BIOLOGICAL IMAGING DEVELOPMENT COLAB

UCSF University of California San Francisco

Perfusion Chamber Setup

Explant and Live-Slice Imaging

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1 Introduction

Setting up a proper perfusion system is vital for a successful explant or live-slice imaging experiment. Please see 'https://bidc.ucsf.edu/intravital-and-explant-imaging' for more information on the available systems at the BIDC.

There are lots of cables, tubes, and hoses needing to be connected, and setting the up the system can seem daunting for new users. Breaking the components into their major sections - **Temperature**, **Solution**, and **Vacuum** - can help streamline the logic of the system and simplify the setup

New users should always train with BIDC personnel to ensure safe operation of the perfusion components.

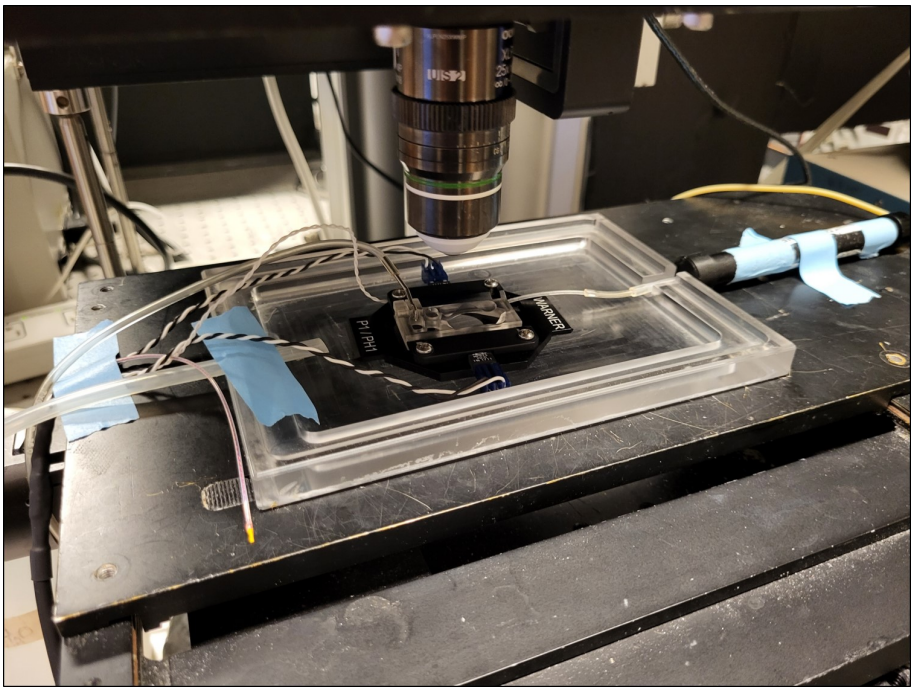

Figure 1: This is what the final setup should look like.

2 Temperature

The Temperature portion of the system consists of two major components - the **sample heater** and the **inline heater**.

The **sample heater** is responsible for keeping the specimen at the appropriate temperature throughout the experiment. This is typically 37° C, but can vary depending on the experiment.

The purpose of the **inline heater** is to ensure the solution is at temperature before it reaches the sample.

Setup:

1. Install the drip tray on the stage.
2. Remove the perfusion chamber from the storage box and inspect for damage.
   Note: The most common issues are corrosion on the leads (wipe off with paper towel or sandpaper if needed) or a cracked coverslip bottom (easily fixed - have BIDC personnel show you how).
3. Connect the sample heater cable to the control box and unravel the cable so it has no kinks or spring tension in it.

4. Connect the electrical leads of the sample heater cable to the chamber ensuring the small cables are 'on top' or away from the drip tray. See Figure: 2.

5. Insert the white temperature probe into the small hole on the side of the chamber. See Figure: 2.

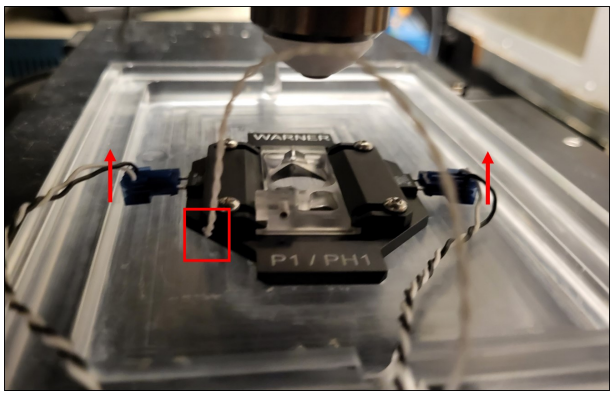

Figure 2: Wires away from the drip tray. White temperature probe inserted.

6. Tape the cable to the table top to reduce the risk of snagging the cable and pulling everything off the stage.

7. Tape the cable and chamber in a way that ensure the system will not move when the stage is moved.

8. Unravel the inline heater component and connect the power cable to the control box.

9. Tape the power cable to the table top to reduce the risk of snagging the cable and pulling everything off the stage.

10. Snake the perfusion hose behind the stage towards the perfusion pump.

11. Turn on the control box and watch that the temperature goes to 37° C. It will likely go above temperature before coming back down. Watch that the sample heater does not go above 45° C. If it does, shut off the controller box, let the heater cool, and retry the connections.

3 Solution

The **Solution** keeps the tissue within a healthy environment including elements such as oxygen content, pH, and salt concentration. It should be perfused across the tissue so that solution evaporation does not imbalance the solution over time.

1. Turn on the water bath and set the temperature to 37° C.

2. Place your solution container in the water bath to raise it to temperature.

   If your solution needs carbogen:

   (a) Spray the carbogen line with 70% ethanol and dry.

   (b) Put the carbogen line into your solution.

   (c) Turn on the carbogen by opening the tank. You should see fine bubbles filling your solution. You **do not** need a raging current.

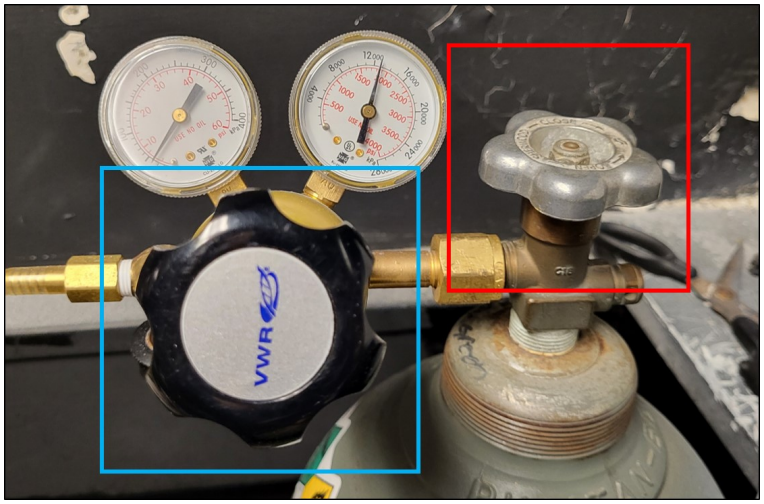

Figure 3: **Red**: Open the tank. **Blue**: Control the flow of the gas.

3. Put the pump tubing section into the peristaltic pump and secure it. See Figure: 4.

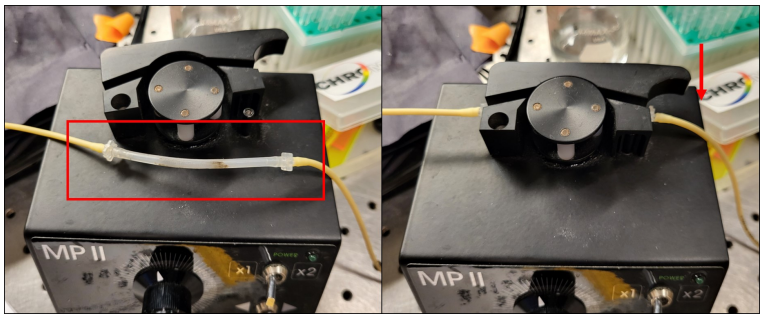

Figure 4: Proper installation of the perfusion line.

4. Put the tubing into 70% ethanol.
5. Put the outlet of the inline heater into/over a waste container.
6. Run the peristaltic pump (fast) and flow the 70% ethanol through the tubing to clean it.
7. Remove the tubing from the 70% ethanol while the pump is still running to empty the tubing.
8. Place the tubing into your solution to perfuse and run it until it comes out of the outlet. This will condition the tubing.
9. Find the speed you would like to perfuse your tissue with and stop the pump.
10. Place the heating unit on the stage with the outlet in the drip tray slot. See Figure: 5.

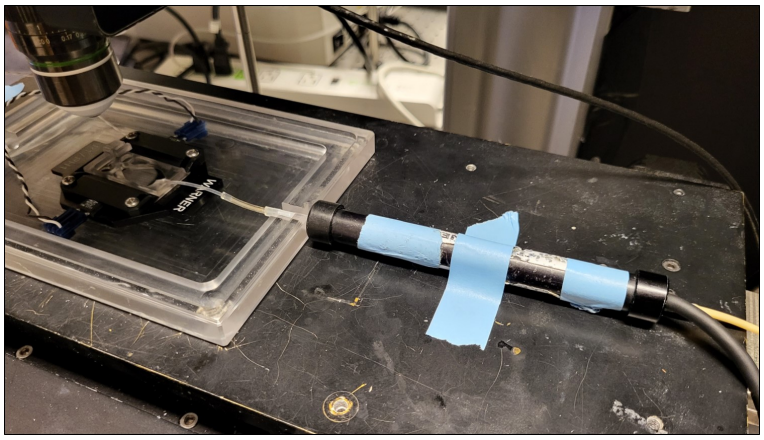

Figure 5: Proper installation of the inline heater.

11. Insert the outlet tube into the side of the perfusion chamber. See Figure: 5.
12. Tape the heating unit onto the stage to secure it. See Figure: 5.

4 Vacuum

The **Vacuum** portion is used to remove the waste solution from the perfusion chamber. It is also connected a second time to remove any overflow so that it does not leak onto the stage.

1. Find the vacuum tubing 'Y' that is hung on pegs over the sink. See Figure: 6.

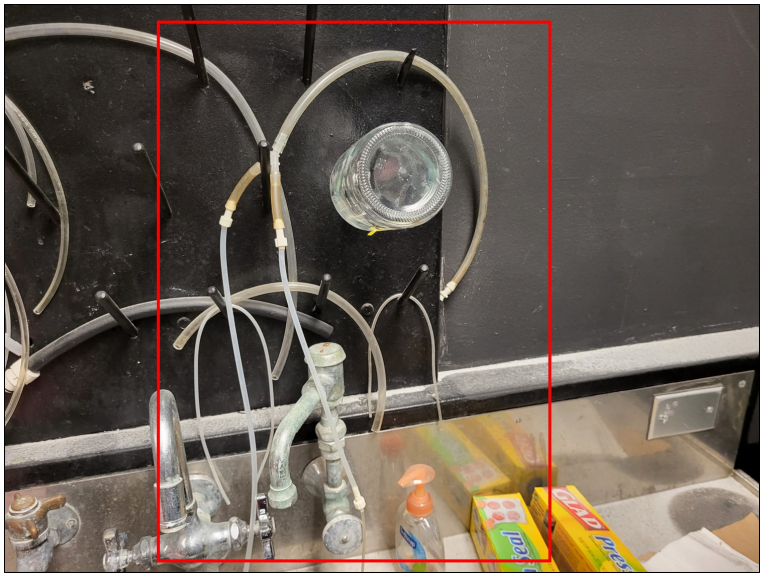

Figure 6: The tubing is hung up by the sink to drain and dry.

2. Use the connector to attached it to the host on the vaccum gauge.
3. Try to remove any kinks in the line and tape it to the table.
4. The larger hose goes into the slot opposite the inline heater. See Figure: 7 **red square**.
5. Tape the large hose directly to the drip tray.

6. Insert the needle into the slot on the perfusion chamber. See Figure: 6 **red line**.

7. The angle of the needle determines the level of the solution in the chamber. You will likely need to adjust this.

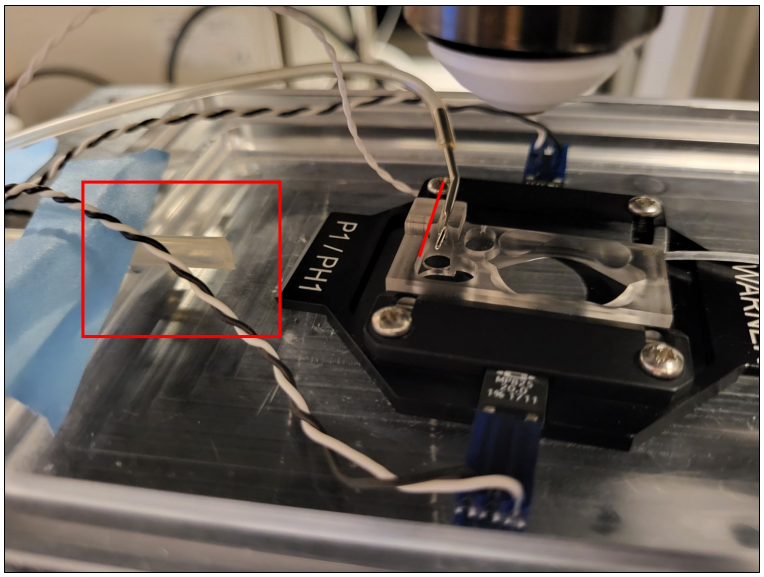

Figure 7: Vacuum tubing at the perfusion chamber.

8. Tape the needle hose to the stage so that it moves as the stage moves.

9. Turn on the vacuum. See Figure: 8.

Figure 8: The regulator should register a pressure when turned on.

Contact the BIDC

The BIDC office is located in Medical Sciences Building Room S1109.
The BIDC office phone number is 415-476-4550.
If you need immediate assistance and no one is available in the office, or it is after business hours, please call the **BIDC Hotline** at 415-745-2432.