USER GUIDE

CONFOCAL PRESSURE MYOGRAPH - 120CP

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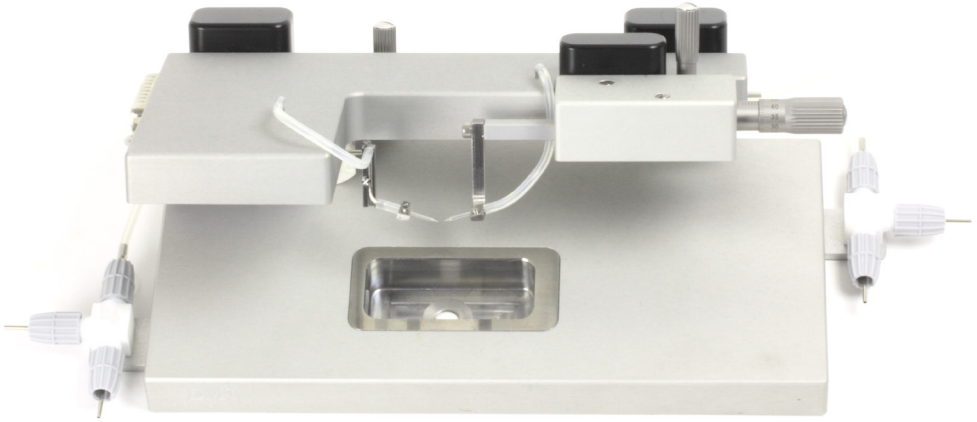

User Guide

Version 1.1

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CHAPTER 1 - CONFOCAL PRESSURE MYOGRAPH OVERVIEW

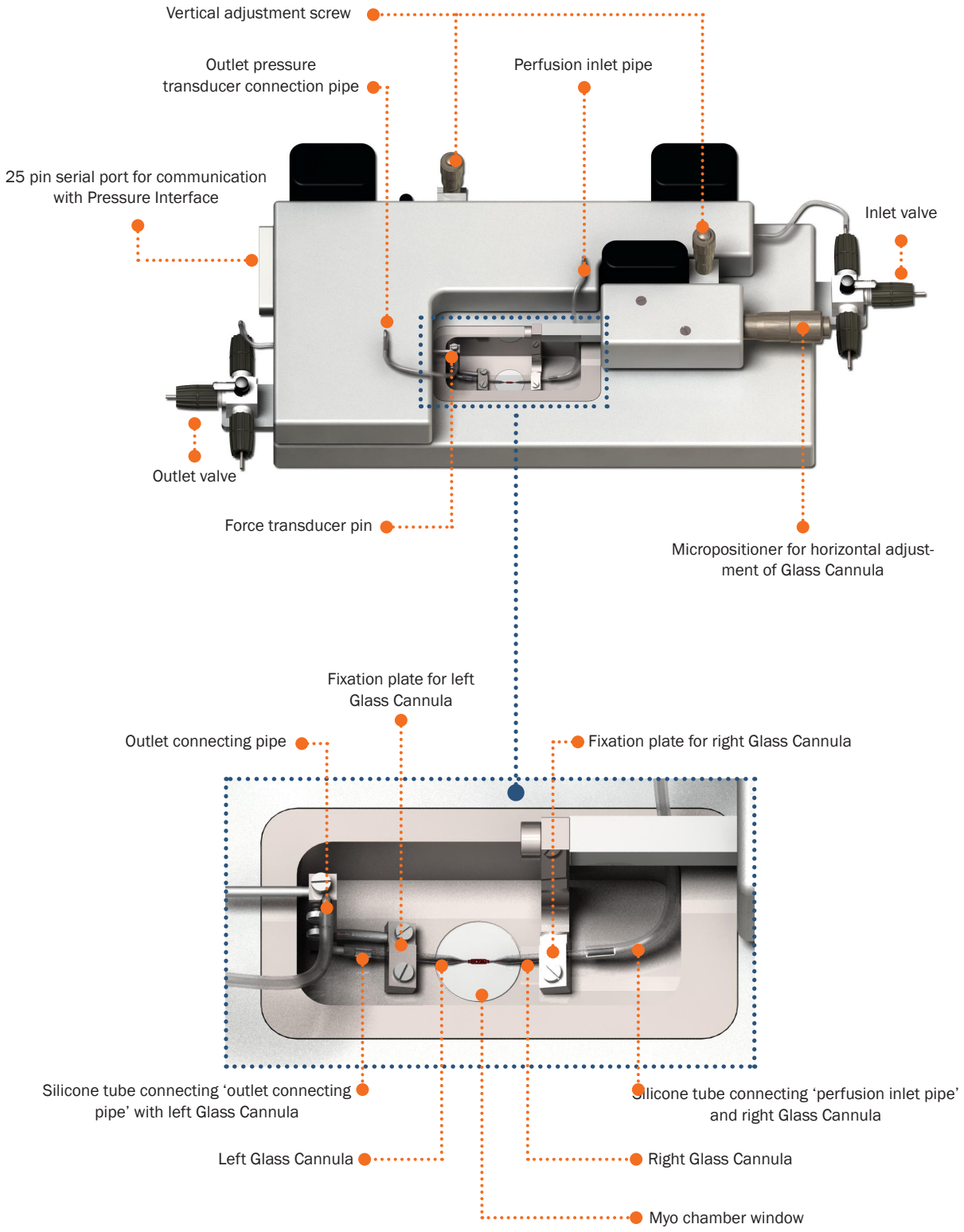


Figure 1.1 *Confocal Pressure Myograph - 120CP with close-up of chamber*

CHAPTER 2 - SET-UP THE CONFOCAL PRESSURE MYOGRAPH - 120CP

2.1 Adjustment of Glass Cannulas

Adjustment of the Glass Cannulas is divided into a pre-experimental alignment and general adjustments done during mounting of a blood vessel or when running an experiment.

General adjustments (see figure 2.1)

General adjustments are performed using the two micropositioners on top of the Confocal Pressure Myograph. The vertical micropositioner ”B” is used to adjust the vertical position of the right Glass Cannula and thereby the vertical position of the mounted blood vessel. The horizontal micropositioner “C” is used to define the length between the two Glass Cannulas and thereby the horizontal stretch of the mounted blood vessel.

Pre-experimental alignment

To make the general adjustments as easy as possible during an experiment, it is important to make an alignment of the Glass Cannulas prior to the mounting of the blood vessel.

Left Glass Cannula (see figure 2.1)

Horizontal positioning of the left Glass Cannula is adjusted by carefully loosening screw “D”. Vertical positioning of the left Glass Cannula is adjusted by carefully loosening screw “E”. Longitudinal positioning of the left Glass Cannula is adjusted by gently loosening of screw “F”.

IMPORTANT

BE VERY CAREFUL NOT TO APPLY TOO MUCH FORCE (>100 GRAM) ON THE FORCE TRANSDUCER PIN WHEN ADJUSTING THE LEFT GLASS CANNULA. THE FORCE TRANSDUCER HAS A MECHANICAL PROTECTION IN ITS LONGITUDINAL DIRECTION OF MOVEMENT, BUT IS VERY VULNERABLE TO FORCE APPLIED ON THE FORCE TRANSDUCER PIN.

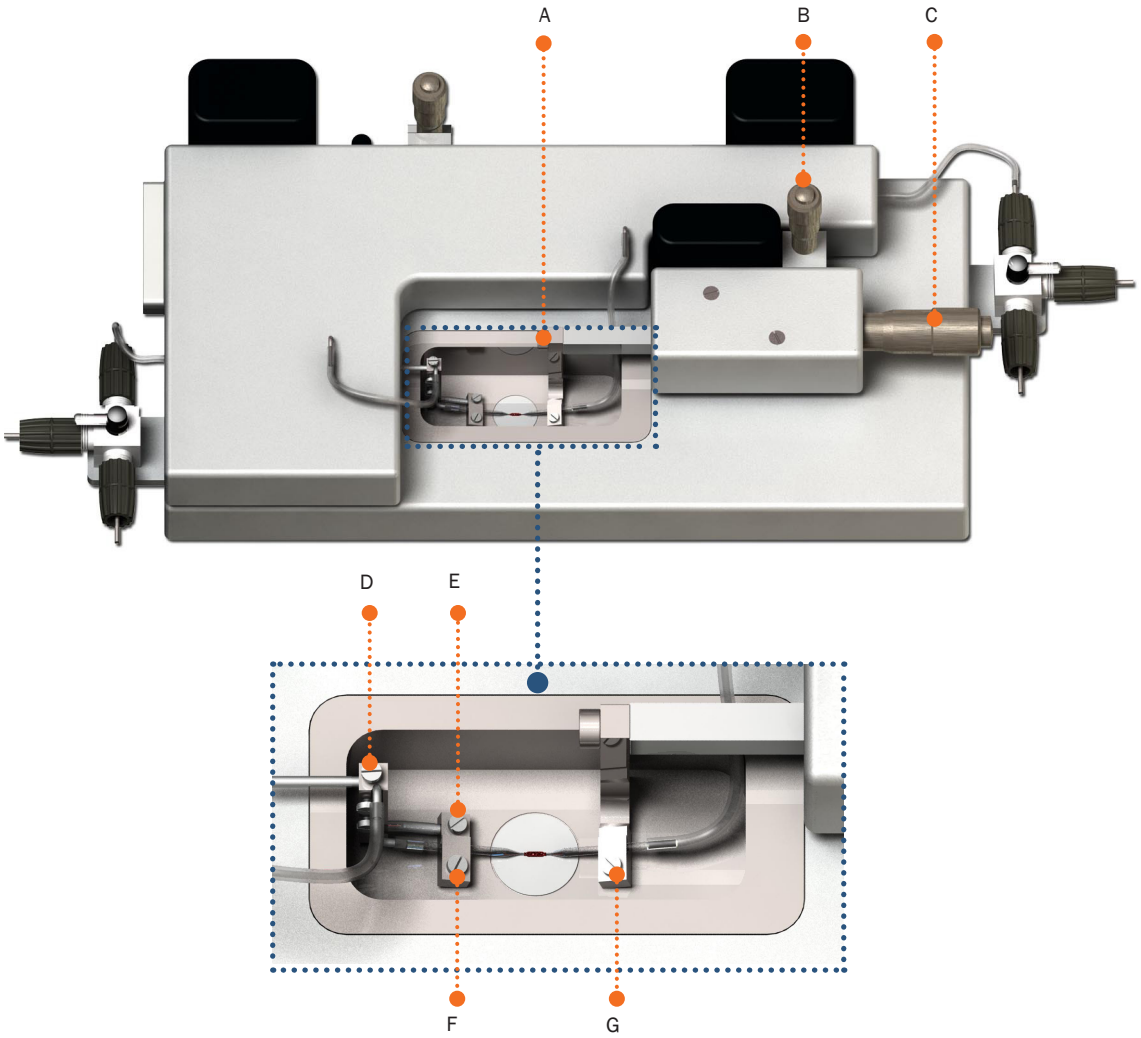


Figure 2.1 *Adjustment of Glass Cannulas*

Right Glass Cannula (see figure 2.1)

Horizontal positioning of the right Glass Cannula is adjusted by carefully loosening screw “A”. Vertical positioning of the right Glass Cannula is adjusted with the micropositioner “C”. Longitudinal positioning of the right Glass Cannula is adjusted by gently loosening of screw “G”.

NOTE
BE CAREFUL NOT TO DAMAGE THE TIP OF THE GLASS CANNULAS DURING THE GENERAL ADJUSTMENT.

2.2 3-way valve adjustments

The 3-way valves on each side of the Confocal Pressure Myograph (at P1 & P2) have three different settings to control the in- or outlet flow to the pressure transducers. The flow settings are illustrated in figure 2.2 and figure 2.3 below.

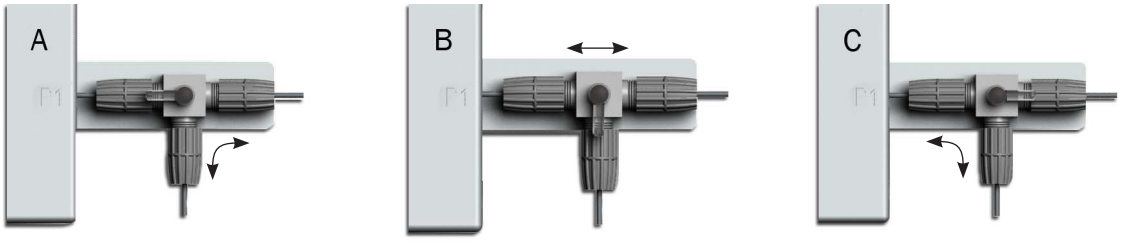


Figure 2.2 *Three-way valve flow settings at P1*
The arrows indicates the flow direction

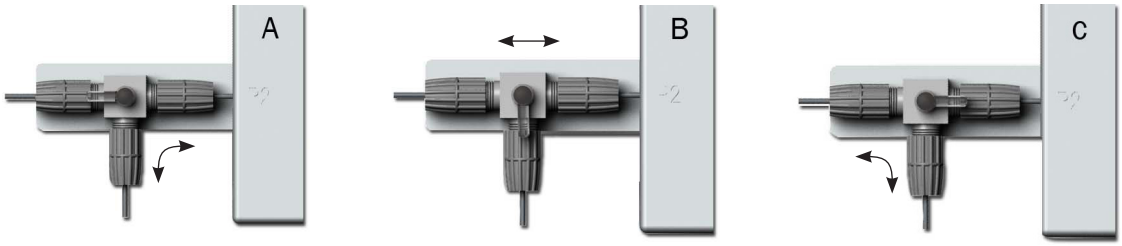


Figure 2.3 *Three-way valve flow settings at P2*
The arrows indicates the flow direction

CHAPTER 3 - EXPERIMENTAL SET-UP

3.1 Mounting protocol for small arteries

This section shortly describes the basic technique of mounting small vessels in the Confocal Pressure Myograph.

3.1.1 Preparation

1. Prepare your PSS.
   60mM KPSS can be used to test contractions, and 16mM KPSS can be used to cause relaxations (usually 16mM KPSS only works in small arteries for relaxation).

2. Make at least 4 loops with nylon suture to use for securing the vessel to the mounting Glass Cannulas. Double loops are best and will prevent the loops from slipping loose once tightened.

3. Pre-heat PSS to 37 °C, making sure the PSS is bubbled with carbogen (5% CO2, 95% O2) or PraxAir (5% CO2, 21% O2, balance N2) for at least 20 to 30 minutes.

4. If desired, rinse the mounting chamber and all tubing that sees buffer with double-distilled H_2O.

5. Fill the P1 reservoir bottle in the Pressure Interface with pre-warmed PSS. DO NOT FILL MORE THAN 250ML. Keep the tubing from the bottle attached to port on top of the bottle marked P1 higher than the bottle, otherwise capillary action will draw buffer from the bottle and will leak all over the bench.

3.1.2 Mounting

1. Isolate small vessel of interest.

2. Clean the vessel as best as possible of perivascular fat and tissue.

3. Place the small artery into a small container of cold or room temperature PSS.

4. Place 10 ml. of cold or room temperature PSS in the mounting chamber.

5. Have a syringe with buffer attached to the P1 3-way valve, but the P1 and P2 3-way valves should be closed to the vessel.

6. Position the sutures appropriately on both Glass Cannulas so that they are ready for use when the vessel is mounted. Place 2 loops on each Glass Cannula in case one breaks or fails.

7. Carefully transfer the vessel from the small container to the mounting chamber. Using a large bore pipette tip and pipette or an eye-dropper, carefully suck up the isolated vessel and transfer it to the chamber. This will minimize handling and potential for damaging the vessel.

8. Continue to mount the vessel on the Glass Cannulas. Start with the P1 side. Once the P1 side of the vessel is mounted on the Glass Cannula, secure the vessel to the Glass Cannula by tightening the loops around the vessel onto the Glass Cannula.

9. Make sure the vessel is mounted in the correct direction. Flow through the vessel is directional, so proximal and distal ends for flow will need to be noted somehow. Mount the proximal end to flow on the P1 Glass Cannula.

10. Once secured, gently rinse the vessel of any blood. Before washing the lumen, gently push some buffer from the syringe through the 3-way valve to the port that is not connected to the vessel so as to bleed out any air that might have been trapped in the valve.

11. Open the valve to the vessel and gently push buffer through to wash out any blood in the lumen. This will also help open the distal end if it is pinched shut. Close the P1 3-way valve so as to keep the vessel from collapsing.

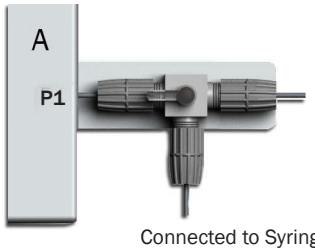
12. Position the P1 Glass Cannula with attached vessel so as to facilitate mounting of the vessel on the P2 Glass Cannula.

13. Gently mount the vessel on the P2 Glass Cannula, without pulling it off the P1 Glass Cannula. Secure the vessel as described in step 7.

14. Once the vessel is secured on the P2 Glass Cannula, open the P1 Glass Cannula and gently push more buffer into the mounted vessel. The vessel should slightly fill and bulge from the small pressure-head. Close the P1 2-way valve to maintain a slightly pressurized vessel.

15. Transfer the mounting chamber to the microscope, and attach all the tubing.

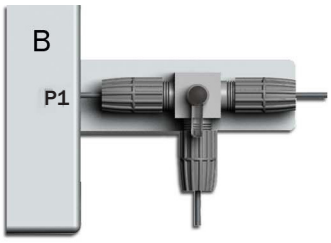
16. Fill the tubing leading from the P1 reservoir bottle (marked P1 at the top of the bottle) with the buffer that has been pre-warmed and pre-bubbled. The easiest way to do this is attach a syringe to the P1 -3way valve as illustrated, and draw buffer from the bottle. The 3-way valve should be positioned the same way.



Connected to P1 Reservoir Bottle

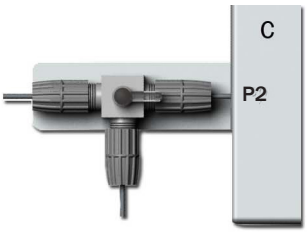
Connected to Syringe - draw buffer from here

17. Once the buffer is drawn through the tubing, turn the 3-way valve as shown.



18. Disconnect the syringe and move it to the P2 side of the chamber, but DO NOT EMPTY THE SYRINGE. Attach to the 3-way valve as shown.

Connected to port of Pressure Interface marked P2

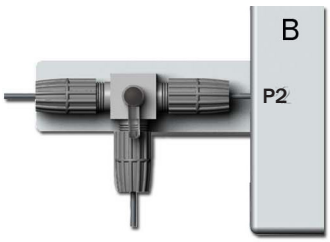


Connected to Syringe filled with buffer

19. Use the buffer that was drawn from the bottle to charge the remainder of the system. The buffer should be pushed gently from the 3-way valve all the way to the waste bottle. When the buffer starts dripping in the waste bottle, stop pushing buffer.

IMPORTANT
BE CAREFUL WHEN CHARGING THIS PART OF THE SYSTEM. EXCESSIVE PRESSURE CAN DAMAGE THE INTERNAL 3RD HIDDEN TRANSDUCER IN THE PRESSURE REGULATOR.

20. Close the 3-way valve as shown below.



21 The system is charged and ready.

3.1.3 Equilibration

1. Turn on the heat, which should be preset to 37 °C.
2. Start aerate PSS in the chamber with oxygen.
3. Set P1 to 20mmHg and P2 to 5mmHg. This will cause a flow gradient and perfuse the vessel with some of the newly charged buffer. Make sure that the charged lines DO NOT have air bubbles, otherwise endothelium will be stripped from the lumen. Perfuse for 2 to 3 minutes.
4. Set P1 and P2 pressure to 10mmHg.
5. Increase pressure by 10mmHg every 5 minutes until target pressure is reached. For cerebral arteries from rats, this may be 60 to 70 mmHg. For mesenteric resistance arteries, this would be 60mmHg.
6. After the first 20 minutes after starting the heat and pressurization, remove the old PSS and replace it with new PSS that should be pre-warmed and oxygenated.
7. Continue to pressurize the vessel.
8. After 40 minutes from the start of heat and pressurization, do another wash, as in step 6.
9. Once 60 minutes have passed, the wake-up or standard-start protocol can be initiated.

3.1.4 Example of wake-up or standard-start protocol

NOTE
SEE APPENDIX 1 FOR BUFFER RECIPES.

1. Once the vessel is equilibrated, remove the old PSS and add 60mM KPSS to cause a contraction.
2. Allow the vessel to contract.
3. Wash the KPSS until baseline diameter is reached.
4. Repeat the KPSS contraction and washes.
5. An agonist such as norepinephrine can be added (10-8M final in the bath) to cause a contraction. However, the vessel should also have developed some myogenic tone. The amount of tone will depend on the vessel used.
6. Once the vessel is contracted, Ach (10-5M final in the bath) can be added to assess endothelial function.
7. Wash the vessel 5 to 6 times over 20 to 30 minutes.
8. Vessel is now ready for experiments.

CHAPTER 4 - CLEANING AND MAINTENANCE

The Confocal Pressure Myograph 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 that the instructions are followed at all times.

4.1 Cleaning the Confocal Pressure Myograph

DMT STRONGLY RECOMMENDS THAT THE PRESSURE SYSTEM AND SURROUNDINGS BE CLEANED AFTER EACH EXPERIMENT.

1. After completing an experiment, remove the vessel mounted on the Glass Cannulas.
2. Remove buffer from the tissue chamber.
3. Turn off heat.
4. Dump any buffers in the bottles (both P1 and P2 bottles). P1 bottle is the bottle that starts with buffer. P2 bottle is the waste bottle. If looking at the Pressure Interface from the front, P1 bottle is on the right and P2 bottle is on the left.
5. Put the bottles back in place and tighten the caps.
6. Make sure all the tubing is connected to the chamber and bottles.
7. Make sure that the system is NOT in No Flow (turn off No Flow if this function was used).
8. If using Glass Cannulas for small vessels, then insert the calibration shunt that came with your system to bypass the Glass Cannulas in the same fashion you would use the shunt to calibrate your pressure transducers.
9. If looking at the cap of the P1 bottle, the frits at the top where the tubing is connected should be clearly marked with “P1”, “4”, and “5”. Detach the tubing from the top of the P1 bottle marked “5”.
10. Attach the Big Ben manometer at the frit marked “5”.
11. Using the Big Ben manometer, push the residual buffer out of the system. Using the Big Ben manometer will allow you to pressurize the system by knowing exactly how much pressure is being used without having to worry about blowing out the P1 and P2 pressure transducers. More importantly, this will protect the hidden P3 transducer in the Pressure Interface. DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANDUCERS!
12. Once the buffer has been cleared from the system and into the waste bottle, fill the P1 bottle with double-distilled water. DO NOT FILL WITH MORE THAN 250 mL OF WATER.
13. Re-attach the P1 bottle top, and make sure it is tight.
14. Using the Big Ben manometer that should still be attached to the “5” frit on top of the bottle, pressurize the bottle using the manometer. DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANDUCERS! Water should be pushed from the bottle through the system. Do this for at least 5 minutes.
15. As an alternative, re-attach the tubing to the “5” frit on top of the P1 bottle. Make sure all the other tubing is connected in the system so that if flow is induced, the water from the P1 bottle will flow from the P1 bottle to the P2 waste bottle freely.
16. Induce flow by setting P1 on the Pressure Interface to 200 mmHg and setting P2 to 50 mmHg. This should cause the water in the P1 bottle to flow through the system to wash the tubing and internal parts of the chamber and pressure regulator. If this method is used to clean the system, then run this flow program for at least 10 minutes.
17. Once the system has been flushed with water, empty the water from the P1 bottle.
18. Re-attach the cap to the P1 bottle, and using the Big Ben Manometer, push the water out from the system by attaching the Big Ben to frit “5” on the P1 bottle. Again, DO NOT EXCEED 250 mmHg! IF YOU EXCEED 250 mmHg, YOU WILL DAMAGE ALL 3 PRESSURE TRANDUCERS!
19. Once the system is emptied, dry the system by continuing to push air through the lines. Push air through for at least 5 minutes.
20. Once air has been pushed through the system, detach the Big Ben manometer from the P1 bottle and reattach the appro-

priate tubing.

21. Detach the tubing from the Pressure Interface (NOT THE BOTTLES) where it is marked P2.

22. Connect the Big Ben manometer here, and push air through to make sure the inside of the regulator is dried. If buffer or water stays in this part of the regulator, it can cause problems that are not covered by warranty, and this step will guarantee that this part of the regulator stays clear.

23. If an 8% acetic acid rinse is performed, then repeat these steps and make sure that the system is thoroughly washed with double-distilled water again to remove the acetic acid.

    NOTE
    AN ACETIC ACID WASH IS NOT NEEDED AFTER EACH USE. USE THIS SPARINGLY. IF ONLY BUFFER HAS BEEN USED IN THE PERFUSION LINE, THEN THERE IS NO NEED TO EVER USE ACETIC ACID TO CLEAN THE TUBING, INTERNAL BUFFER LINES OF THE CHAMBER, AND INSIDE THE PRESSURE INTERFACE.

IMPORTANT I
BE VERY CAREFUL NOT TO DAMAGE PRESSURE TRANSDUCER P3 WHEN FLUSHING THE BUFFER CIRCUIT. THERE ARE NO READINGS FROM P3 VISIBLE ON THE PRESSURE INTERFACE. DMT THEREFORE RECOMMENDS THAT THE CONFOCAL PRESSURE MYOGRAPH ALWAYS BE FLUSHED FIRST. IN THIS WAY IT IS POSSIBLE TO GET A FEELING OF HOW MUCH "FLUSHING PRESSURE" THAT IS EQUAL TO 150 mmHG.

IMPORTANT II
NEVER FLUSH THE AIR CIRCUITS (PORT NO. 2, 3 AND 4) ON THE PRESSURE INTERFACE BOTTLES WITH ANY KIND OF FLUID.

4.2 Cleaning the Confocal Pressure Myograph Chamber and Glass Cannulas

DMT STRONGLY RECOMMENDS THAT THE CONFOCAL PRESSURE MYOGRAPH CHAMBER AND SURROUNDINGS BE CLEANED AFTER EACH EXPERIMENT.

After a "normal" experiment use the following procedure to clean the Confocal Pressure Myograph chamber and supports:

The chamber and Glass Cannulas can be cleaned at the same time while the systems is being flushed if the flow program is used as described in step 15 above.

1. To clean the chamber, fill the chamber with double-distilled water and wash at least 3 times.

2. To clean the Glass Cannulas, one can use a syringe and needle. An 18-guage needle with the sharp bevel removed works the best.

3. Fill the syringe with air.

4. To clean the Glass Cannulas, attach the syringe with needle at the point in the chamber indicated at figure 4.1.

5. Make sure the Glass Cannulas are not aligned. If they are still aligned, pushing the air into the Glass Cannulas will only cause the buffer or water to shoot into the Glass Cannula straight across from it.

Detach tubing here and insert end of blunted 18-gauge needle attached to syringe into tubing to clean P2 Glass Cannulas.

Detach tubing here and insert end of blunted 18-gauge needle attached to syringe into tubing to clean P1 Glass Cannulas.

Figure 4.1 Chamber close-up

By attaching the syringe at these points, the P1 and P2 pressure transducer is bypassed, and water or air can be pushed through the Glass Cannulas as hard as the user likes.

6. Push the air from the syringe through the Glass Cannula. This should push out any buffer in the Glass Cannula.

7. Remove the syringe and fill with double-distilled water.

8. Re-attach as in step 4 above.

9. Push water through the Glass Cannula to wash.

10. Remove the syringe, remove the water from the syringe, and reattach the syringe as in step 4.

11. Push air through the Glass Cannula to dry.

12. Repeat steps 3 through 11 on the P2 Glass Cannula. Attach the syringe at the point in the chamber as indicated in figure 4.1.

Using this cleaning procedure will minimize damage to the pressure transducers, will prevent clogs from occurring in the Pressure Interface, and will minimize clogs in the Glass Cannula.

IMPORTANT
ACETIC ACID MAY BE USED MORE LIBERALLY IN CLEANING THE CHAMBER AND GLASS CANNULAS. IT IS IMPORTANT TO THOROUGHLY WASH THE ACETIC ACID FROM THE GLASS CANNULAS, OTHERWISE PRECIPITATE WILL FORM IN THE GLASS CANNULAS AND CLOG THEM, REQUIRING THAT THE GLASS CANNULAS BE CHANGED MORE OFTEN.

If the chamber still have salt build-up then continue with the following procedure:

1. Fill the Confocal Pressure 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 Confocal Pressure Myograph chamber and Glass Cannulas 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 Glass Cannulas with 96% ethanol or a weak detergent solution (e.g. Treepol).

4. To remove more resistant or toxic chemicals, incubate the Confocal Pressure Myograph chamber and Glass Cannulas with 1M HCl for up to 1 hour. In exceptional cases incubate the chamber and supports with an up to 3M HNO_3 solution for about 15 minutes.

5. Wash the Confocal Pressure Myograph chamber and Glass Cannulas several times with double distilled water.

IMPORTANT NOTES
BE VERY CAREFUL USING STEP 3 AND 4 REPEATEDLY AS STRONG REAGENTS CAN CAUSE EXTREME DAMAGE TO THE CONFOCAL PRESSURE MYOGRAPH.
BE VERY CAREFUL NOT TO DAMAGE THE GLASS CANNULAS 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 Confocal Pressure Myograph chamber for 30 minutes with 20µl of a 2mM 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 Confocal Pressure Myograph chamber several times with double distilled water.

4. Incubate the Confocal Pressure 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 Confocal Pressure Myograph chamber with an 8% acetic acid solution for 10 minutes and continue the mechanical cleaning with a swab-stick.

6. Wash the Confocal Pressure Myograph chamber several times with double distilled water.

4.3 Maintenance of Confocal Pressure 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. Afterwards, remove the cover from the Confocal Pressure Myograph chamber and turn on the vacuum pump and vacuum valve 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.

4.4 Maintenance of force transducer

The force transducer is one of the most delicate and fragile components of the Confocal Pressure Myograph. Therefore careful handling of the force transducer is of most importance to prevent it being damaged.

The left Glass Cannula in the Confocal Pressure Myograph is connected to the transducer pin coming through a small hole in the transducer house located on top of the Confocal Pressure Myograph, as illustrated in figure 4.2 below. To prevent the buffer from running into the transducer house, the hole is filled with high vacuum grease.

As a part of 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 (SEE FIGURE 4.2 BELOW).
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 TRANSDUCERS.

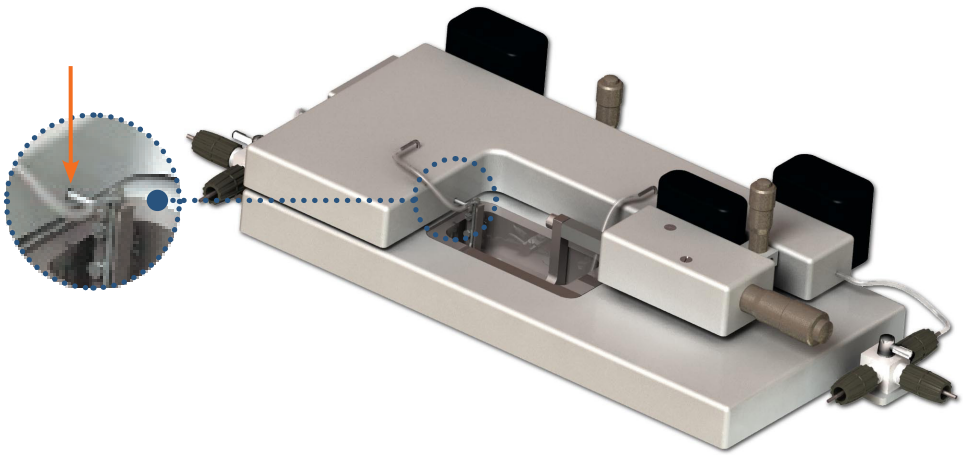


Figure 4.2 *Transducer pin hole sealed up with high vacuum grease*

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

4.5 Pressure transducer and Glass Cannulas

To prevent the transducers and the tubing inside the Confocal Pressure Myograph from being blocked by buffer salt deposits after an experiment, DMT recommends that the system shoul be flushed using the following procedure.

1. Keep the Confocal Pressure Myograph connected to the Pressure Interface to enable continous monitoring of the pressure readings from P1 and P2.

2. Fill a small syringe with 8% acetic acid and flush both pressure transducers and Glass Cannulas by connecting the syringe needle to the 3-way valve for the individual pressure transducers and Glass Cannulas using a small piece of silicone tube.

IMPORTANT
BE VERY CAREFUL NOT TO APPLY A TOO HIGH PRESSURE WHEN FLUSHING AS THIS MAY DAMAGE THE PRESSURE TRANSDUCERS. KEEP AN EYE ON THE PRESSURE TRANSDUCER READINGS ON THE PRESSURE INTERFACE WHEN FLUSHING AND MAKE SURE THAT THE PRESSURE DOES NOT EXCEED 150 mmHg.

3. Flush the pressure transducers and Glass Cannulas as described in step 2. Repeat the procedure two to three times.

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)	14.9	0.625	1.25	2.50	5.00
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

(1.0 M solution)

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 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:

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
$NaHCO_3$	(84.01)	14.9	0.625	1.25	2.50	5.00
Glucose	(180.16)	5.5	0.5	1.00	2.00	4.00
EDTA	(380)	0.65	0.125	0.25	0.50	1.0
$CaCl_2$	(110.99)	40	20mL	40mL	80mL	160mL

(1.0 M solution)

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 dissolved. This takes about 15 minutes at room temperature.

Before use:

5. Dilute the 25 x PSS stock solution 1:25 using double distilled H_2O.

6. Add:
   1.091 g/L Glucose
   2.100 g/L $NaHCO_3$

7. 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	4.37	8.73	17.46
KCl	(74.557)	60	2.24	4.47	8.95	17.89
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)	14.9	0.625	1.25	2.50	5.00
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

(1.0 M solution)

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 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.

NOTES