

Protocol 1: Manufacturing Reusable Flow Chambers.

Glass slides for reusable flow chambers can be ordered from a local glass manufacturing facility. With ultrasonic milling modify regular microscope slides (75 mm x 25 mm, 1.0 mm thick) to make two grooves 15 ± 1 mm long, 1.0 ± 0.1 mm wide and 0.8 ± 0.05 mm deep. Distance between the closest ends should be 14 ± 1 mm; this distance is optimal for a chamber assembled with 22 x 22 mm coverslip.

1.1. Place a 100 mm long polyethylene tube (O.D. 0.61 mm) in each groove in the slide, leaving ~ 5 mm overhangs at the inner ends of the grooves. Fix the tubes inside the grooves with cyanoacrylate adhesive, embedding the tubes completely inside the grooves.

1.2. Fill the grooves with epoxy glue, while avoiding spilling the glue inside the tubes. Let the glue dry for ~ 1 day.

1.3. With a sharp razor blade cut the solidified glue mass 3-4 mm from the distal end of each attachment site, removing the parts proximal to the center of the slide. The tubes should remain inside their grooves. Removing the proximal parts will also cut and remove the inner overhangs, creating a flat surface with two tube openings.

1.4. Fill a syringe with water and test whether the tubes are working properly. If the liquid flows freely, put a drop of epoxy glue (~ 5 mm in diameter) at the outer ends of the grooves, dry for 1 day. This will make chambers more durable, so they can be used repeatedly for many months.

Note 1: To make a chamber for an inverted microscope, the slides should be modified additionally to make two small holes at the opposite ends of the grooves. Insert the tubes through the holes in the slide, bend the tubes and fit them tightly inside the grooves. Follow steps 1.2-1.4, but remove the epoxy glue from the entire surface, which will be used to make a flow chamber.

Note 2: To reduce a chamber volume, use milling to make two indentations 0.050 ± 0.005 mm deep, leaving the central part of the slide 5.0 ± 0.5 mm wide and slightly elevated. When the flow chamber is assembled (as described below), place the double-sided tape inside these indentations.

Note 3: To reuse these modified slides, after finishing the experiments remove the coverslip and double-sided tape using a razor blade. Remove the sealant by peeling it off and by wiping the slide with 70% ethanol. Place the slide in a container with 1-2% of a lab dishwashing detergent, attach tubing to a peristaltic pump and perfuse 50-70 ml, follow with equal volume of deionized water, dry and store in a dust-free compartment.