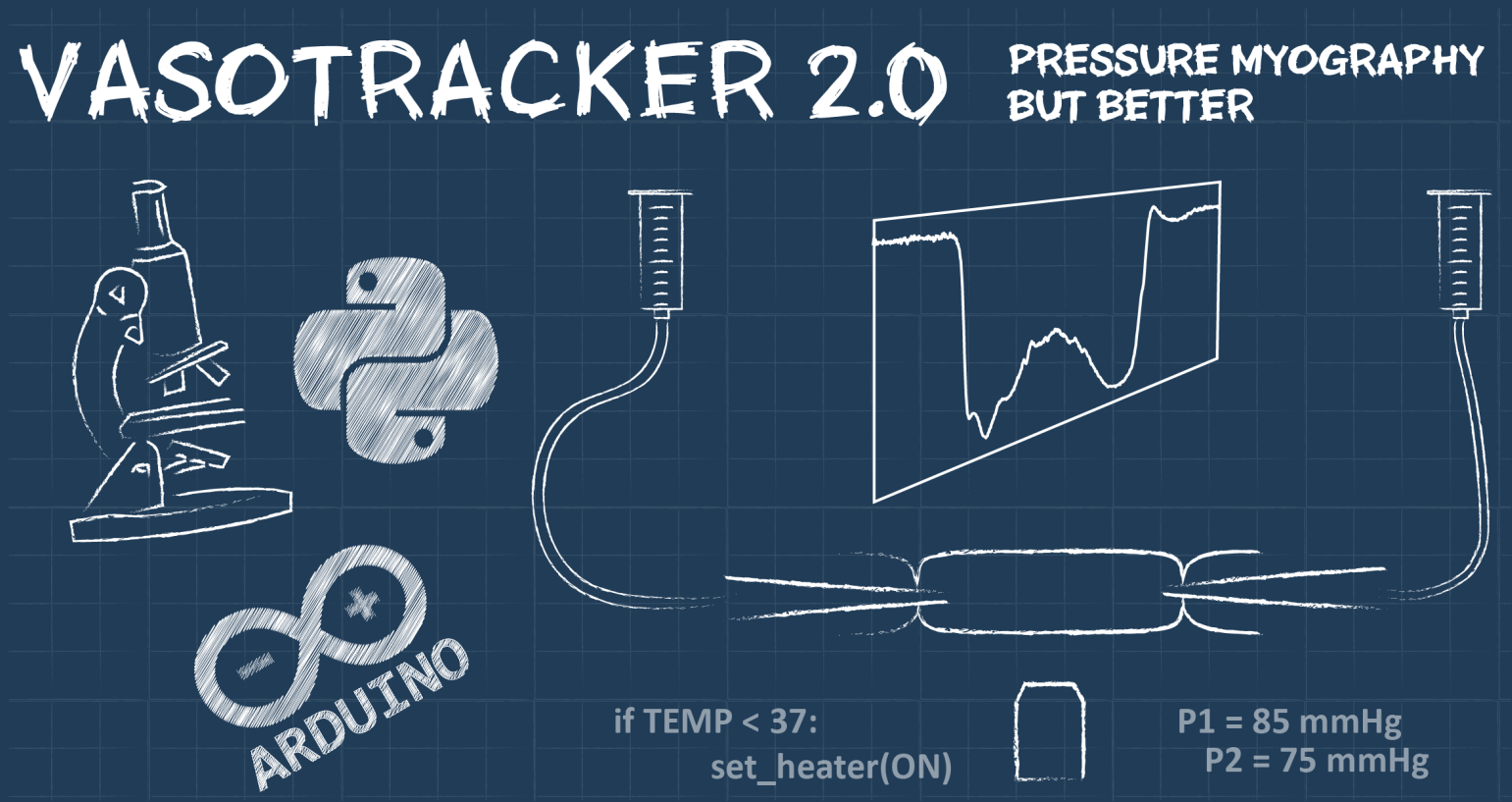
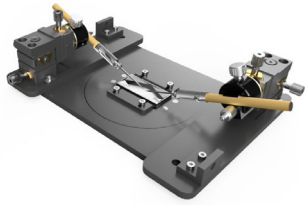


Bath Chamber User Manual

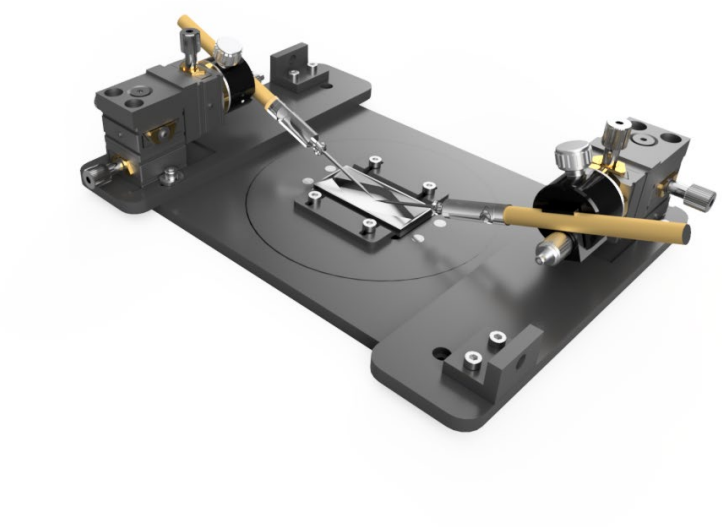
2024



Developed by Calum Wilson & Matthew Lee at the University of Strathclyde

Table of Contents

Overview



The VasoTracker 2.0 Pressure Myograph Bath Chamber

The VasoTracker 2.0 Pressure Myograph Bath Chamber is an open-source, low-cost imaging chamber for pressure myography experiments. The chamber consists of a durable base and a low-volume (~2 ml) vessel chamber insert, optimized for efficient reagent use and precise environmental control through superfusion.

Key Features

- **Low-Volume Chamber**: The vessel chamber insert is designed for efficient use of reagents and environmental control, with only ~2 ml of volume.
- **Precision Positioning**: Independent XYZ positioning of both cannulas is enabled by dual pipette holders mounted on miniaturized 3-axis translation stages, ensuring accurate and flexible manipulation of vessels.
- **Precise Vessel Positioning**: The angled cannula design allows for easy vessel positioning near the coverslip, supporting low-working distance objectives on inverted microscopes, as well as water-dipping objectives on upright systems.
- **Durable Materials**: The base can be machined from POM-C, and the chamber insert from acrylic, making it durable and adaptable to various experimental conditions.
- **Magnetic Attachments**: Superfusion plumbing is attached securely with flexible magnetic holders, making it easy to manage and adjust.

Citing VasoTracker

VasoTracker began life with the support of the Wellcome Trust and the British Heart Foundation to facilitate research on blood vessel function. Our ability to continue supporting, developing, and maintaining VasoTracker depends on further grant funding. If you use VasoTracker in any way, please cite VasoTracker in your scientific publications. For citation details, please visit: https://vasotracker.com/publications/

Parts List

VasoTracker Pressure Myograph Chamber Parts

CNC Machined Components	Supplier	Product #	Qty	Price	Total	Supplier Link
01_Nikon_Baseplate**	VasoTracker	-	1	£250.00	£250.00	VasoTracker GitHub
02_Chamber_Base**	VasoTracker	-	1	£130.00	£130.00	VasoTracker GitHub
03_Chamber_low_volume**	VasoTracker	-	1	£140.00	£140.00	VasoTracker GitHub
04_Clamp**	VasoTracker	-	2	£97.00	£194.00	VasoTracker GitHub
05_Tubing Connector**	VasoTracker	-	2	£99.00	£198.00	VasoTracker GitHub
Prices scale with quantity!						
Prices correct 10/2024		**Total**			**£912.00**	

3D Printed Components	Supplier	Product #	Qty	Price	Total	Supplier Link
Magnetic Plumbing Holder	VasoTracker	-	2	£1.00	£2.00	VasoTracker GitHub
Prices correct 10/2024		**Total**			**£2.00**	

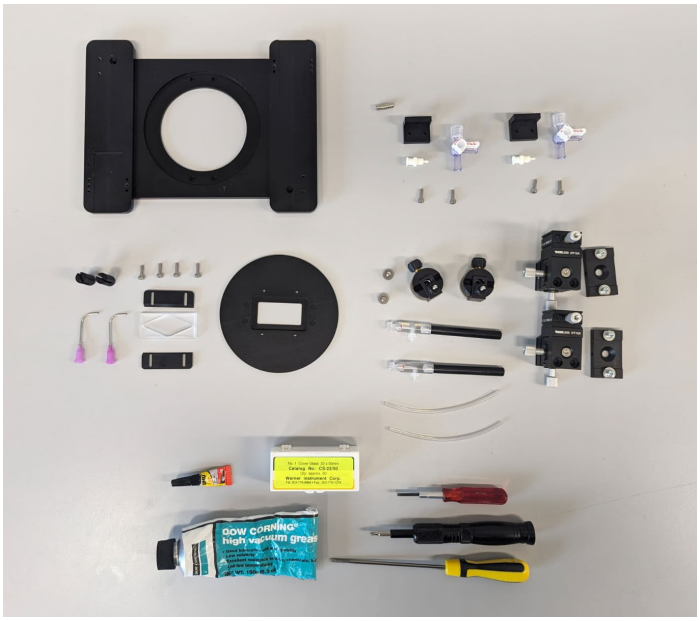
Commercial Components	Supplier	Product #	Qty	Price	Total	Supplier Link
Micromanipulators	Thorlabs	DT12XYZ/M	2	£288.00	£576.00	Thorlabs
Rotatable cannula clamps	Siskiyou	MXC	2	£105.35	£210.70	Siskiyou
1.5 mm glass cannula holders	WPI Inc	MPH3	2	£64.00	£128.00	WPI Inc
Handle for cannula holder	WPI Inc	2505	2	£31.00	£62.00	WPI Inc
Perfusion plumbing	Needlez	NB16G1.5B90	2	£0.68	£1.36	Needlez
Prices correct 10/2024		**Total**			**£978.06**	

Consumables & Miscellaneous Supplies	Supplier	Product #	Qty	Price	Total	Supplier Link
Coverglass (50 mm by 24 mm)	MarienFeld	MARI0102222	1	£107.00	£107.00	VWR
50x Magnets	Amazon	5 mm ⌀ x 1 mm	1	£5.59	£5.59	Amazon
50x Magnets	Amazon	5 mm ⌀ x 2 mm	1	£3.99	£3.99	Amazon
Superglue	Amazon	Gorilla Glue	1	£3.37	£3.37	Amazon
M3 Socket Screws	Amazon	NB16G1.5B90	1	£10.99	£10.99	Amazon
M4 Socket Screws	Amazon	MARI0102222	1	£10.99	£10.99	Amazon
Prices correct 10/2024		**Total**			**£141.93**	

Tubing & Connectors	Supplier	Product #	Qty	Price	Total	Supplier Link
Masterflex 1.22 ID tubing (100 ft)	VWR	MFLX06449-31	1	£201.00	£201.00	VWR
Masterflex 1.52 ID tubing (100 ft)	VWR	MFLX06460-36	1	£157.00	£157.00	VWR
Female luer (1/16" barb; pack of 25)	VWR	GY-45502-00	1	£28.90	£28.90	VWR
Male luer (1/16" barb; pack of 25)	VWR	MFLX45504-00	1	£20.40	£20.40	VWR
Luer stopcock (pack of 10)	VWR	MFLX30600-02	1	£34.60	£34.60	VWR
Threaded Luer (pack of 25)	VWR	MFLX45508-30	1	£28.90	£28.90	VWR
Prices correct 10/2024		**Total**			**£470.80**	

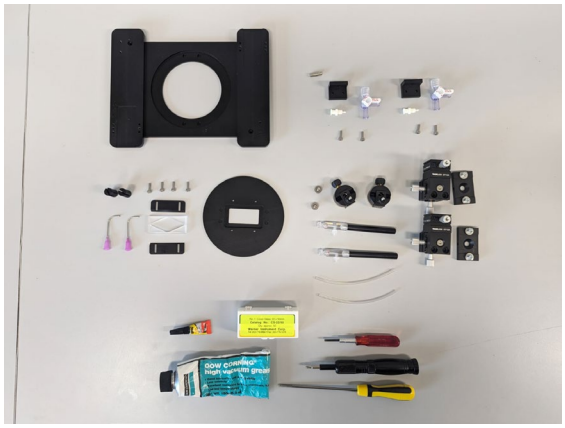
Total Price					**£2,504.79**	

Building the VasoTracker Bath

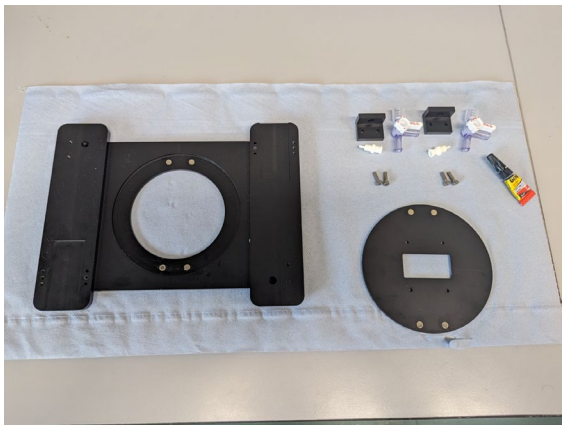


Step by Step Guide

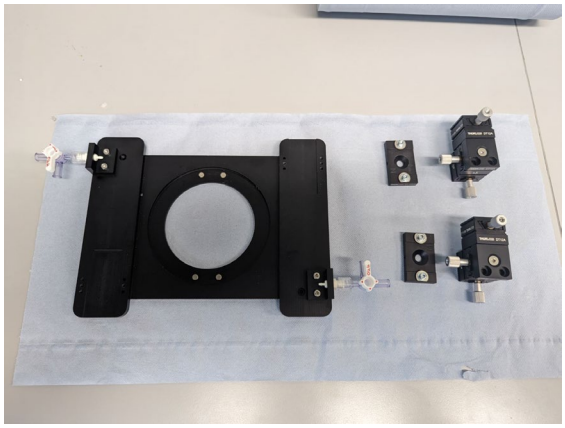
1. **Gather your supplies**



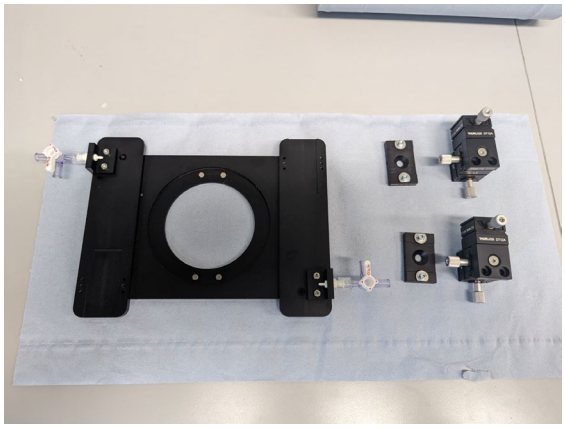
2. **Attach magnets:** Secure magnets into the baseplate and chamber base using superglue (8 magnets in total,4 in baseplate, and 4 in chamber base). Ensure correct orientation of the magnets!



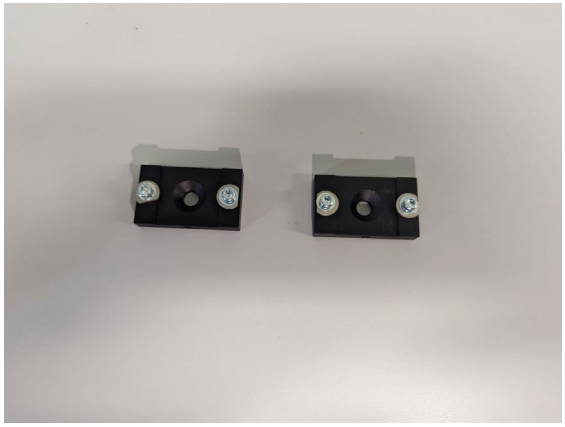
3. **Mount the tubing connectors:** attach one on each side of the bath using M3 screws. Connect a threaded leur and stopcock to each.



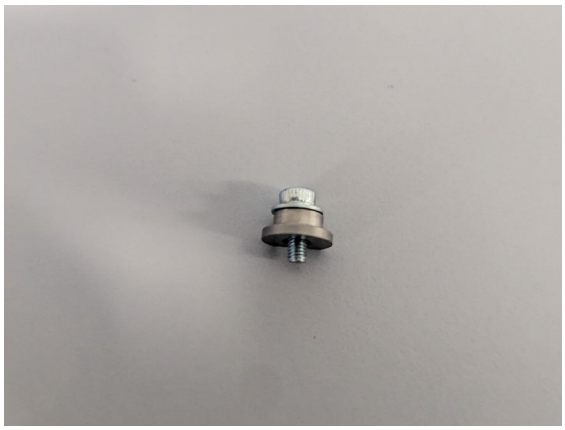
4. **Attached the 3-axis positioners onto the baseplate.**



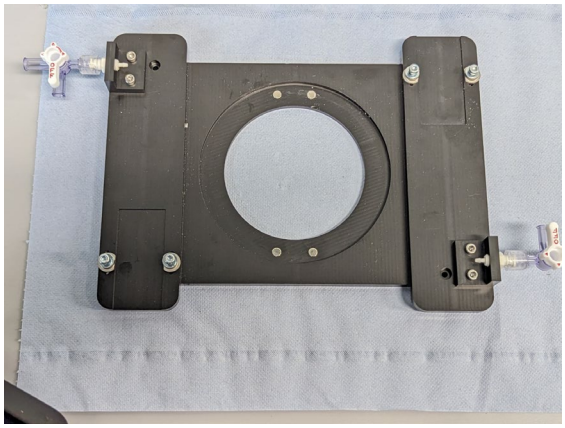
- **4A. Remove the base from each positioner.** Keep the screw assemblies!



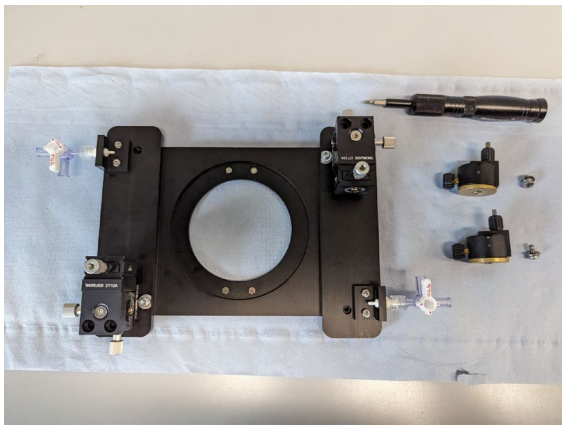
- **4B. Rotate the nuts on the screws so that the narrow end is facing the screw cap.**



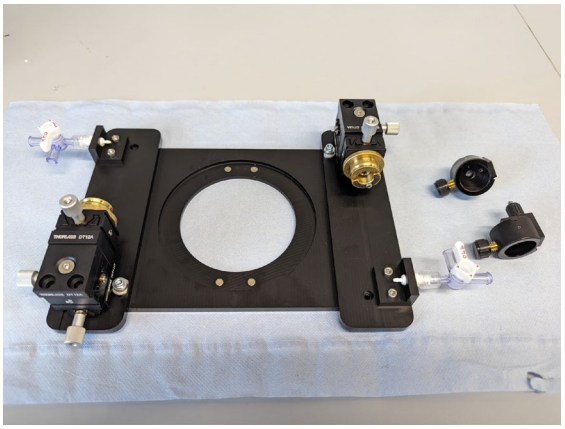
- **4C. Mount the four screw assemblies onto the baseplate.** Do not tighten fully.

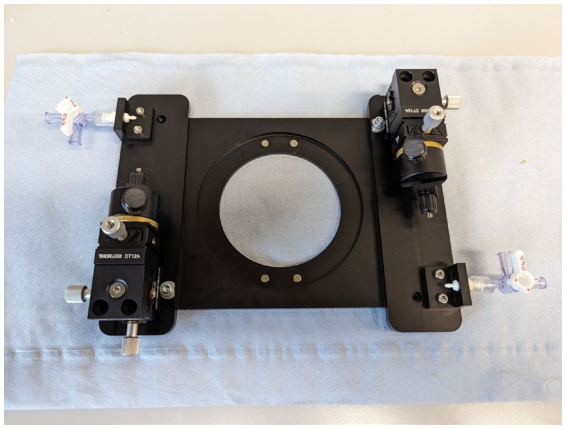


- **4D. Slide each of the positioners into position.** Secure each using the four screws.

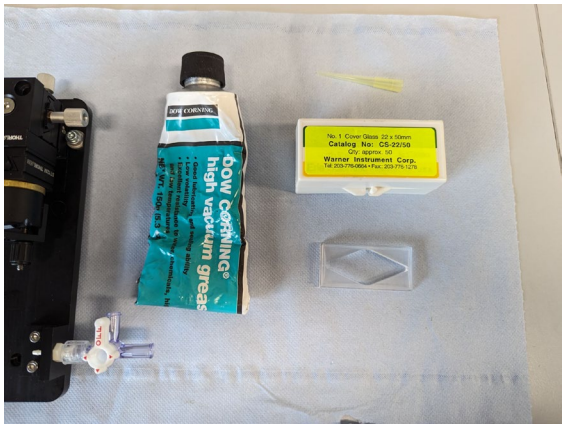


- **4E. Disassemble the rotatable probe clamps then mount one to each of the positioners:**

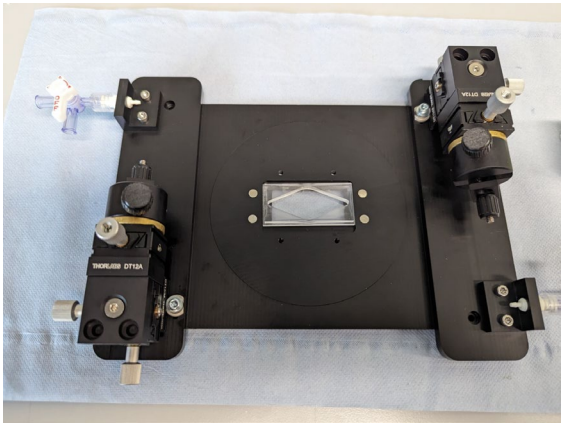


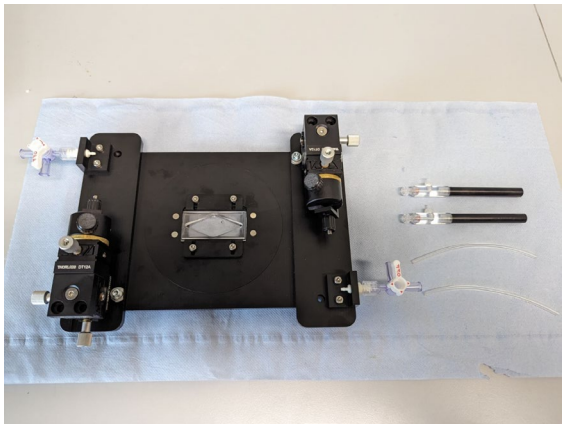


5. **Prepare the bath chamber:** mount a coverslip to the bath chamber using vacuum grease.

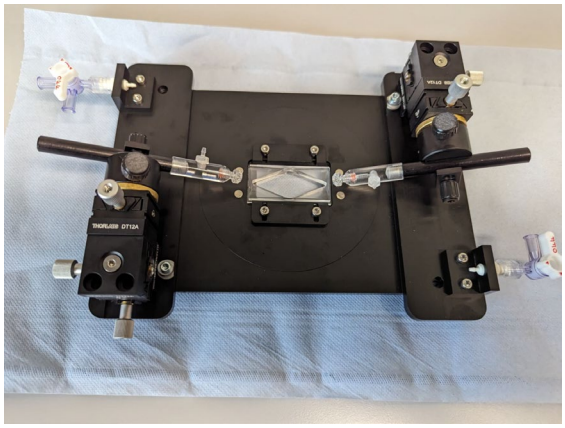


6. **Place the chamber base into the baseplate (the magnets will snap it into place). Then mount the chamber in the center and secure with the screw clamps.**

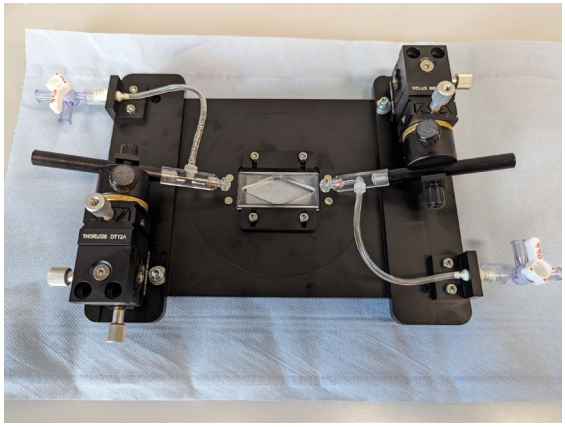




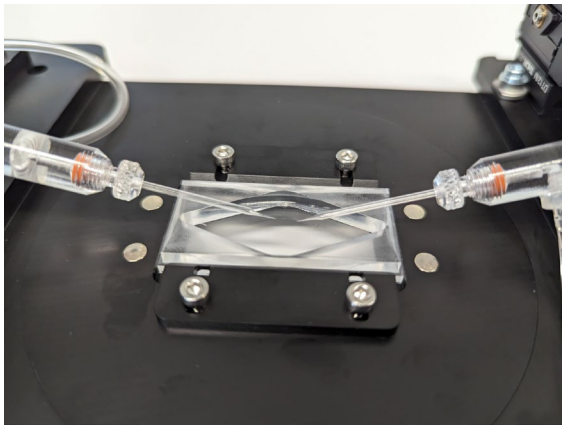
7. **Attach the cannula holders to the rotatable clamps.**



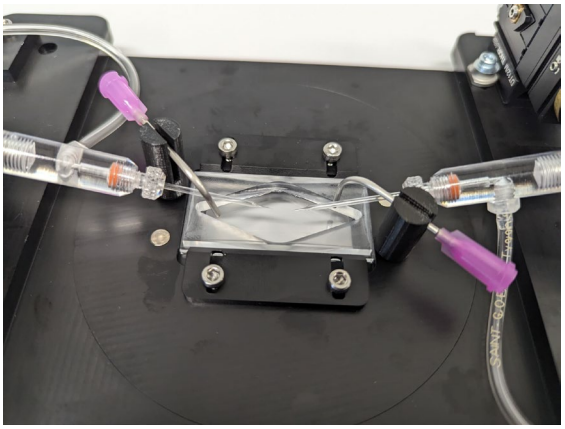
8. **Connect the cannula holders to the luer tubing connectors.** Use either 1.22 mm for a lower dead volume, but 1.52 mm inner diameter tubing also works.

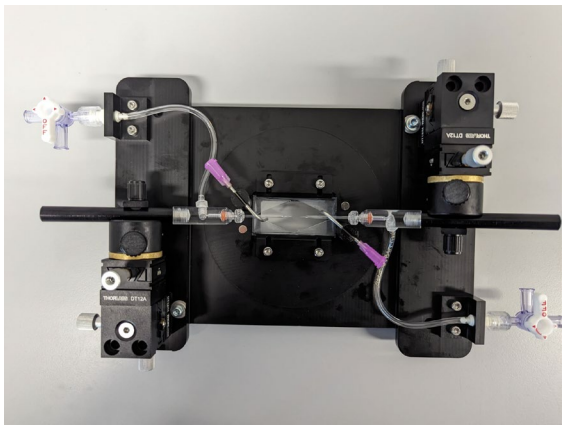


9. **Insert pulled glass cannula (1.5 mm OD; matched to specific cannula holders) and center in the bath.**



10. **Attach perfusion plumbing holder.** Glue magnets into the bottom of the 3D printed perfusion plumbing holders and mount onto the bath.





11. **Perform some experiments with your new VasoTracker bath!**

Replacing the Glass Cannula

12. **Remove the Cannula Holder from the Chamber:**

- Loosen the rotatable clamp securing the cannula holder and gently rotate it out of the bath chamber.

Tip: Detaching the tubing connected to the cannula holder may facilitate easier removal.

13. **Stabilize the Cannula Holder:**

- Once the cannula holder is removed from the bath, tighten the rotatable clamp to hold it securely in the horizontal position.

14. **Remove the Old Glass Cannula:**

- Loosen the thumb screw on the cannula holder and carefully remove the old cannula.

15. **Prepare a New Glass Cannula:**

- Use a pipette puller (e.g., Sutter P-97) to pull a new glass cannula to the appropriate dimensions.
- **Adjust the Length:** Trim the new cannula to the appropriate length.

Tip: Score the cannula with a diamond-tipped scribe before snapping to achieve a clean end. Fire polish the end with a flame for smoothness. Cutting the new cannula to a length similar to the old one simplifies realignment in the chamber.

16. **Insert the New Cannula:**

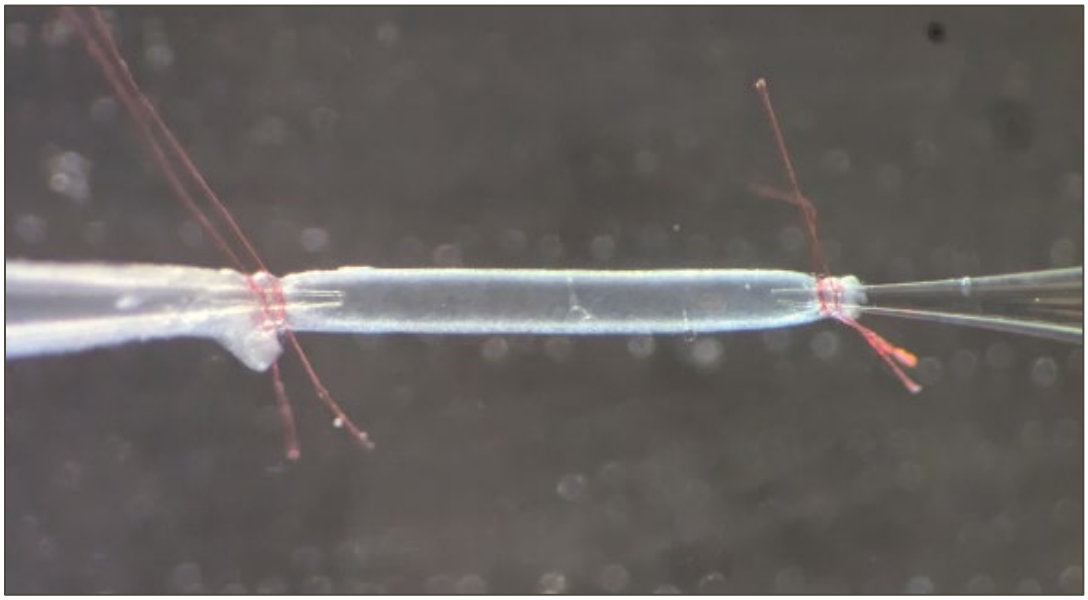
- Gently insert the new cannula into the holder and secure it by tightening the thumb screw.

17. **Reinsert the Cannula into the Bath Chamber:**

- Loosen the rotatable clamp, carefully rotate the cannula holder back into the bath chamber, and ensure the new cannula does not contact the bottom of the chamber.

Tip: Under a dissection microscope, use the VasoTracker 3-axis positioners to precisely realign the cannula in the center of the bath.

Preparation of Isolated Blood Vessels



Mounting the Vessel onto the Cannula

1. **Prepare the bath chamber:**
    - Fill the bath chamber with your physiological saline of choice.
    - Flush the tubing/cannula with physiological saline using a 5ml syringe attached to the leur stopcocks. Leave the syringe attached to one of the cannula (you will mount the first end of your vessel on this cannula).
2. **Prepare Ties:**
    - Cut enough threads or fine suture material for both ends of the vessel. Loop one or two suture threads over the first cannula in advance to simplify attachment.

Tip: A double overhand knot provides more stability than a single knot. Using two threads on each end can further enhance stability. Some people use suture threads, but we like to pull apart cotton thread and use individual strands for our ties.

3. **Tie vessel onto first cannula:**
    - Gently cannulate one end of the vessel, securing it to the cannula with your ties.

Tip: If possible, leave a small piece of fat or branch attached to the vessel. This allows you to maneuver the vessel by gripping the fat/branch with forceps, helping to avoid potential damage to the vessel itself.

4. **Flush blood from the vessel lumen:**
    - Use the attached syringe, very gently flush the lumen of the vessel to remove any blood.
5. **Prepare the second cannula:**
    - Align the second cannula with the other end of the blood vessel and loop one or two ties over it.
    - Ensure the luer stopcock is in the closes position.
6. **Attach the vessel to the second cannula:**
    - Mount the vessel onto the second cannula, position the suture threads over the vessel and cannula, and secure by tightening.
7. **Straighten the Vessel:**
    - Gently pressurize the blood vessel and use the 3-axis positioners to straighten the vessel without stretching it.

Tip: With a bit of practice, you can perform this step with a syringe. But inexperienced users should pressurize the vessel using a pressure servo system, or gravity-driven pressure control. See next step.

Pressurizing the Vessel

1. **Place the myograph chamber on your microscope stage:**
    - It may be useful to visualize the vessel using the VasoTracker software.
2. **Connect the Pressure Control:**
    - Connect your pressure control system (servo or gravity-driven) to the 3-way valves. Keep the valves (on the chamber) closed initially, allowing physiological saline to flow through the tubing, and the open end of the valve not heading to the vessel, into a waste receptacle

> **Tip:** Flowing saline through the pressure system ensures that air bubbles are removed from the system. If any get stuck in the tubing, give it a tap to dislodge and allow enough saline to flow through to remove any air bubbles.

3. **Start Perfusion and Temperature Control:**
    - Connect the inflow and outflow tubing for perfusate circulation and temperature monitoring. Start the perfusate system and maintain the temperature using your chosen control method.
4. **Pressure Adjustment:**
    - Using either manual or automated control (using VasoTrackers automated pressure control, see VasoTracker software), gradually increase the pressure.
    - Begin with low pressure (~20 mmHg) and increase to the desired experimental pressure, typically 60 - 120 mmHg, depending on the vessel type and study objective, at 10-20 mmHg increments, allowing a 5-minute equilibration between each increment.
5. **Monitor Vessel Diameter:**
    - Use the VasoTracker software to track the vessel's diameter throughout the pressurization process.
    - The vessel may need to be stretched (using the 3-axis positioner) to ensure it is not kinked or curved.
6. **Check for Leaks and Adjustments:**
    - Once at the desired pressure, close of the vessel by closing both 3-way valves. The diameter should remain stable. A decrease in diameter indicates a leak in the system. If there is a leak:
        - Ensure all tubing connections are tight and there are no visible leaks.
        - Ensure the ties attaching the vessel to the cannula are tight and with no leaks.
        - Visibly inspect the vessels for any leaks, this may indicate damage to the vessel or small branches.

Checking the viability of the blood vessel

Once the vessel reaches the target pressure and diameter, leave to equilibrate for 30 – 60 minutes and then perform a viability check before continuing with any experiment. The steps below are one example viability protocol, but there are many other suitable procedures (e.g. using a depolarizing saline containing a high concentration of K+)

1. **Induce Contraction with Phenylephrine (PE):**
    - Add enough phenylephrine to the perfusate bath to reach a final circulating concentration of 1 µM, and constrict the blood vessel.
    - Allow the vessel to contract fully until a stable plateau is reached.
2. **Wash with PSS:**
    - Wash the PE out using PSS buffer until the vessel returns to its baseline diameter.
    - Repeat the PE contraction to confirm reproducible contraction responses.
3. **Assess Endothelial Function with Acetylcholine (ACh):**
    - Once the vessel reaches the contracted state with PE, add acetylcholine (ACh) to reach a final circulating concentration of 1 µM.
    - Allow the vessel to dilate fully until a stable plateau is reached. Measure the level of vasodilation.
4. **Wash with PSS:**
    - Wash the PE and ACh out using PSS buffer until the vessel returns to its baseline diameter.
5. **Ready for Experimental Use**:
    - Assess the level of vasoconstriction/vasodilation achieved, and if acceptable then continue with your experiments. If the responses are not acceptable, it is time to get a new vessel mounted.

Tip: VasoTracker software makes it easy to assess percentage vasoconstriction and percentage vasodilation. Simply set the “reference diameter” at the beginning of your viability test and VasoTracker software will display the current diameter as a percentage of this reference value.

www.vasotracker.com