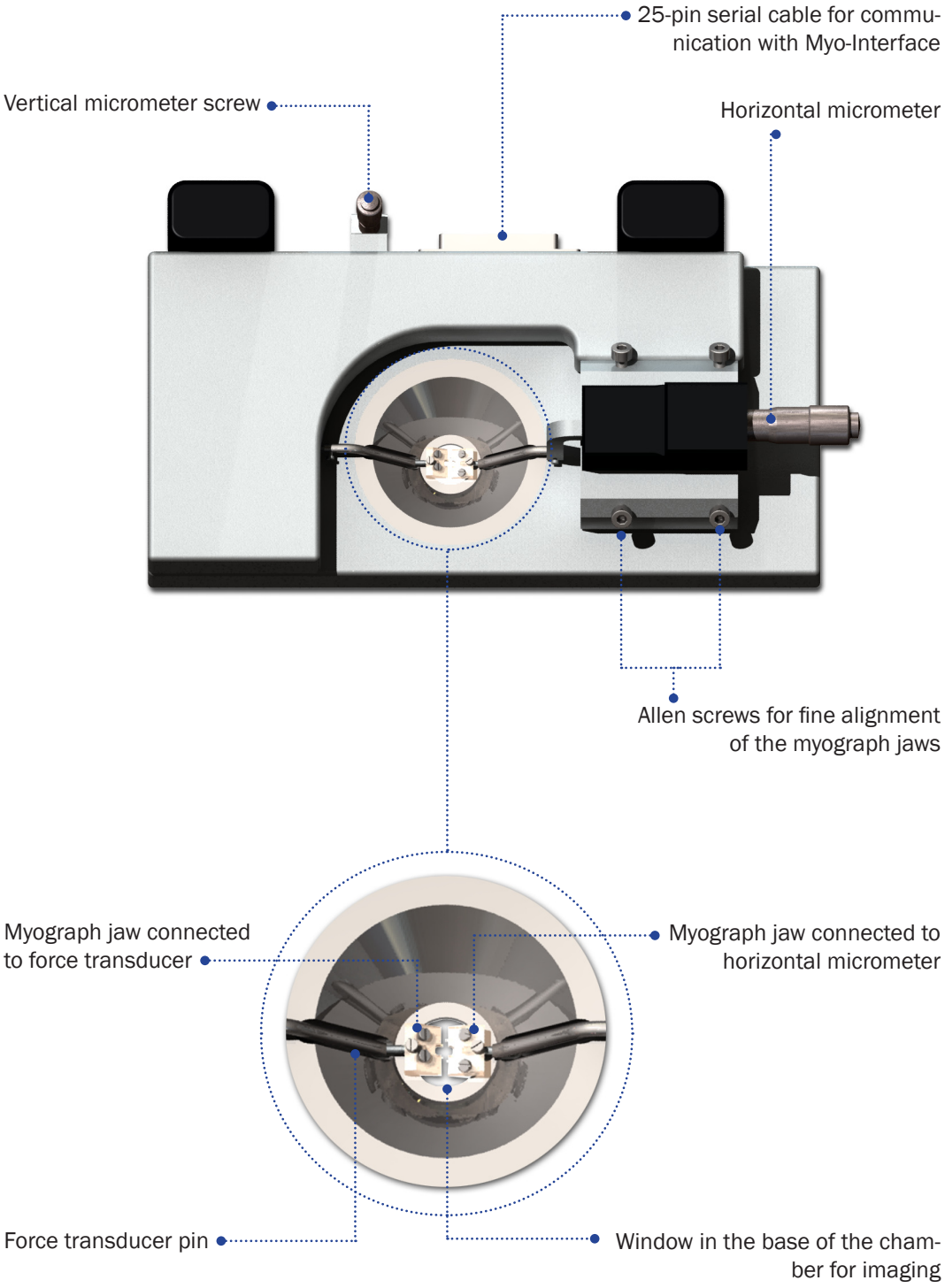


Figure 1.1 *The confocal wire myograph unit with close-up detail of the chamber*

1.2 Myo-Interface

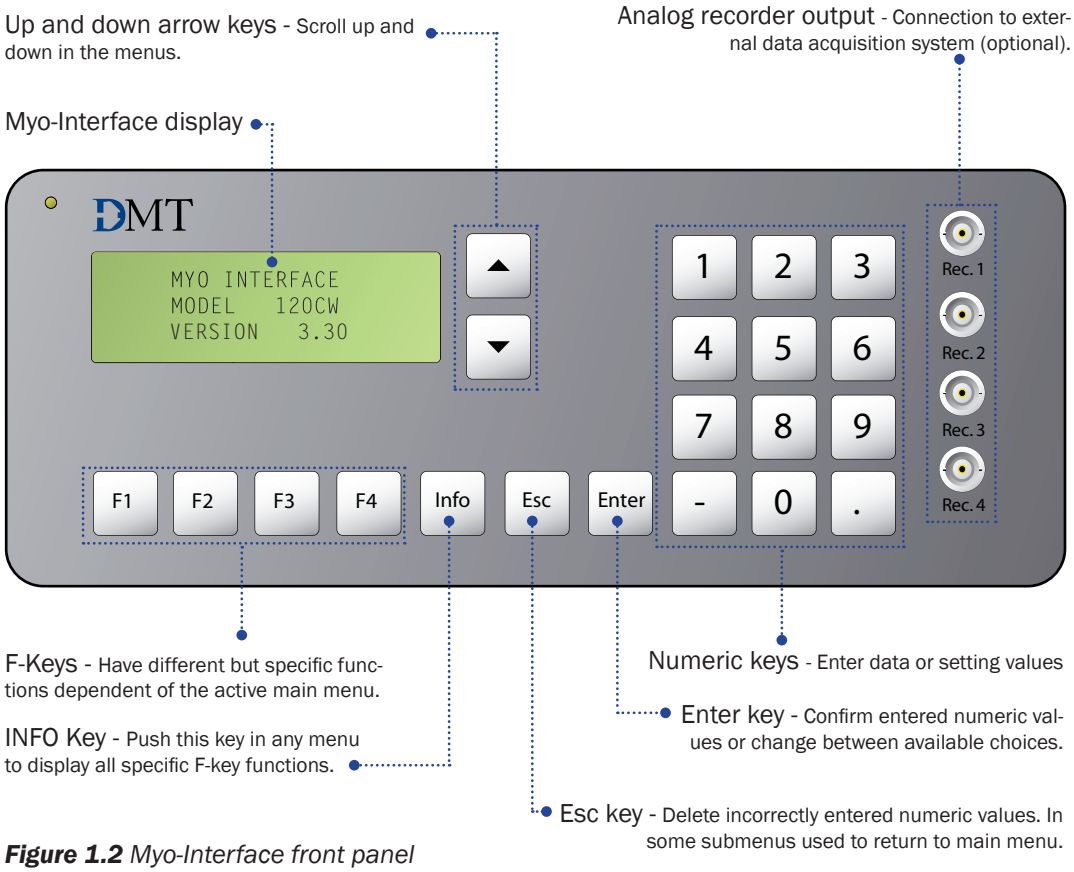


Figure 1.2 *Myo-Interface front panel*

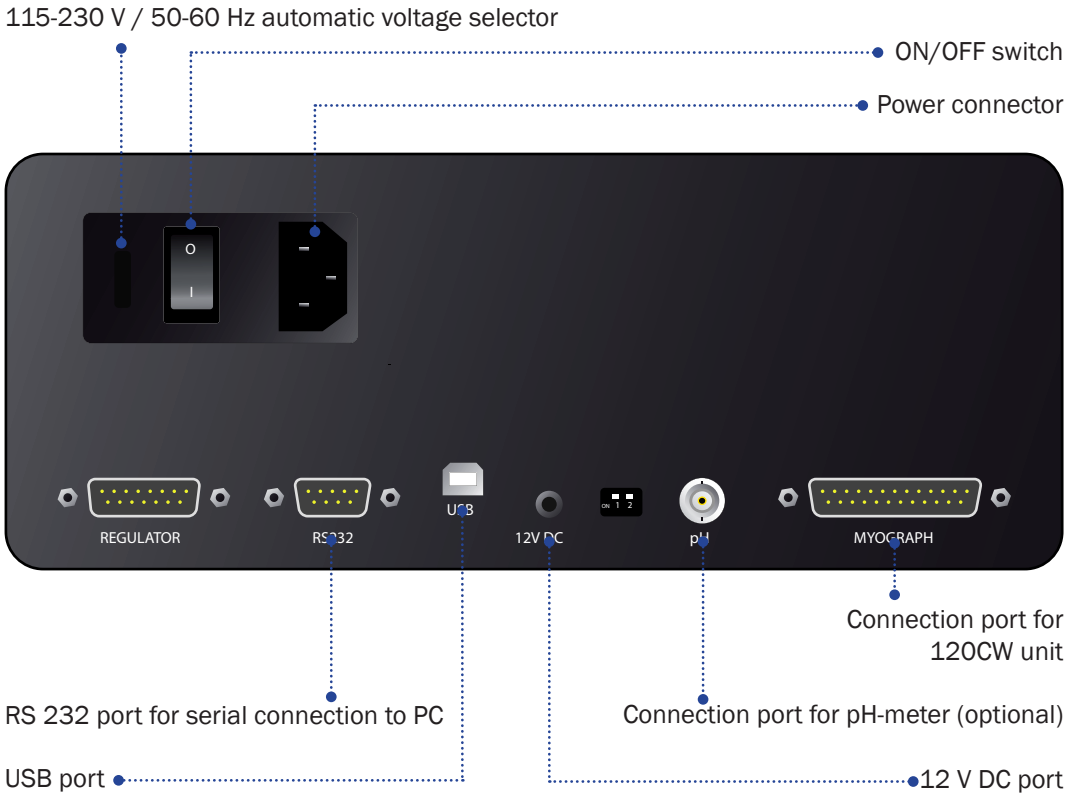


Figure 1.3 *Myo-Interface rear panel*

Chapter 2 — Setting up

2.1 The complete myograph 120CW system

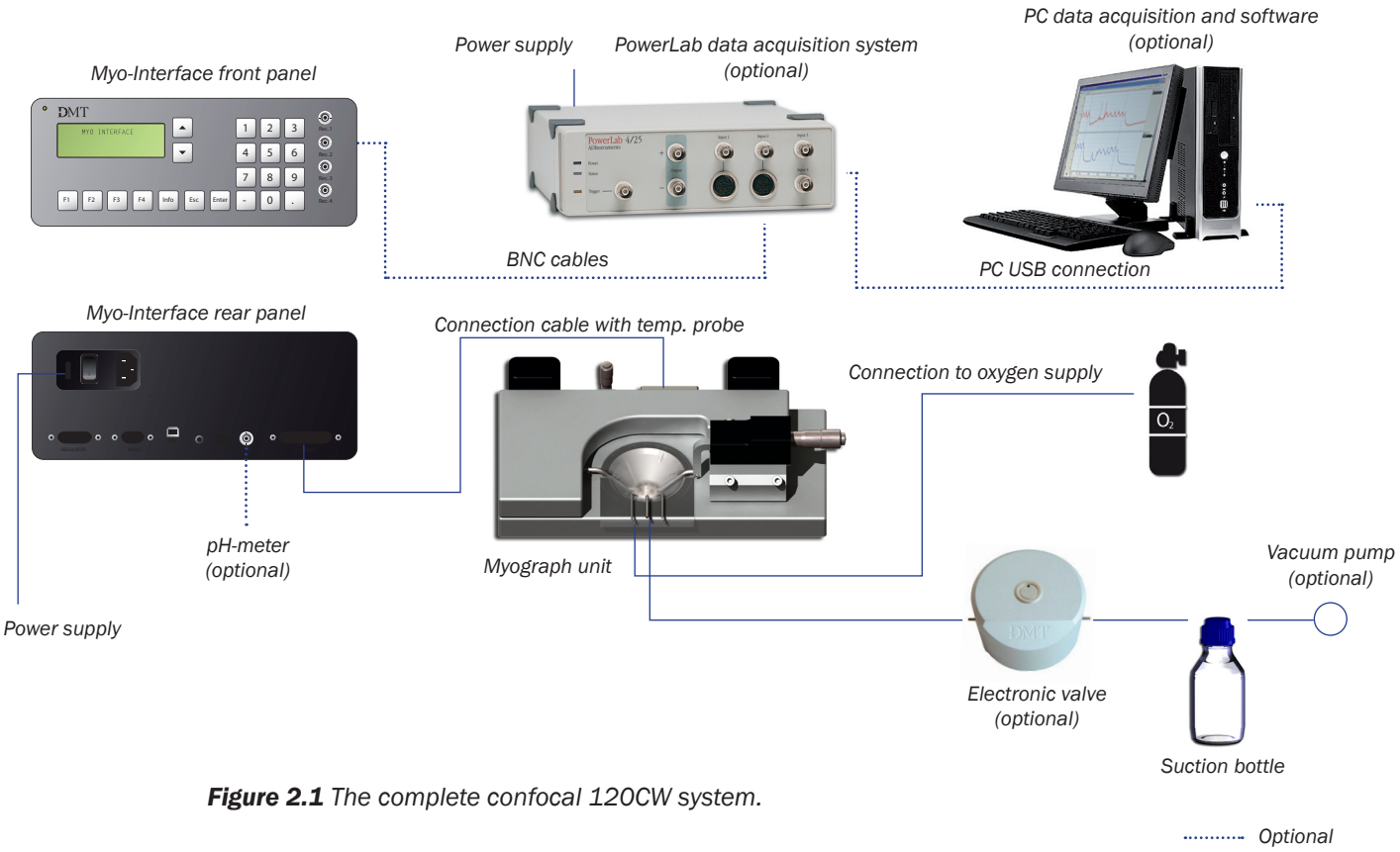


Figure 2.1 *The complete confocal 120CW system.*

2.2 Setting up step-by-step

The chapter contains a complete step-by-step description of how to set-up a complete myograph system as illustrated in fig. 2.1.

1. **Myograph unit – Myo-Interface connection:**
   Connect the myograph 120CW unit to the Myo-Interface using the grey 25-pin connection cable. The end of the cable with the temperature probe is placed into the myograph chamber to monitor actual temperature.

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

   I. **Direct PC connection:**
   Connect the Myo-Interface to one of the COM-ports on the PC using the serial cable delivered with the Confocal wire myograph 120CW system.

II. **PowerLab (optional):**
Connect the Myo-Interface to the PowerLab unit using BNC cables. Connect Rec 1 on the Myo-Interface to Input 1 on the PowerLab. Rec 2 to Input 2, and so forth. Connect the PowerLab unit to one of the USB-ports on the PC using the USB cable delivered with the PowerLab system.

3. **Oxygen supply:**
Connect the small pipes on the myograph chamber cover to an adjustable oxygen supply using small silicone tubing (internal diameter 1.5 mm).

4. **Suction connection:**
Connect the large pipe on the myograph chamber cover to a vacuum pump via a suction bottle and the vacuum valve as illustrated in fig. 2.2. The internal diameter of rubber tubes for connection to the pipes on the chamber cover is 2 mm. An electronic vacuum valve is available from DMT.

5. **Electrode (optional):**
Connect the pH electrode to the pH port on the backside of the Myo-Interface and install the software flash update as described in the provided procedure. Perform a pH calibration as described in chapter 3.

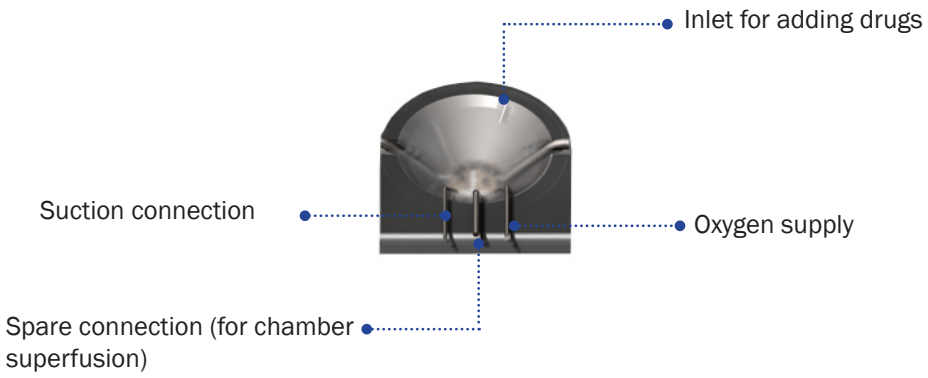


Figure 2.2 *Chamber cover*

2.3 The first weight calibration

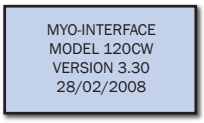
Prior to the shipment of the Confocal wire myograph system, it has gone through two days of continuous testing including a weight calibration. However, in order to ensure that the myograph is operating at an optimum, DMT recommends that a new weight calibration is performed before the confocal wire myograph system is used.

The weight calibration procedures are described in detail in chapter 4.

Chapter 3 - The Myo-Interface

3.1 Turning on the Myo-Interface

When the 120CW Myo-Interface is switched on, the display shows that the system is starting up and initializing, after which the start-up message depicted to the right is shown.
The display automatically shows the Main menu after several seconds. Otherwise, press F1 to proceed to the Main menu.
When the start-up message is active, the ▲ and ▼ keys can be used to adjust the display contrast setting.



3.2 Menus and submenus

The layout and relationship between the various menus and associated submenus in the Myo-Interface are depicted in the schematic fig. 3.1.

General navigation

The following controls are used to display the various menus, choose varying menu options and change values :

F1	Change to the next menu
F2 – F4	Have varying functions depending on the current active menu
Info	Push this key in any menu to display all specific associated F-key functions
Numeric keys	Enter data or setting values
Enter	Confirm entered numeric values or change between available choices
Esc	Delete incorrectly entered numeric values In some submenus used to return to main menu
▲ – ▼	Scroll up and down through the display, as only four lines are capable of being displayed at a time. Being in the top line of a submenu, use ▲ to change to the previous main menu.

The active line in the menu is indicated by a > symbol.

Main menu

The Main menu displays the current values from the force transducer, the actual temperature probe reading and heating setting (in °C), the heating status as well as the pH probe reading (optional).

Temperature

To change the temperature setting, move the Set. Temp line to the top of the display (the > symbol is displayed in that line). Use the numeric keys to enter a new temperature setting and press Enter to confirm.

Heating

To turn on the myograph heating move the Heat is line to the top of the display and use the Enter key to switch between on and off.

Press the Info key and the display shows the F-key options associated with the Main menu. Choose one of the F-keys to proceed with the following options or press Info again to return to the Main menu:

F1 Change to the next main menu (Recorder).

F2 Opens the Valve Activation submenu. An optional electronic vacuum valve is available for the system, which can be activated here.

F3 Opens the Zero Force submenu (press F1 to zero the force or F4 to cancel)

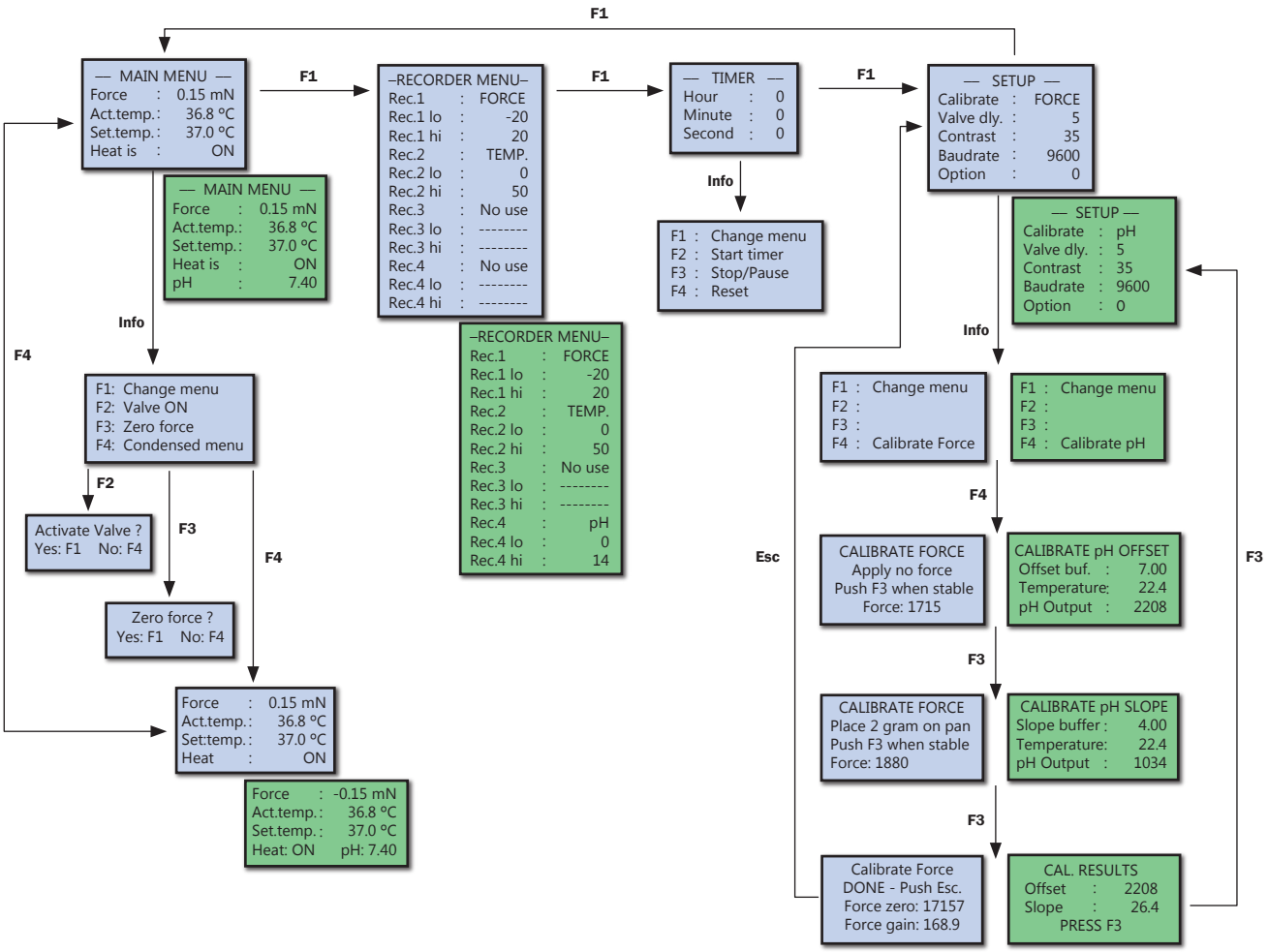


Figure 3.1 *Menu overview: the green panels indicate the menu appearance/options when the optional pH-meter is installed.*

F4 Displays a condensed Main menu. Press F4 to toggle between the condensed and the normal view.

Recorder menu

The 120CW Myo-Interface has four analog output ports on the front panel for connection to a data acquisition system. These default settings for these output ports are:

Rec. 1 Myograph force (FORCE) output
Rec. 2 Temperature (TEMP.) output
Rec. 3 pH reading output (if, installed)
Rec. 4 No output.

The output order can be programmed to whatever order you desire. Make the recorder line of interest active (uppermost in display) and use the Enter key to toggle between the available signals.
The full-scale output from the Myo-Interface is one volt. In the Recorder menu it is possible to change the associated values for each analog output that correspond to 0 V (lo) and 1 V (hi).

Timer menu

The Timer menu makes it possible for the Myo-Interface to act as a timer: the interface can sound an alarm after a predefined countdown. To set the countdown time,

move the line to be programmed (hour, minute or second) to the top of the display. Use the numeric keys to enter the time value and press Enter to confirm.
To control the timer function, press the Info key and the display shows the F-key options for the Timer menu. Choose one of the F-keys to proceed with the following options or press Info again to return to the Main menu:

F1 Proceed to the Setup menu.
F2 Start the countdown (the time values are entered in the Timer menu).
F3 Stop or pause the countdown.
F4 Reset the entered time values or to turn off the alarm.

Setup menu

Calibrate Displays the signal to be calibrated (either force or pH).
Valve dly The time in seconds that the electronic valve (optional to the system) remains open when activated.
Contrast The actual contrast setting in the display.
Baudrate Data transmission rate from the Myo-Interface to a PC via the RS-232.
Option The option line allows access to submenus using specific access codes.

Use the numeric keys to change the valve delay or display contrast settings and press Enter to confirm.
Press the Info key and the display shows the F-key options available for the Setup menu. Press Info again to return to the Setup menu or one of the F-keys to proceed with one of these options.

F1 Press F1 to proceed to the Main menu.
F4 Calibrate Force (or pH)

Having chosen in the Setup menu which signal to calibrate, press F4 to initiate the calibration process. Press Esc to return to the Setup menu if you do not wish to calibrate.

Force calibration

This procedure is described in detail in Chapter 4.

pH calibration (optional)

Having chosen to calibrate the pH settings, press F4 to initiate the calibration. In the Calibrate pH Offset display, the first line shows the value of the offset buffer (first buffer solution), which is always 7.00. The second line shows the temperature of the buffer solution. The temperature is an important parameter in the calibration formula and is obtained automatically by placing the myograph temperature probe in the buffer solution. The third line shows the output from the pH probe as raw data from the A-D converter.
Place the pH-meter electrode and temperature probe in the offset buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press F3 to proceed.
The first line now displays the value of the slope buffer (second buffer solution), which is always 4.00. Place the pH and temperature probes in the slope buffer solution and turn on stirring. When the relative pH output in the bottom line is stable, press F3 to proceed.
The pH calibration is now finished. The parameters are stored in the internal memory of the Myo-Interface. Press F3 to return to the Setup menu.

Chapter 4 - The confocal wire myograph unit

Chapter 4 contains a complete explanation of how to adjust, calibrate and maintain the Confocal wire myograph unit to ensure the equipment is always performing to its highest standard.

4.1 Adjustment of supports

A successful mounting of any kind of tubular tissue segment in the confocal wire myograph is very dependent on perfectly matching supports. The supports are matched prior to the shipment but daily use of the myograph system and greasing of the transducer pinhole will over time create a need for an adjustment of the supports.

Adjustment of the supports is performed using the following step-by-step procedure. Please note that the amount of force on the screws should be very little to avoid breaking the threads. The procedure is illustrated in fig. 4.1.

1. Carefully loosen screw (A) on the top of the support connected to the force transducer. Align the horizontal support and carefully tighten the screw again.
2. Loosen screw (B) on the top of the support connected to the linear slide. Align the horizontal support matching the force transducer connected support as carefully as possible and gently tighten the screw again.
3. Loosen screw (C) on the linear slide to roughly match the linear slide support to the force transducer support in the horizontal plane as illustrated in fig. 4.1. Tighten the screw before proceeding with step 4).
4. The plate on which the linear slide is mounted is balanced on top of a small stainless steel ball making it possible to finely adjust the linear slide support in all vertical and horizontal planes using the four Allen screws (D). Use the four Allen screws to make the final horizontal and vertical (fig. 4.1) adjustments to match the linear slide support to the force transducer support. The correct matching of the supports is illustrated in fig. 4.1.1.

Important:
Be careful not only to continuously tighten the Allen screws when doing the final adjustments. Always remember to loosen the Allen screw placed diagonal to the Allen screw being tightened otherwise there is a high risk of damaging the myograph frame.

Special designed jaws:

The myograph are supplied with jaws for inverted scopes but can be used with normal stainless steel jaws to mount small vessels on an upright system. For experimental work requiring electrical field stimulation a set of plastic jaws with attached platinum electrodes is available.

Chamber:

The chamber has a conical shape and can thus be used with a small amount of buffer liquid. The chamber can be customized to operate with volumes of 3 mL or less.
The conical milling on the bottom outside of the chamber allows objectives on inverted microscopes to be raised to the thin glass window to accommodate very low working distance objectives.

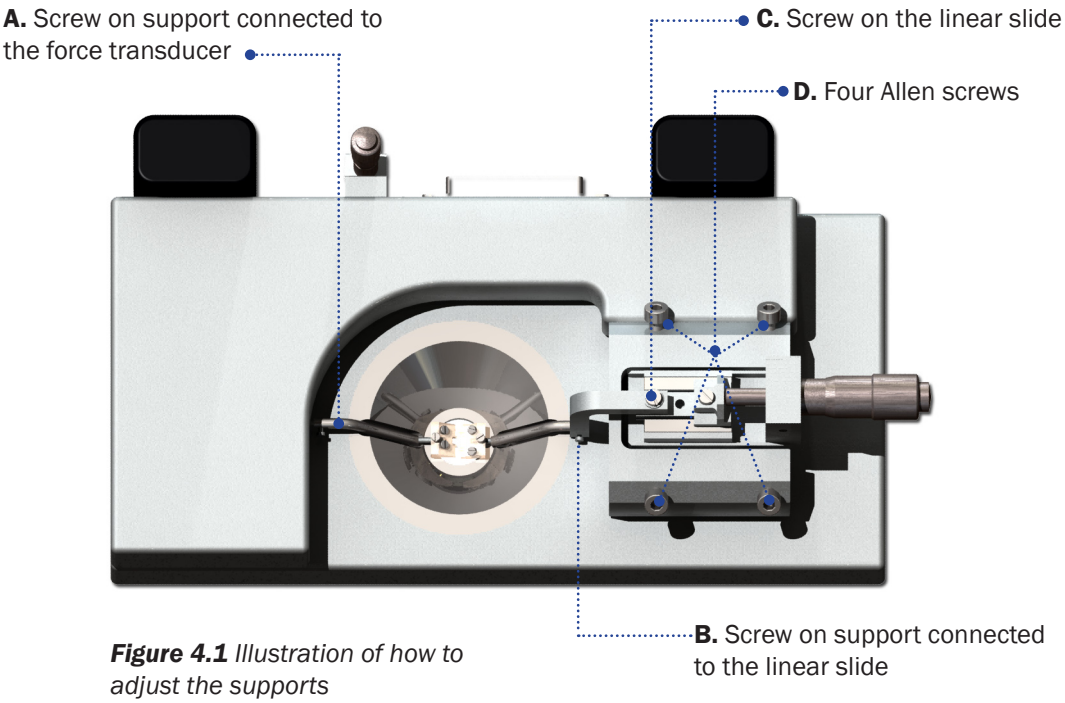


Figure 4.1 *Illustration of how to adjust the supports*

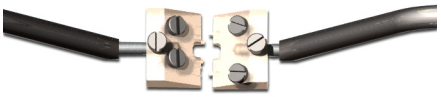


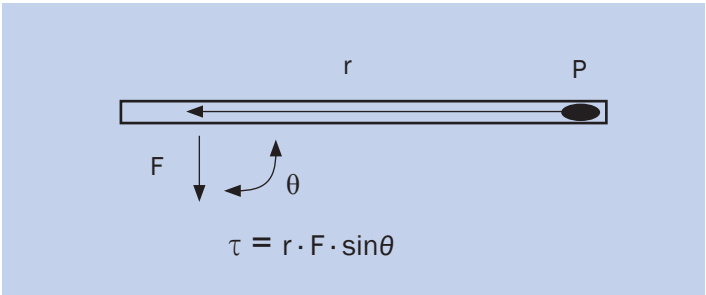
Figure 4.1.1 *Illustration of the reverse supports*

4.2 Calibration of force transducer

As a part of the general maintenance of the myograph, DMT recommends that the myograph is weight calibrated at least once every month. DMT also recommends that the myograph is weight calibrated every time the system has been moved or has not been used for a long period of time.

4.2.1 Principles of weight calibration

Weight calibrating the force transducer is based on simple physics: the net torque acting on a balance when applying a certain amount of weight. The magnitude of the torque τ about a point of rotation P is defined by:



where r is the distance from the point of rotation to the point on the object where the force **F** is acting with the angle of θ. Applying the physics to the weight calibration setup is illustrated in fig. 4.2.

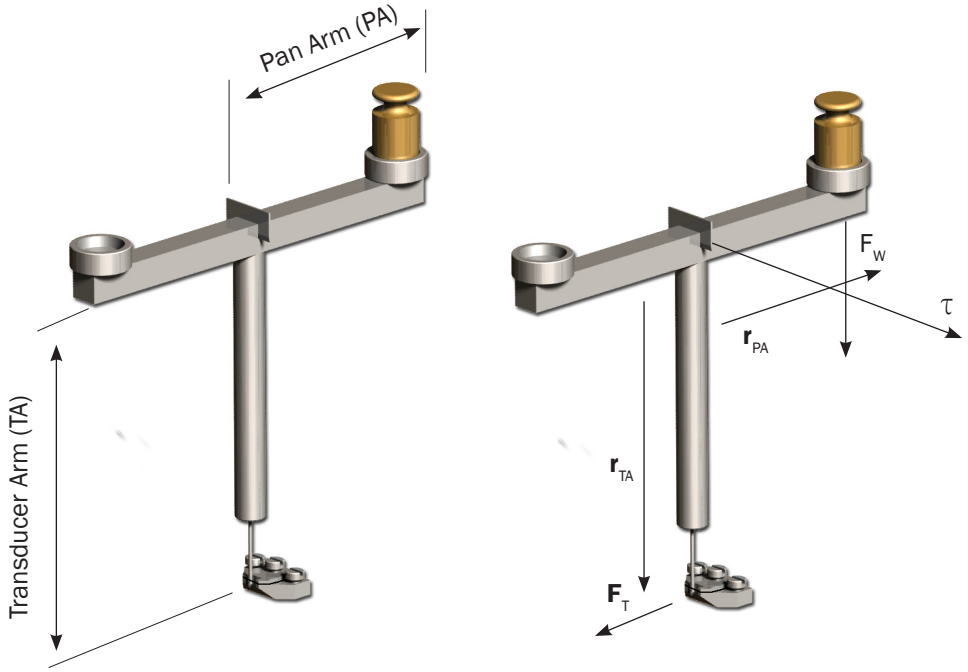


Figure 4.2 *Theoretical principle of the weight calibration*

Applying the weight on the pan arm creates a net torque acting at the "center of gravity" resulting in a force FT acting on the force transducer. The following two equations describe the forces working in the weight calibration system:

1. $\tau = r_{PA} \cdot F_W \cdot \sin\theta_1 = r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1$
2. $\tau = r_{TA} \cdot F_T \cdot \sin\theta_2$

where r_{PA} is the length of the "pan arm". F_W is the force acting on the "pan arm" when applying the weight. F_W is equal acceleration of gravity times the mass of the weight. r_{TA} is the length of the "transducer arm" and F_T is the force acting on the force transducer.

The net torque acting at "center of gravity" is constant for the weight calibration setup, which makes equation 1 and 2 equal, making it possible to calculate the force acting on the force transducer:

$$r_{TA} \cdot F_T \cdot \sin\theta_2 = r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1$$

$$F_T = \frac{r_{PA} \cdot (m_{weigt} \cdot g) \cdot \sin\theta_1}{r_{TA} \cdot \sin\theta_2}$$

As the length of the "pan arm" is 2 cm, the length of the "transducer arm" is 4 cm, the weight is 2 g, both angles are 900 and the acceleration of gravity is 9.81 ms^{-2}, the force acting on the force transducer is:

$$F_T = \frac{2\ cm \cdot (2\ g \cdot 9.81\ ms^{-2}) \cdot \sin 90}{4\ cm \cdot \sin 90}$$

$F_T = 9.81\ g \cdot m \cdot s^{-2}$ As 1N is equal $1kg \cdot m \cdot s^{-2}$, F_W is equal to: $F_T = 9.81\ mN$

4.2.2 Weight calibration procedure

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

1. Fill the myograph chamber with double distilled water and move the jaws apart. Mount a 40 µm stainless steel wire on the jaws connected to the force transducers.

2. Place the calibration bridge, balance and weight randomly on the myograph unit allowing it to be warmed up together with the myograph unit. Turn on the heating in the Main menu on the Myo-Interface.

3. After approximately 20-30 minutes the whole system will have reached the target temperature (normally 37°C). Place the warmed calibration bridge and balance on the myograph unit as illustrated in fig. 4.3. **Note:** the weight should not be placed on the balance yet.

4. Make sure that the tip of the transducer arm on the balance is placed in the gap between the wire and the jaw as illustrated in fig. 4.4. Carefully move the calibration bridge until the tip of the transducer arm is placed freely in the gap, which means it does not touch either the wire or the jaw.

5. Go to the Setup menu on the Myo-Interface and choose to calibrate the myograph force transducer. Press F4 to start calibration.

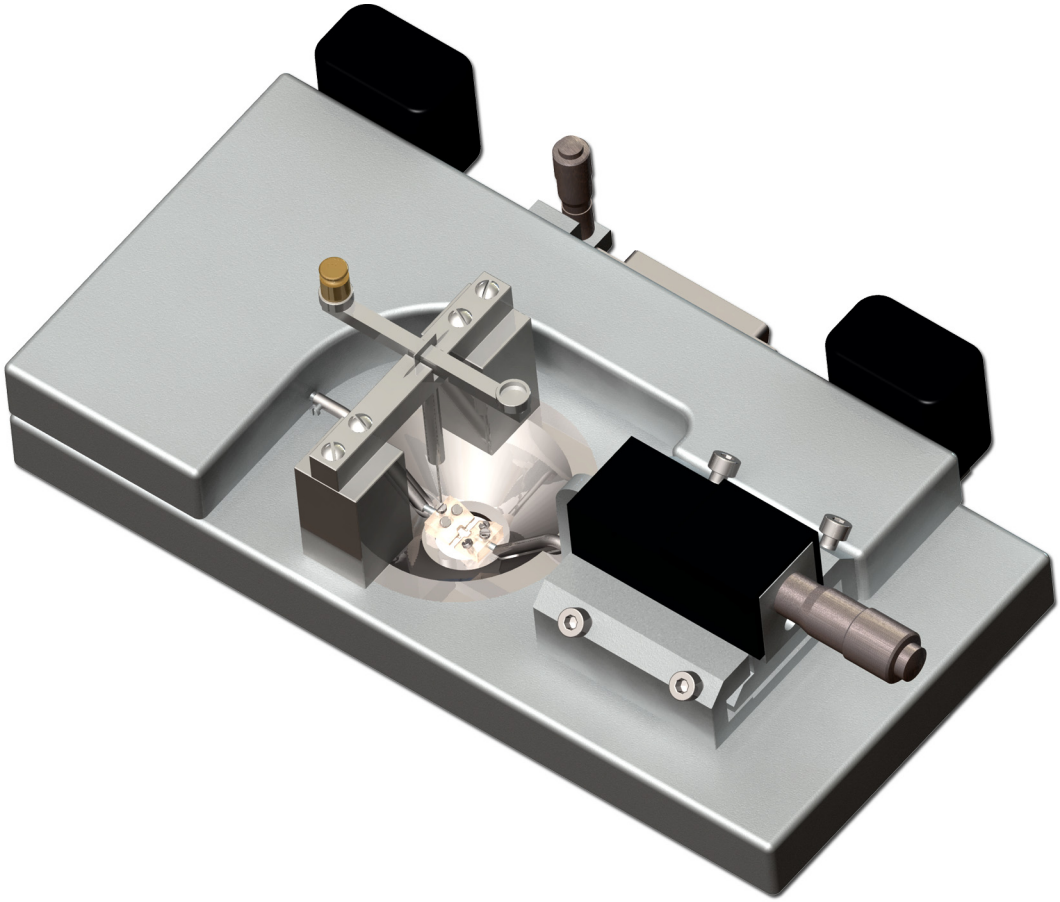


Figure 4.3 *Weight calibration setup - showing placement of the calibration bridge and balance (with weight in place).*

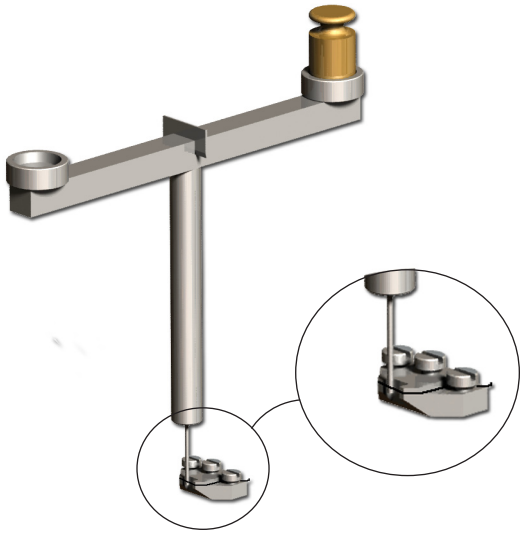


Figure 4.4 *Illustration of how to fit the balance between the wire and the gap in the support*

6. Make sure that absolutely no force is applied on the force transducer by checking that the tip of the transducer arm is not touching either the wire or jaw. Also check that the relative force reading in the display is stable. Press F3 to proceed with calibrating.

7. Carefully place the 2 g weight on the pan as illustrated in figs. 4.3 and 4.4. The force applied on the force transducer should mimic the stretch created by the contraction of a mounted ring preparation. Wait until the relative force reading is stable. Press F3 to finish the calibration.

8. Press Esc and go to the Main menu on the Myo-Interface. The force reading on the Myo-Interface should now be very close to 9.81 mN. Carefully remove the weight and proceed from step 3 to calibrate the other myograph.

If the force reading is different from 9.81 mN then try to calibrate the force transducer once again starting with step 3.

9. After calibrating, carefully remove weight, balance and calibration bridge. The myograph is now ready for use.

4.3 Checking the force transducer

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

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

4.3.1 Simple force transducer check

1. If the force reading on the Myo-Interface appears unstable, then first check that the Myo-Interface and the Confocal Myograph Unit are properly connected through the 25-pin grey cable.

2. If the force reading still appears unstable, then perform a new weight calibration of the force transducer as described earlier in this chapter.

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

- If the value is 0 or above 6500, then the force transducer is broken and needs to be changed.
- If the reading is between 1–499 or 3001–6250 then contact Danish Myo Technology for further instructions.

4.4 Changing myograph window glass

The glass in the myograph chamber window is fixed in placed and kept waterproof by a thin layer of high vacuum grease on the circular edge between the glass and the myograph chamber base. Daily use of the myograph objectives that require water or other immersion solvents will mean that the glass needs to be replaced daily prior to mounting.

The following procedure describes how to change the myograph window glass:

1. Carefully loosen the glass from the myograph chamber edge using small forceps or similar tool.
2. Clean the edge at the bottom of the myograph chamber to remove any remaining old grease.
3. Carefully apply a small amount of high vacuum grease around the edge using a wood stick or similar equipment. Take a new window glass using forceps and gently push it into place. Before starting a new experiment make sure that the complete edge is sealed up with high vacuum grease to keep the myograph window waterproof.

4.5 Myograph maintenance

The Confocal Wire Myograph System 120CW is a very delicate and sophisticated piece of research equipment. In order to keep it working at its best, DMT recommend that the following sections are read carefully and the instructions are followed at all times.

4.5.1 Myograph chamber pipes

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

4.5.2 Force transducer

The force transducer is the most delicate and fragile component of the myograph system. Therefore careful handling is necessary.
One of the jaws is connected to the transducer pin. To prevent the buffer from running into the transducer house the hole is filled with high vacuum grease.
As part of the daily maintenance, it is very important to inspect the greasing of the transducer hole before starting any experiment. Insufficient greasing causes damage and malfunction of the force transducer.

Important:

- DMT recommends that the high vacuum grease, sealing up the transducer hole, be changed at least once a week.
- DMT takes no responsibilities for the use of any other kinds of high vacuum grease than the one to be purchased from DMT.
- DMT takes no responsibilities for any kind of damage applied to the force transducer.

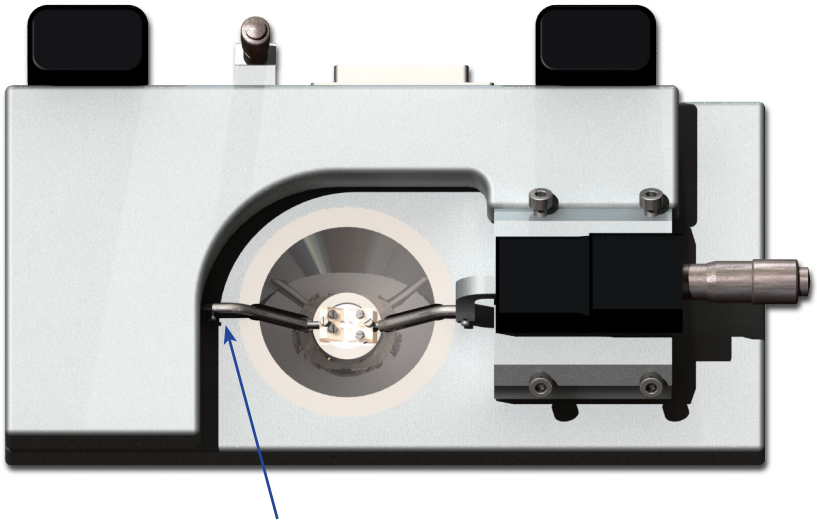


Figure 4.6 *Transducer pin hole to be sealed up with high vacuum grease*

4.5.3 Linear slides

Check the linear slides (underneath the black cover) for grease at least once a week. In case of insufficient lubrication, grease the slides with the original enclosed grease for linear slides at the places marked by the arrows in fig. 4.7.

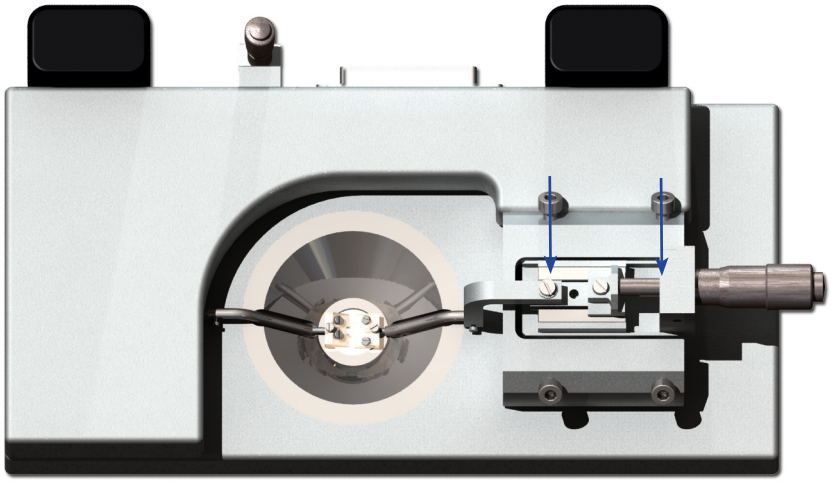


Figure 4.7 *Greasing points on the linear slides*
(note: only use the grease marked with "Grease for linear slides)

4.5.4 Myograph cleaning

DMT strongly recommends that the myograph chamber and surroundings be cleaned after each experiment.

After an experiment use the following procedure to clean the myograph chamber and supports:

1. Fill the myograph chamber to the edge with an 8% acetic acid solution and allow it to stand for a few minutes to dissolve calcium deposits and other salt build-up. Use a swab stick to mechanically clean all chamber surfaces.

2. Remove the acetic acid and wash the myograph chamber and jaws several times with double distilled water.

3. If any kind of hydrophobic reagent have been used, which might be difficult to remove using step 1 and 2, then try incubating the chamber and jaws with 96% ethanol or a weak detergent solution.

4. To remove more resistant or toxic chemicals, incubate the myograph chamber and glass cannulas with 1M HCl for up to 1 hour. In exceptional cases incubate the chamber and supports with a up to 3M HNO_3 solution for about 15 minutes.

5. Wash the myograph chamber and supports several times with double distilled water.

Important Notes:

- Be very careful using step 3 and 4 repeatedly times as strong reagents can cause extreme damage to the myograph unit.
- Be very careful not to exert any force on the myograph jaws during the cleaning procedure.
- After cleaning, **ALWAYS** check that the greasing around the transducer pin is sufficient to keep out the buffer solution from the transducer compartment.

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

1. Incubate the myograph chamber for 30 minutes with 20µl of a 2 mM T-1210 Tetrakis-(2-pyridylmethyl)-ethylenediamine solution dissolved in double distilled water.

2. Use a swab-stick to mechanically clean all the affected surfaces during the last 15 minutes of the incubation period.

3. Wash the myograph chamber several times with double distilled water.

4. Incubate the myograph chamber with 96% ethanol for 10 minutes while continuing the mechanical cleaning with a swab-stick.

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

6. Wash the myograph chamber several times with double distilled water.

Important Notes:

- In exceptional cases it may be necessary to remove the supports for individual cleaning to make sure that all surfaces are clean.

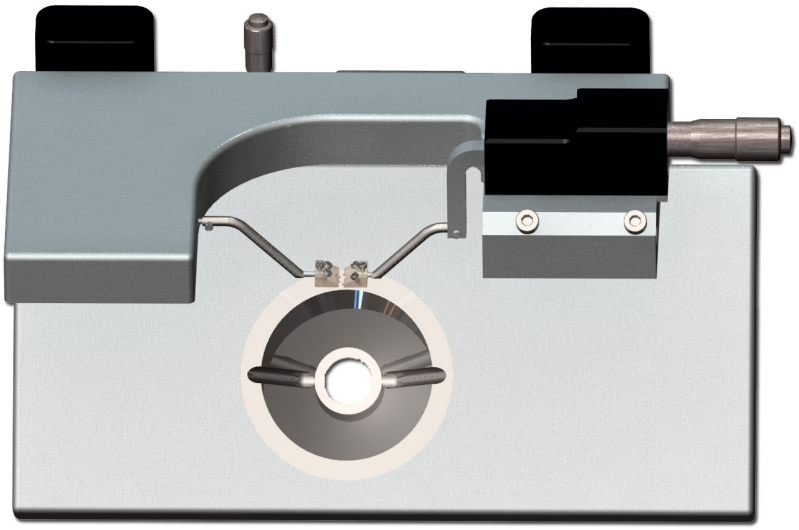


Figure 4.8 *The top part of the myograph can be tilted for easier access when cleaning.*

Chapter 5 — Getting started

Chapter 5 contains some practical protocols and procedures describing how to quickly get started using the Confocal wire myograph system. The chapter mostly addresses users who are not yet familiar with the wire myograph technique. However it may contain some hints that will also be useful for the experienced myograph user.

5.1 Dissection protocol for small mesenteric arteries

The wire myograph technique is versatile in that a large variety of physiological and pharmacological studies of ring preparations from different species are possible. The wire myograph is predominantly used for investigation of small blood vessels and as an example this chapter describes the dissection of rat mesenteric arteries.

1. A laboratory rat is euthanized in accordance to the local national law and regulations. A midline laparotomy is performed to expose the mesenteric bed.

2. Use scissors to remove about 10 cm of intestine together with its feeding vasculature, including part of the superior mesenteric artery. Be careful not to damage the vasculature during this procedure. The proximal end of the intestine section must be about 10 cm from pylorus. Make a cut in the proximal end of the intestine for later identification.

3. Place the excised intestine section in a Petri dish (about 9 cm in diameter) coated with a 5 mm thick layer of Sylgard at the bottom to hold the fixing pins. Immediately fill the Petri dish with cold PSS, which has been well aerated with carbogen. The dissection is performed without further oxygenation of the PSS. The cold temperature ensures the artery is relaxed during the mounting procedure.

4. Pin down the proximal end of the intestine section on the left-hand side of the Petri dish without stretching the vessels. Pin down the remaining of the intestine section in an anti-clockwise direction. In this configuration (proximal end at the left side, distal end at the right side and running anti-clockwise from proximal to distal side) the feeding vasculature is on the far side of the intestine and the veins are usually uppermost.

5. Select the vessel segment to be investigated (fig. 5.1). First time myograph users are recommended to start dissecting and mounting vessel segments from the first or second branch from the superior mesenteric artery (internal diameter ~200-300 µm).

6. Use high quality forceps and ocular dissection scissors to dissect the vessel segment of interest. Start cutting through the mesenteric membrane along both sides of the vessel, about 1-2 mm from the vessel. To avoid accidentally cutting the artery always cut along the length of the vessels and never perpendicular to them (fig. 5.2A-B).

7. Dissect away as much adipose tissue as needed around the vessels to distinguish between the artery and vein. The artery can easily be identified by the following characteristics (fig. 5.3):

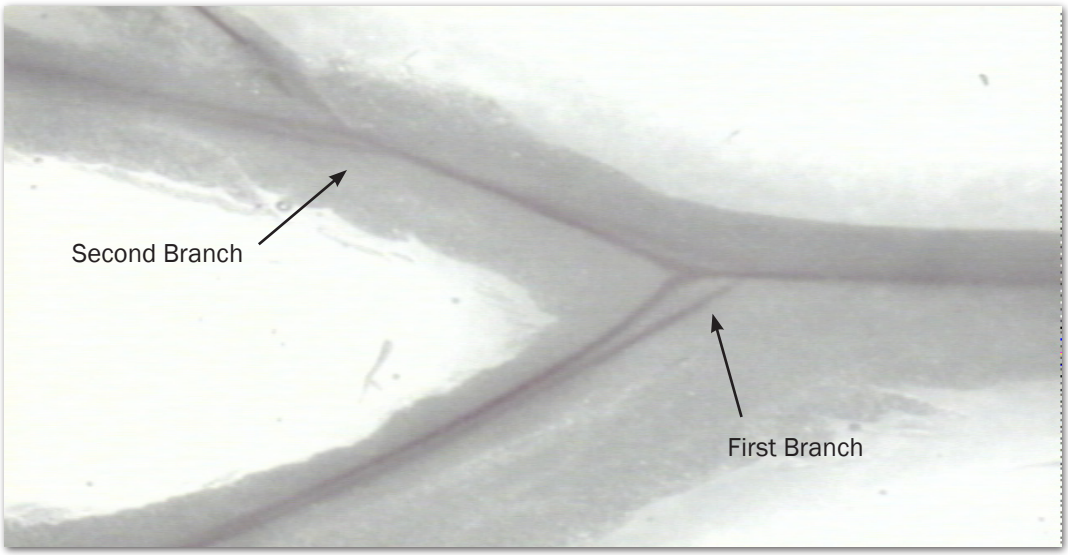


Figure 5.1 *Branching of the mesenteric arteries*

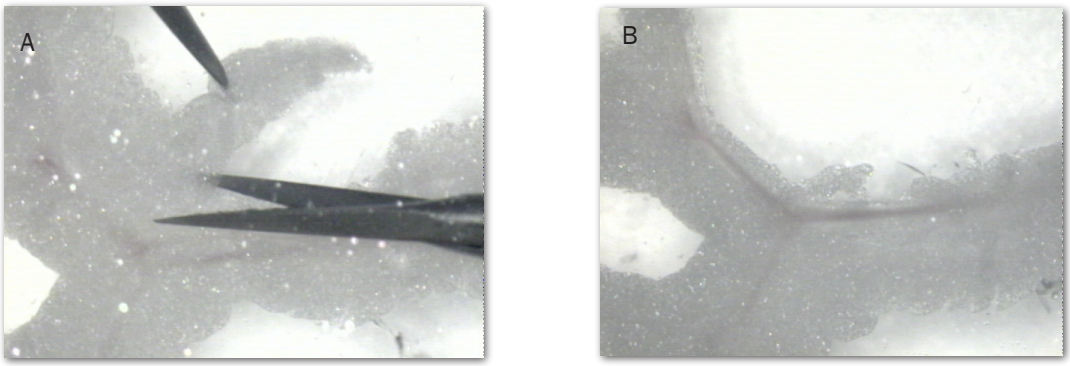


Figure 5.2 *Removal of adipose tissue around the area of interest*

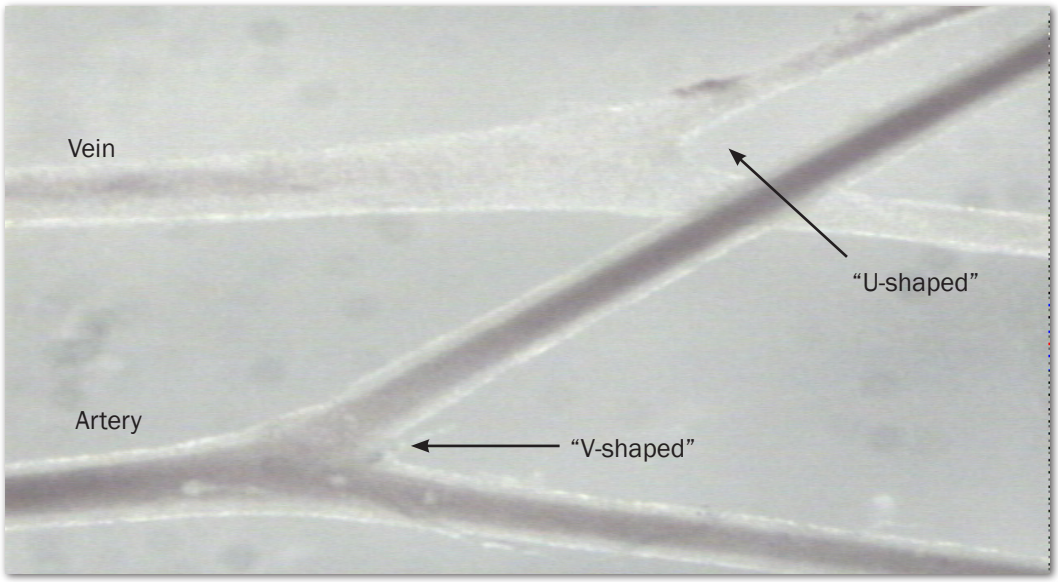


Figure 5.3 *Distinguishing between artery and vein*

- The branch points of arteries are V-shaped whereas those of veins are more U-shaped.
- The arterial wall contains a thick layer of smooth muscle cells compared to the vein wall, which only contains a single or a few layers of smooth muscle cells. The histological difference is clearly visible in the stereomicroscope.
- If you still have difficulty and the vein and artery still contain some blood then try to move the blood forward by very gently squeezing the vessels with a forceps. In the artery the blood will run back quickly whereas in the vein the blood will run back very slowly if it even does so. Note, it is important that you perform this on vessels other than those you will use as this procedure damages the vessels.

8. Dissect away the vein using scissors to cut the adipose and connective tissue between the artery and vein. One method is to cut the vein in one position and afterwards gently to pull the vein away from the artery. In this way a fine membrane of connective tissue becomes visible between the adipose tissue and the artery. Carefully cut the fine membrane to remove the vein and adipose tissue while avoiding any direct contact between the scissor and artery (fig. 5.4 A-B).

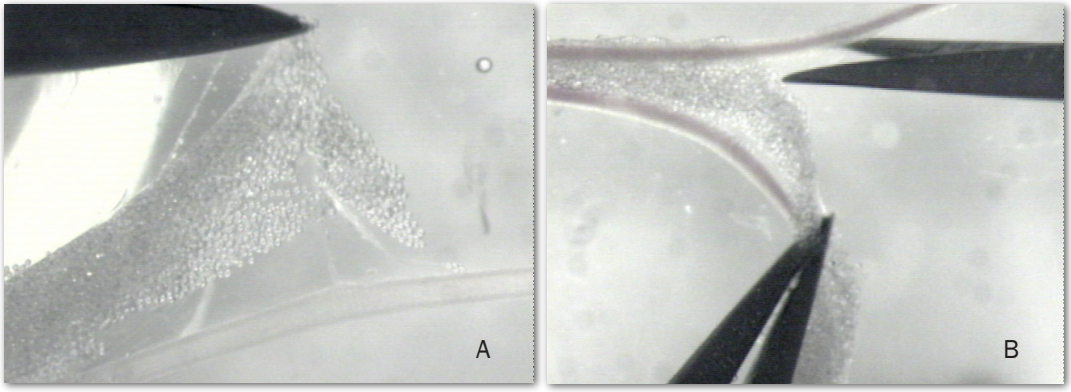


Figure 5.4 *Removal of vein*

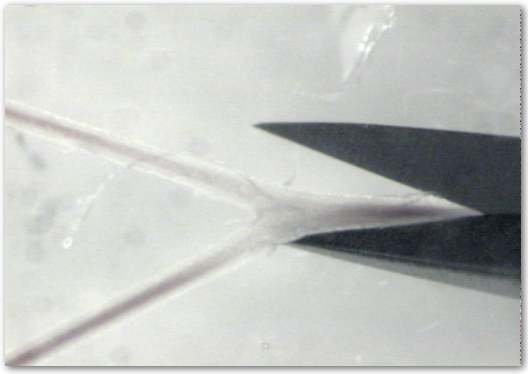
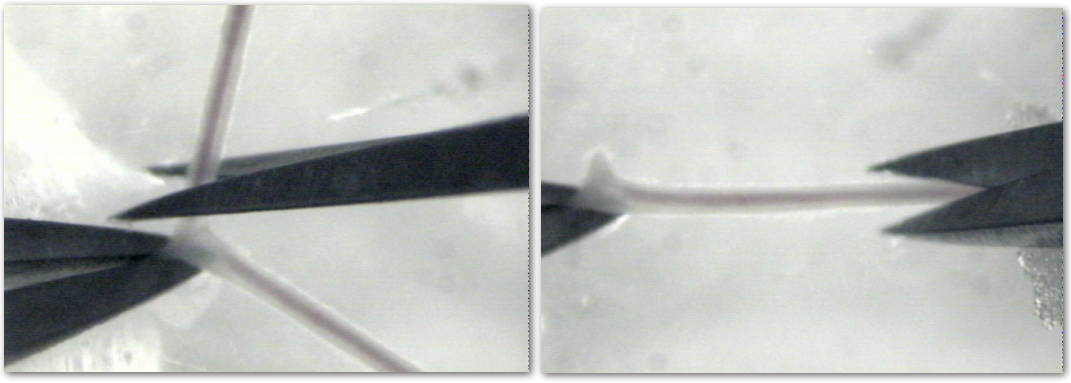


Figure 5.5 A, B and C *Cutting free the artery to be studied*



9. Clean the artery by removing any remaining adipose or connective tissue. Gently pull away adipose or connective tissue to make the connective tissue membrane become visible. Cut the membrane to remove the tissue.

10. Cut the distal end of the artery section to be investigated. Afterwards cut the proximal end while ensuring that the vessel segment has the correct length (fig. 5.5A-C).

5.2 Mounting protocol for small arteries

The difficulty of mounting ring preparations in any wire myograph mostly depends on the lumen size. The myograph technique is usable for ring preparations with internal diameter >100 µm using 40 µm stainless steel wires. 25 µm tungsten wires are available for ring preparations with an internal diameter as low as 60 µm. For large diameter vessels, mounting pins (200 µm and larger) are an available option.

The confocal myograph reverse mounting jaws are shaped with pegs at the bottom, to position the vessel as close as possible to the objective of the inverted confocal microscope. The special jaws (with a vessel mounted) are depicted in fig. 6.2. The pegs make the mounting of the vessel a little more difficult than in the normal wire myograph. Another difference from the normal wire myograph is that the mounting supports for the jaws are longer and thus less rigid. Slight pressure on the jaw can result in a significant stress upon the mounting supports. On the transducer side this results in stress upon the transducer pin, which could damage the transducer. Furthermore, there is a risk you will push the jaws out of alignment. For these reasons the demands upon your carefulness and awareness are increased in these procedures.
These instructions hopefully will help you to succeed with the mounting procedure. Experience with mounting in a normal myograph is an advantage and will ease the process.

Procedure:
Prior to mounting

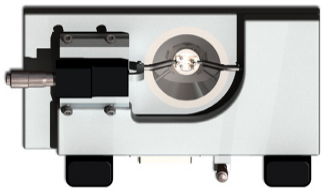
1. A vessel (size 150–500 µm) is dissected as described in 5.1.

3. Before mounting, it is important that the cover slip (diameter 19 mm, the thickness may need to be optimized to your optics) at the bottom of the myograph is clean. It is recommended that the glass be changed every day. To ease this process, the top part of the myograph with the heads can be tilted up and out of the way: this also permits easy access for cleaning. To remove the old glass, press up gently from below. Remove the old grease and fix the new glass with a little grease (high vacuum grease). It is important to remove any air bubbles between the glass and the chamber to ensure a good, water-tight seal.

4. It is also important that the mounting heads are carefully adjusted and aligned, so that there are no disturbances in the force recording, when the heads are close together. The alignment procedure is described elsewhere in the manual (Chapter 4). For the mounting procedure the following equipment is used: a stereomicroscope (magnification 4-40×) and a fibre light source, two pairs of fine forceps, a pair of fine scissors (trabecular type), a small screwdriver and wire.

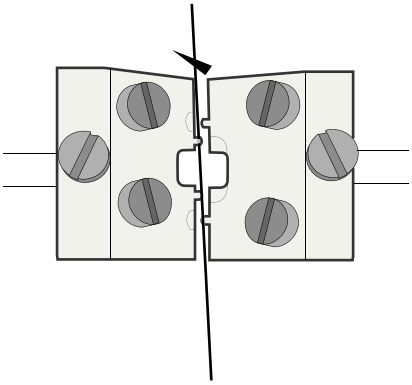
5. To have as much space as possible, move the supports up with the vertical micrometer screw (1.3) before you start the mounting procedure.

6. Place the myograph in front of you, with the micrometer support to your left. The head with the shortest distance between the pegs should be attached to the micrometer arm.



7. Close heads partly.

8. Hold a wire with the forceps one-third from the top. Place the wire between the heads underneath the top left peg and over the lower right peg.

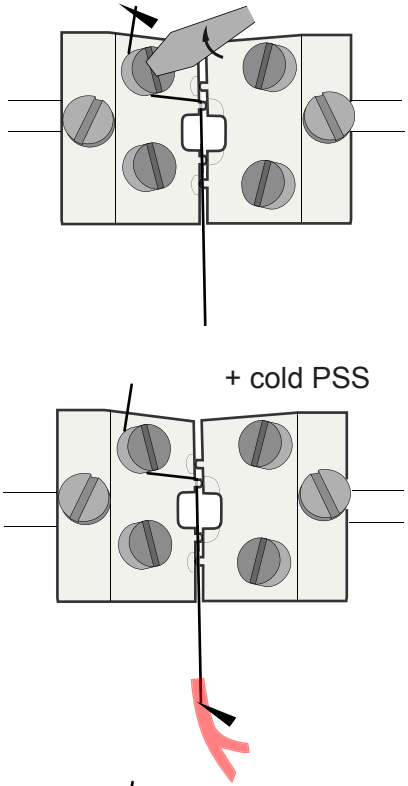


9. When the wire is in position, the heads are moved together and the top end of the wire is bent up and fixed by the top left screw. Remember always to place the wires clockwise under the screw heads, so that tightening of the screws will tighten the wires too.

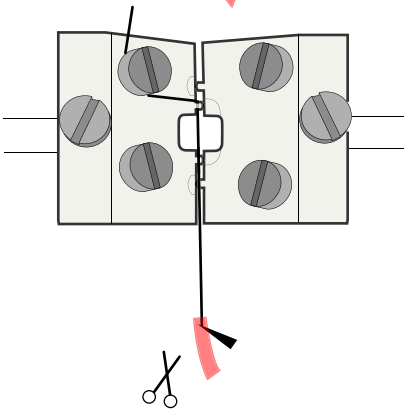
10. The protruding wire should be straight, and long enough to reach the lower left screw. Excess wire can be cut off.

11. Fill the chamber with cold PSS and transfer the vessel to the myograph chamber.

12. Holding the vessel close to the proximal end with the forceps, the vessel is mounted onto the wire.

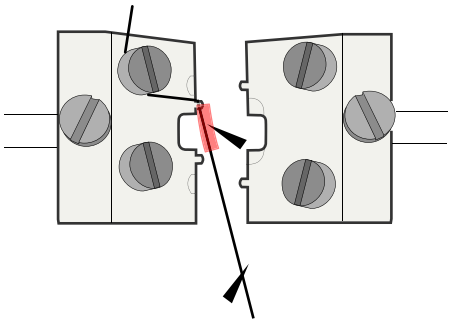


13. The vessel can now be cut off to give it the right length to fit the gap between the shortest pegs (2 mm). Add a little bit more = the width of one peg. (Explanation follows in step 15).



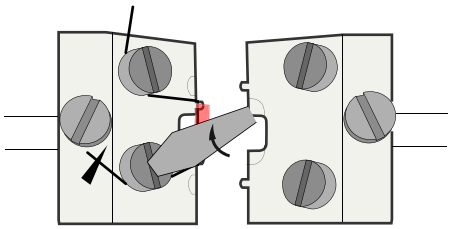
14. The jaws are now moved widely apart and the wire is released, so the vessel can pass the pegs without touching them and thereby be damaged.

15. The vessel is pulled gently up the wire and the wire is positioned underneath the top left peg.

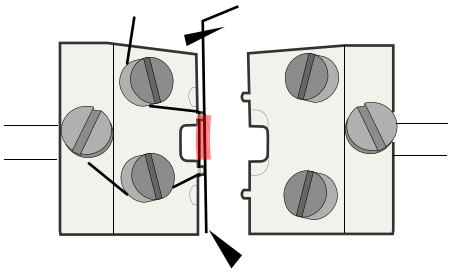


Note: When the proximal end of the vessel is fixed between the wire and the peg this prevents the vessel slipping down and becoming compressed when the second wire is inserted. The vessel should not be fixed at the distal end. A fixation at the distal end would hinder the vessel to move freely in the longitudinal direction.

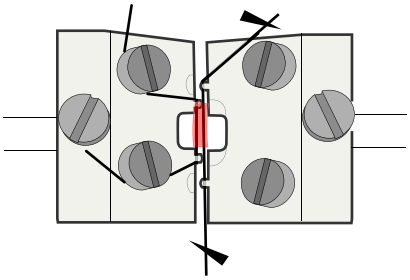
16. The wire is fixed with the lower left screw and at the same time is pulled to run tight from the screws along the head and underneath the left pegs. The vessel should run parallel to the front of the head.



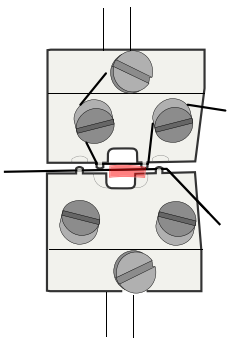
17. A long wire (25 mm) is bent one-third from the top in a 90° angle. This wire is guided into the vessel along the first wire from the top end of the vessel. When the wire is through the whole length of the vessel, it is cautiously pulled through the rest of the way, until it passes the lower right peg and enough wire is through to reach the lower right screw.



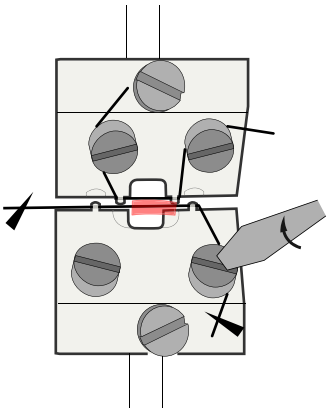
18. The jaws are screwed closely together. The top end of the wire, with the 90° angle, should point upwards between the jaws and the wire should run underneath the right side pegs.



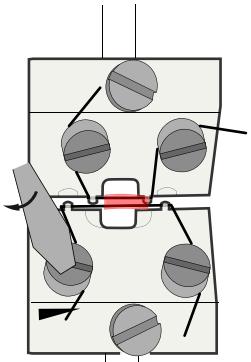
19. To ease the coming steps, which maybe the most critical ones for a viable preparation, the myograph is turned 90 degrees, so the vessel runs from right to left.



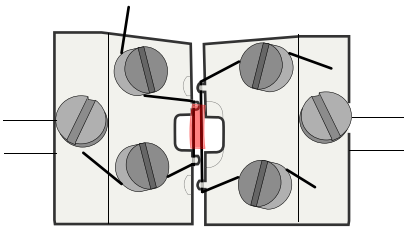
20. The top end of the second wire is bent towards the top right screw (the screws are still named as if the myograph was not turned 90°.) and fixed below the screw head. Take care that the vessel is not stretched or moved in the longitudinal direction. You might prevent this by holding the other end of the wire with your second pair of forceps.



21. The lower end of the wire now needs to be fixed to the lower right screw. Take care that the second wire is mounted parallel to the first wire and (of course) on the outside of it. Remember again that the wire should be straight and tight.



22. By looking in the microscope and at the same time watching the force signal, the height of the left wire is adjusted to allow it to move freely when the wires are moved together or away from each other. The height of the wire is adjusted by adjusting the Allen keys on the micrometer side.



Move the myograph to the confocal microscope and attach bubbling to the bath. Connect the myograph to the Myo-Interface and turn the heating on. Zeroing of the micrometer (X_0) can now be done (wires moved together until they are just touching) and a normalization performed (as described in the next section).
After the artery is normalized, contractility tested and loading with the fluorescent dye completed, move the heads downwards with the vertical micrometer to position the artery just above the cover slip. Once you can see the jaws are close to the cover slip, follow the force reading on the Myo-Interface. It is important to now advance slowly and carefully - too much downwards force and you will put unnecessary strain on the transducer and risk cracking the cover slip. When the artery is touching the bottom of the chamber, the force reading with increase. At this point, move the artery up slightly. The artery is now positioned for imaging and the objective should be able to focus on the vessel.

Note that movement artifacts can occur when the artery is stimulated with agonists or depolarized with potassium. This factor can be somewhat avoided by the use of ratiometric dyes. Contraction can be inhibited by wortmannin, which covalently binds to the myosin light-chain kinase. Alternatively, the artery can be hyperpolarized with potassium channel openers thereby preventing the opening of voltage-sensitive calcium channels and contraction.

5.3 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 set 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 100 mmHg = 13.3 kPa.

Principles of the normalization procedure

In practice the normalization is performed by distending the segment stepwise and measuring sets of micrometer and force readings (fig 5.13, step 1-4). 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 100 mmHg, IC_{100} is calculated from the point of intersection using the Laplace relation (fig. 5.14). IC_1 is calculated from IC_{100} by multiplying a 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 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.

Appendix 6 contains a complete description of the mathematical rationale and calculations of the normalization procedure.

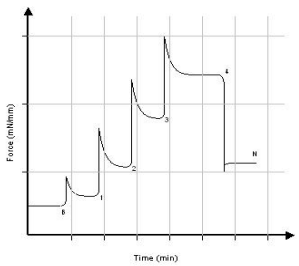


Figure 5.13 *Illustration of the stepwise normalization procedure*

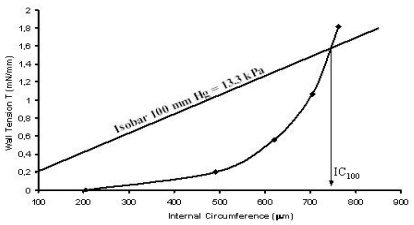


Figure 5.14 *Illustration of the exponential curve fitting and determination of IC_{100}*

5.4 Standard start

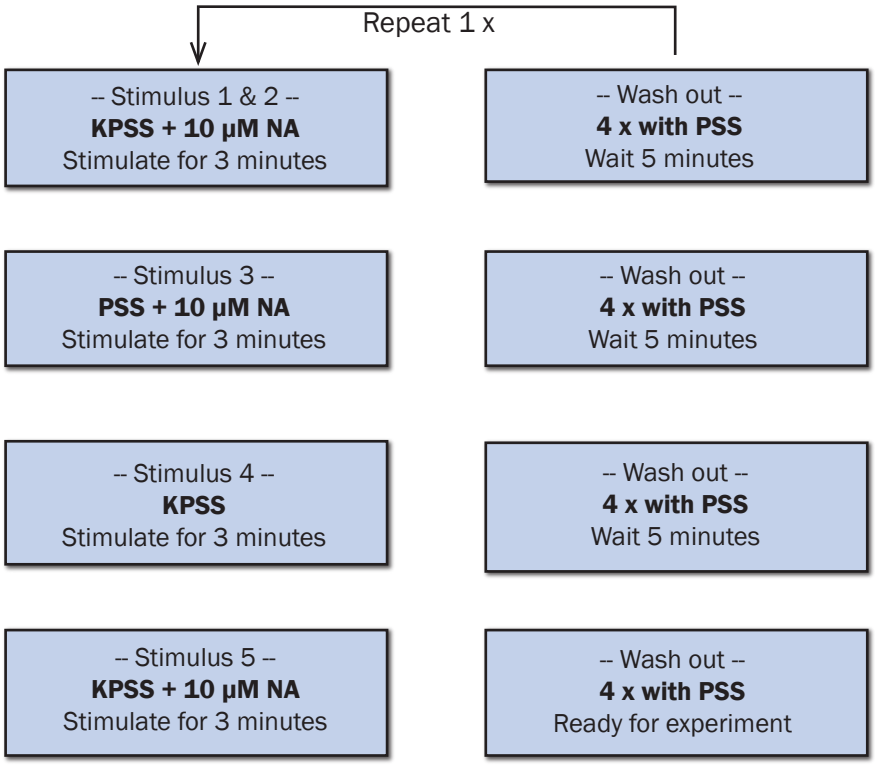
The purpose of performing a standard start is to:

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 13.3 kPa = 100 mmHg).

The standard start is performed after the vessel segment has been heated, equilibrated and normalized. The present procedure is suitable for rat mesenteric arteries. Another procedure may be needed for other animal species and tissue or vessel types.

Principles of the standard start 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 10 µM noradrenaline to give a maximum contractile response. The third stimulus is performed using a mixture of PSS and 10 µM noradrenaline to give a maximum pure agonist mediated (α-adrenoceptor) contraction. The fourth stimulus is performed using KPSS to give a depolarising contractile response (this stimulus also includes a component from neurally released noradrenaline). The final stimulus is performed using a mixture of PSS and 10 µM 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 at the end of this chapter.



5.5 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.

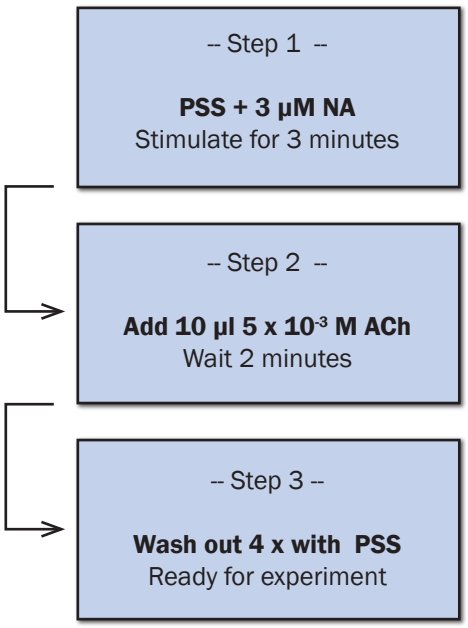
The procedure can be performed after the vessel segment has been heated, equilibrated and normalized. Preferably the procedure should be done after performing a "standard start" to make sure that the vessel segment is viable.

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

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.



5.6 Buffer recipes

Physiological saline solution (PSS)

To make 1 L of PSS:

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	118.99	6.95
KCl	74.56	4.69	0.35
$MgSO_4$ - $7H_2O$	246.48	1.17	0.29
KH_2PO_4	136.09	1.18	0.16

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
$CaCl_2$ - $2H_2O$	147.02	2.50	0.37

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L**
$NaHCO_3$	84.01	25.00	2.10
EDTA	372.24	0.03	0.01
Glucose	198.77	5.50	1.09

1. Dissolve the chemicals in approximately 100 mL double distilled H_2O as three individual solutions as described in the table above. Gently heat solution 3 to dissolve the EDTA.
2. Solution 1 is added to a graduated bottle and the bottle is filled with double distilled H_2O to a final volume of 500 mL.
3. Solution 3 is added to the graduated bottle, which afterwards is filled with additional double distilled H_2O to a final volume of about 850 mL.
4. Aerate the solution with carbogen (95% O_2 + 5% CO_2) for about 20 minutes.
5. Solution 2 is added and the graduated bottle is filled with additional double distilled H_2O to reach the final volume of 1000 mL. Continue the carbogen bubbling until the pH of the buffer solution reaches 7.4.

25x concentrated PSS

To make 1 L concentrated PSS:

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	118.99	173.85
KCl	74.56	4.69	8.75
$CaCl_2$ - $2H_2O$	147.02	2.50	9.20

Solution 2

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
$MgSO_4$ - $7H_2O$	246.48	1.17	7.23
$KH2PO_4$	136.09	1.18	4.02

Solution 3

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
EDTA	372.24	0.03	0.25

1. Dissolve the chemicals for solution 1 in about 800 mL double distilled H_2O in a 1000 mL graduated bottle. Dissolve the chemicals for solutions 2 and 3 in 75 mL double distilled H_2O in individually cylinders. Gently heat solution 3 to dissolve the EDTA.
2. Solution 2 and 3 is added to solution 1 and the graduated bottle is filled with additional double distilled H_2O to reach a final volume of 1000 mL.

Before use:

3. Dilute the 25 x PSS stock solution 1:25 with double distilled H_2O.
4. Add
   1.091 g/L Glucose
   2.100 g/L $NaHCO_3$
5. 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)

To make 1 L of KPSS:

Use the recipe for regular PSS but replace the desired concentration of NaCl with KCl. For example, to make 60 mM KPSS;

Solution 1

Chemical	**MW (g/mol)**	**Conc. (mmol/L)**	**Conc. (g/L)**
NaCl	58.44	64.86	3.79
KCl	74.56	58.82	4.39
$MgSO_4$ - $7H_2O$	246.48	1.17	0.29
KH_2PO_4	136.09	1.18	0.16

Calcium-free physiological saline solution (Ca-free PSS)

To make 1 L of Ca-free PSS:

Use the recipe for regular PSS but omit the $CaCl_2$ and add EGTA (to buffer the residual Ca^{2+} in solution). The concentration of EGTA in the PSS should be 1-5 mM to ensure sufficient buffering.

Appendix 1 — Terms of warranty

Warranty
DMT A/S warrants to the original user that myograph systems manufactured by DMT A/S will be free from defects in materials and workmanship for a period of three years after the date of delivery. DMT A/S will repair or replace any defective part, subject to the conditions, limitations and exclusions.

Exclusions
Force and pressure transducers, separately or part of myograph systems manufactured by DMT A/S, are disclaimed from any warranty.

Limitations
This warranty shall not apply to equipment subjected to accidental damage, improper use, alteration, or deterioration.

Warranty on third-party products will be as determined by their respective manufacturer.

DMT A/S shall not be liable for consequential, incidental, special, or other direct or indirect damages resulting from economic loss or property damage sustained by you or any end user from the use of the products sold or services rendered hereunder.

Warranty returns
A “Return Material Authorisation” (RMA) number is required for all returns. This number should be clearly indicated on all returned myograph systems.

Products damaged due to improper or inadequate packaging when returned for RMA purposes are not granted warranty coverage.

Appendix 2 — Service check

A myograph working at optimal performance is extremely important for success when studying small blood vessels or other small tubular tissues. To make sure that our customers always are dealing with first class myographs, DMT offers a Myograph Service Check at a very favourable price.

The Myograph Service Check includes a complete separation for inspection of all mechanical and electronic parts. The myograph is then reassembled, adjusted and finally all electronic and mechanical parts are tested.

Please note that the service does not include replacement of transducers or any other needed spare parts. Please contact DMT for information about prices.

Appendix 3 — Shipping instructions

If the myograph system needs to be sending back for service or repair, please read the following shipping instructions very carefully.

Before you start packing the myograph system, please remember that you are dealing with very delicate equipment and therefore care must be taken. DMT recommends that each part of the myograph system be wrapped individually (i.e. with bubble wrap) and placed together in a large box (preferable the box you once received the myograph system in). Place the wrapped items in the middle of the box and fill out the surroundings with chips of expanded polystyrene.

Important:
Before closing the box, make sure that no enclosed items can be shaken around as transport by road or air from time to time can be quite roughly.

Address the box to:
DMT A/S
Skejbyparken 152
DK-8200 Aarhus N
Denmark

Make sure that all four sides of the box are marked “fragile” or similar. Make an indication on the top of the box that it contains goods returned for repair/service.

Customers outside the EC must further enclose a pro forma invoice stating that the box contains goods being returned for repair or service.

If arranging transportation through a courier, please keep in mind the high value of the myograph system and that a standard insurance provided by the courier in most cases is insufficient to cover damage or loss of the myograph system. In most cases an additional insurance coverage is needed.

Appendix 4 — Myograph accessories and spare parts

This section contains a complete register of equipment needed to set-up a Confocal Wire Myograph System. In addition the chapter contains a list of special myograph 120CW accessories and spare parts.

Besides the main focus on development and manufacturing, DMT has specialised in offering our customers first class laboratory equipment needed for a confocal wire myograph set-up at very competitive prices. Please contact DMT Sales Department for further product information and prices.

A4.1 General myograph equipment

This section contains a complete and yet very useful checklist of laboratory equipment needed when setting up a basic wire myograph system:

- **Zeiss Axiovert 40CFL microscope** that is ready for future fluorescence upgrade. Item # MZA-8000

- **Dissection stereo microscope** Item # DSM-9953
  (Including ocular micrometer and stage micrometer) DMT recommends the Zeiss Stemi 2000 StereoMicroscope.

- **2 Dissection and mounting forceps** Item # DF-3000
  DMT recommends Dumont Medical No. 5, tip (0.10 mm x 0.06 mm).

- **Pipettes**
  DMT recommends CappAero™ pipettes.

- **Light source** Item # LS-9955
  DMT recommends Schott Cold Light Source, either Model KL 200 or Model KL 1500.

- **Water bath including heater** Item # WBH-9951
  DMT recommends Julabo 5L.

- **1 pair of ocular Scissors** Item # DS-1000
  DMT recommends the Geuder G-19745 8cm straight scissors.

- **Glass bottle 2 L**

- **Vacuum pump** Item # VP-9952
  DMT recommends a membrane vacuum pump having a volume of at least 6 L/min.

- **Dissection petri dish** Item # PD-2000
  DMT recommends a ~9 cm glass Petri dish coated with a 5 mm Sylgard polymer layer.

A4.2 Myograph 120CW system accessories

This section contains a list of special accessories available for the Confocal Wire Myograph System 120CW:

- **ADI PowerLab data acquisition system**
  Including Chart data acquisition and analysis software.
- **DMT Normalization module** DMT Item #
  Chart add-on software.
- **pH-meter** DMT Item #
  Including pH electrode and Myo Interface pH software flash update.
- **25 µm tungsten wire** DMT Item #
  For mounting of ring preparations, with internal diameter down to 60µm.
- **Electronic vacuum valve** DMT Item # VV-9937
- **Standard PC system** DMT Item #
- **DMT CS-100 (2 Channel) & CS-200 (4 Channel) Current Stimulators**
  DMT Item #CS-100 & CS-200 Combined pulse and train generator.

A4.3 Wire Myograph 120CW system spare parts

This section contains a complete list of standard available spare parts for the myograph 120CW. For parts not listed in this section or for special parts, which may need to be custom-made, please contact DMT for further information.

- **Force transducer** DMT Item # FT-12110
- **Roll of 40 µm stainless steel wire** DMT Item # SSW-123456
- **High vacuum grease and grease for linear slides** DMT Item # HVG-1000
- **Reverse mounting jaws (stainless steel)** DMT Item # RJ-120
- **Reverse mounting jaws (plastic) with in-built platinum stimulation electrodes**
- **Mounting pins (200 µm wire thickness)**
- **Calibration kit** DMT Item # GK-1050

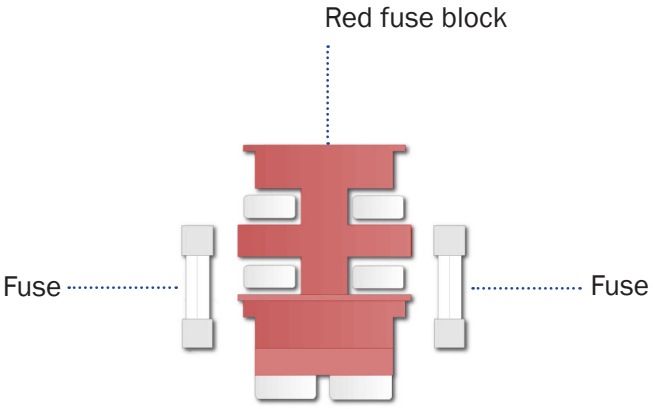
Appendix 5 — Fuse replacement

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

When a fuse blows and needs to be changed, it is imperative that the replacement fuse is equal to the one blown.
The 310A system uses: T1.6A / 250 V, 6.3 x 32 mm
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. Use a small screwdriver to open the voltage selector block.
2. Remove the red fuse block.
3. Remove the existing fuses.
4. Insert the new fuses.
5. Replace the fuse block back into the voltage selector block
   Note: ensure that the correct voltage for your country is displayed.



Appendix 6 — Normalization theory

The importance of making a normalization before initiating an experiment with any tubular tissue segment is described in Chapter 5. In this appendix the mathematical rationale and calculations underlying the normalization procedure are described in detail.

Mathematical calculations

Let (X_i, Y_i) be the pair of values representing the micrometer reading and force reading respectively characterising each step in the normalization procedure. Y_0 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_1 and a_2 are the vessel end points when 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 fig. 5.14 in Chapter 5. 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 100 mmHg isobar.

The normalized internal circumference IC_1 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 X_1 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 7 — Calibration of eyepiece reticule

Principles of ocular calibration

The purpose of calibrating the eyepiece reticule is to determine a conversion factor (δ) allowing the microscope to be used for measuring vessel segment lengths mounted in the wire myograph.

Several types of eyepiece reticules are available for such a purpose. The most simple and yet very useful type is a horizontal scale as illustrated in fig. A7.1.

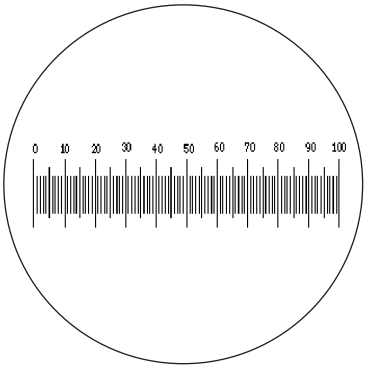


Figure A7.1 *Horizontal eyepiece reticule scale*

The basic principle is to use the eyepiece reticule, typically consisting of 50-100 divisions, to measure the length of an object in terms of reticular divisions spanned by the object. Having the conversion factor specific for the eyepiece reticule and used magnification, the length of the object in mm is easily calculated.

All reticules need to be calibrated in order to determine the specific conversion factor characteristic for the particular eyepiece reticule and the used magnification. For such purpose a special devise called a stage micrometer is needed. A stage micrometer is simply a microscope glass slide having a scale engraved on the surface. A typical micrometer scale is 2.00 mm long engraved with divisions of 0.01 mm equalling 10 µm per division. However micrometer glass slides with less fine divisions are also useful for calibrating a stereomicroscope to be used with the wire myograph.

Ocular calibration procedure

1) Decide which microscope magnification is to be used for the segment length measurements. Use the largest possible fixed magnification where the eyepiece reticule scale still covers the whole gap of the myograph jaws.

2) Place the stage micrometer on the microscope stage and focus on it. Fit one of the division lines on the stage micrometer to one of the division lines of the reticule scale very precisely. While keeping the stage micrometer absolutely fixed on the microscope stage, find another position on both scales where the division lines also fit precisely. Read the position of the two “fit points” on both scales and fill in the values in the ocular calibration sheet. Repeat the procedure twice.

Ocular calibration sheet

Date:	**Operator:**
Microscope (type and #):	
Ocular type:	
Magnification:	**Front lens:**

	Stage micrometer			**Reticular scale**		
Measurement	Position 1	Position 2	Length	Position 1	Position 2	Length
1	div.	div.	mm	div.	div.	div.
2	div.	div.	mm	div.	div.	div.
3	div.	div.	mm	div.	div.	div.
Mean			mm			div.

Calculations

1) Calculate the length between the two positions on the stage micrometer by subtracting the value of position 1 from the value of position 2. Multiply the length in divisions with the length of each division to get the length in mm.

2) Calculate the length between the two positions on the reticule scale by subtracting the value of position 1 from the value of position 2.

3) Calculate the mean length value of both the stage micrometer and the reticular scale.

4) Calculate the conversion factor:

$$\delta = \frac{\text{Mean Stage Micrometer Length (mm)}}{\text{Mean Reticule Scale (div)}}$$

Appendix 8 — How to read a millimeter micrometer

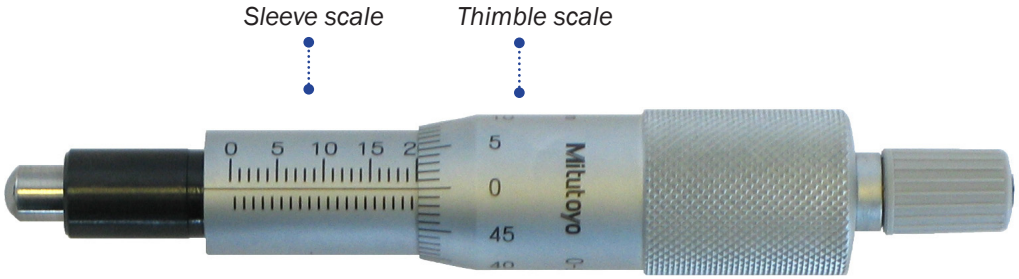


Figure A8.1 *Overview of the micrometer parts (actual reading 20000 µm = 20 mm)*

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

Thimble scale
The thimble is divided into fifty equal parts, and one complete rotation of the thimble is indicated by the smallest division on the sleeve, which equals 0.5 mm. Each divison on the thimble scale is 10 µm. 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 10000 µm (10 mm).
2. Note that there is no mark completely visible between the 10 mm mark and the thimble.
3. Read the value on the thimble corresponding to the intersection with the horizontal line on the sleeve.

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

Figure A8.2 *Example 1: reading = 10380 µm*

Example 2

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

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

Figure A8.3*. Example 2: reading = 16780 µm*

Appendix 9 — System specifications

Vessel size:	>60 µm
Vessel alignment:	Manually / X, Y & Z
Chamber / shape:	Single bath / conical
Chamber volume:	Max. 10 mL
Chamber material:	Acid-resistant stainless steel
Chamber cover:	With connection for suction and gassing
Force range:	±100, ± 200 mN
Force resolution:	0.01 mN
Micrometers:	Manual precision
Weight calibration:	Manual
Heating:	Built into chamber, independent of superfusion
Temp. range:	Ambient temp. to 50°C
Temp. resolution:	0.1° C
Temp. probe:	External
Output reading:	Force (mN)
Analog output:	Up to four outputs, 1.0 V full scale for all acquired signals, user-de fined
Serial output:	Serial interface - RS232 / RS485
Voltage:	100 to 240 VAC (auto) 50/60 Hz

Optional accessories:

pH-meter (enabled in the interface)

Range:	pH 0 - 14
Temp. correction:	0–50°C

Notes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