Additional information:

Since the chamber operates with very small fluid volumes, fluid evaporation will be much more noticeable than with perfusion systems that use higher fluid volumes. We recommend reducing fluid evaporation with the following two methods: 1) Re-humidify the oxygen or carbogen from the tank and 2) direct an airflow of moist air from a humidifier at the chamber.

Oxygen or carbogen are dehumidified when the manufacturer fills the gas tanks to reduce internal corrosion of the tank, making these gases very dry and effective at evaporating solutions. We strongly recommend re-humidifying the gas before using it to bubble the ACSF in the recording chamber. This can easiest be done by dispersing the gas through a bubbling stone into a container with distilled water, and then connecting the (gas) outflow of that container to the recording chamber. Be careful when designing such a system and keep in mind that it may explode due to the gas pressure if not designed or operated properly.

To further reduce evaporation, a typical household indoor humidifier can be placed near the recording chamber, and the air/mist stream directed at the recording chamber, thereby "bathing" the chamber in a steam of moist air.

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User Manual

Recording chamber for small fluid volumes

Electrophysiological recordings from brain slices are typically performed in small recording chambers that allow for the superfusion of the tissue with artificial extracellular solution (ECS), while the chamber holding the tissue is mounted in the optical path of a microscope to image neurons in the tissue. Most existing recording chambers continuously add fresh or reconditioned ACSF through one port of the chamber, while removing an equivalent amount of spent ACSF through a second port.

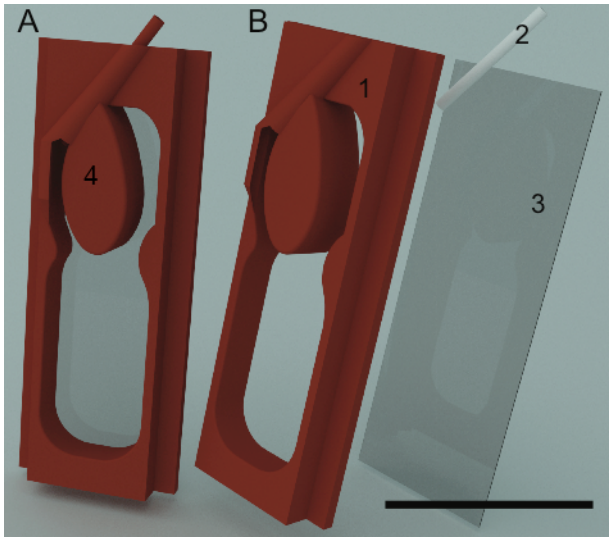

Figure 1

The key difference of this chamber is that it eliminates all inflow or outflow ports, associated tubing and external holding containers, thereby reducing the total amount of ACSF required for proper fluid circulation and reconditioning. The total amount of ACSF required for proper function of this chamber is between 1.5 to 2.5 mL total.

The re-oxygenation / re-carbogenation of the ACSF is performed

directly in the recording chamber. Besides oxygenation/carbogenation, pressurized gas also circulates ACSF in the chamber in a signature figure 8 pattern that supports higher flow speeds around the edges of the chamber while supporting much lower flow speeds near the center where the tissue section is located. To properly function, the fluid volumes, gas pressure, and location of the gas inflow tubes must be correctly adjusted.

Assembly of the chamber:

Figure 3

1) Mount cover glass on bottom of chamber. Use one of the included cover glasses (object 3 in image 1) and mount onto the bottom of the chamber using the included silicone sealant. Make sure a thin (!) layer of sealant is present around all four sides of the chamber and bottom on the "peninsula (object 4 in image 1)" for solid attachment and to avoid leakage of ACSF out of the chamber.

2) Mount gas inflow tube. Use the included piece of brown tubing (object 2 in image 1) and insert into the hole in one of the shorter sidewalls of the chamber. When doing this, flip the chamber over to observe the length of insertion of the tube through the bottom glass. Insert the tube all the way into the round halfpipe (typically the roof of the chamber) until it protrudes through the halfpipe by about 1 millimeter (images 2 and 3). Try to install the gas tube as close to the half pipe as possible (meaning pointing up as high as possible in the final orientation), such that the gas will exit the tube near the top meniscus of ACSF when in use. Seal the tube around the entry hole (on the outer side of the short wall) with silicone sealant, thereby arresting the gas pipe in its location as well as preventing leakage of ACSF out of the entry hole.

3) Let sealant dry overnight.

4) Insert one part of the included Luer-lock connectors into one end of the included larger diameter clear tube, and install the other end of the clear tube over the smaller gas inflow pipe that is now mounted to the recording chamber. Use the other Luer lock connector to connect the chamber to a gas tank via the second stage of a standard gas regulator.

Use of the chamber:

5) Install the chamber in the optical path of a microscope securely and in a flat (horizontal) position.

6) Make sure the gas is running through the aeration tube before adding ACSF (at 50 – 65 mBar per second stage of the gas tank regulator).

Figure 2

7) Next, slowly fill the chamber with ACSF making sure that the liquid surface does not get any higher than the outlet of the tube. Next, place a tissue section into the center and place a harp or other slice holder onto the section as needed.

8) The gas inflow will start to circulate the ACSF in the desired figure 8 pattern. Make minor adjustments to the gas pressure to account for different fluid volumes in the chamber; for higher volumes, use slightly higher gas pressures.

9) Work with the tissue section as you would in any other chamber.