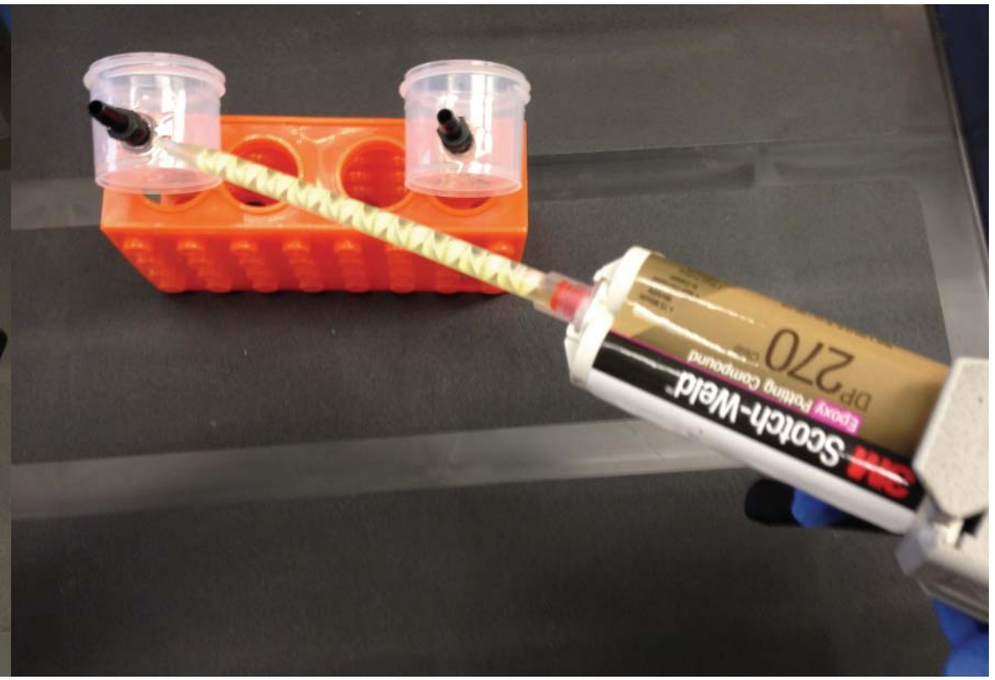
ETC
Chamber Fabrication

Deisseroth Lab 4.17.13
Based on Chung et al 2013
http://www.nature.com/nature/journal/vaop/ncurrent/full/nature12107.html
http://clarityresourcecenter.org/

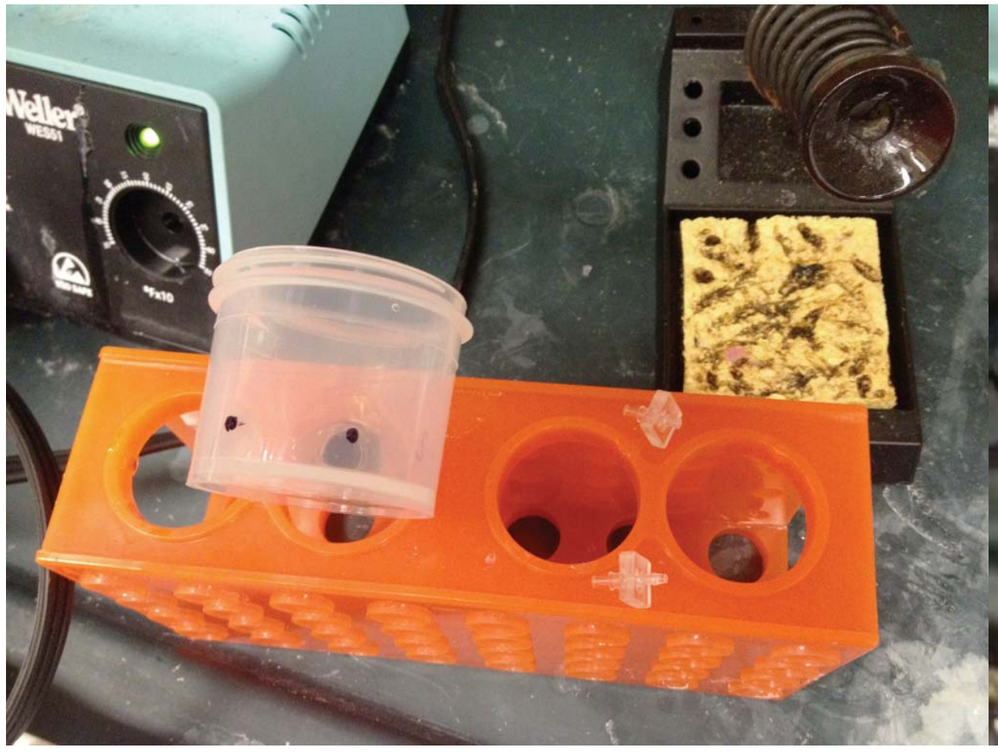
Start with a 60 mL nalgene bottle.
Mark areas to be soldered for inlet(bottom) and outlet(top). Make sure they are on the same plane and on opposite sides of the bottle when screwed tightly shut.

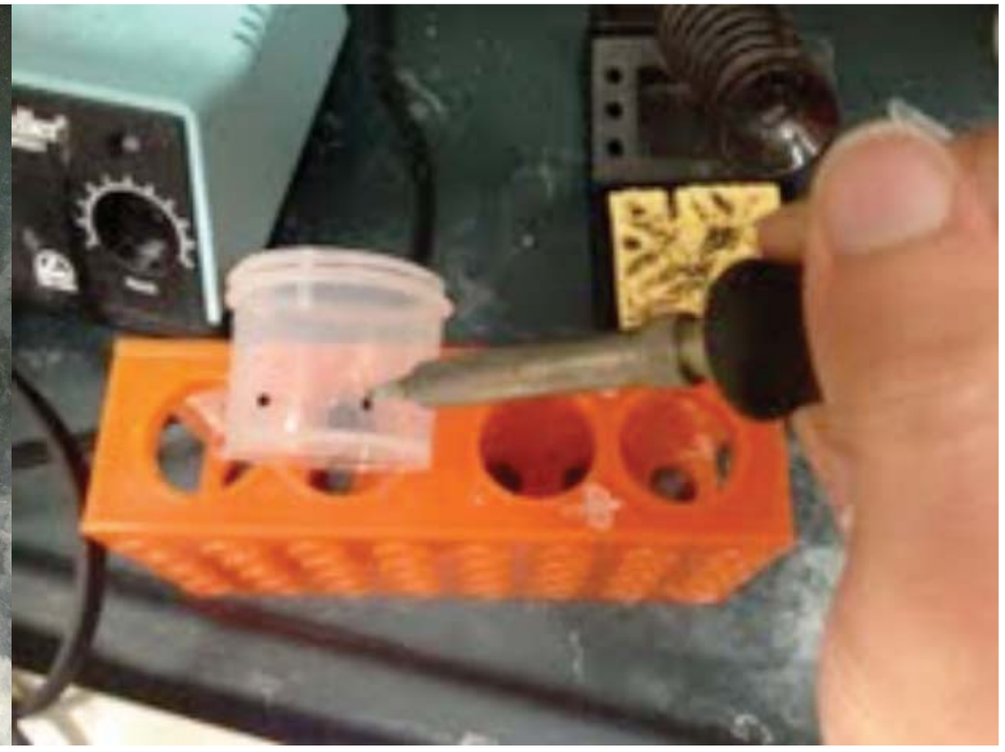
Solder holes into Nalgene bottle and screw in barbed fitting while plastic is melted.

Apply epoxy to the junctures(inside and outside bottle) of the barbed wire fittings. Avoid getting epoxy into the inlet and outlet. Let dry overnight with each epoxy application.

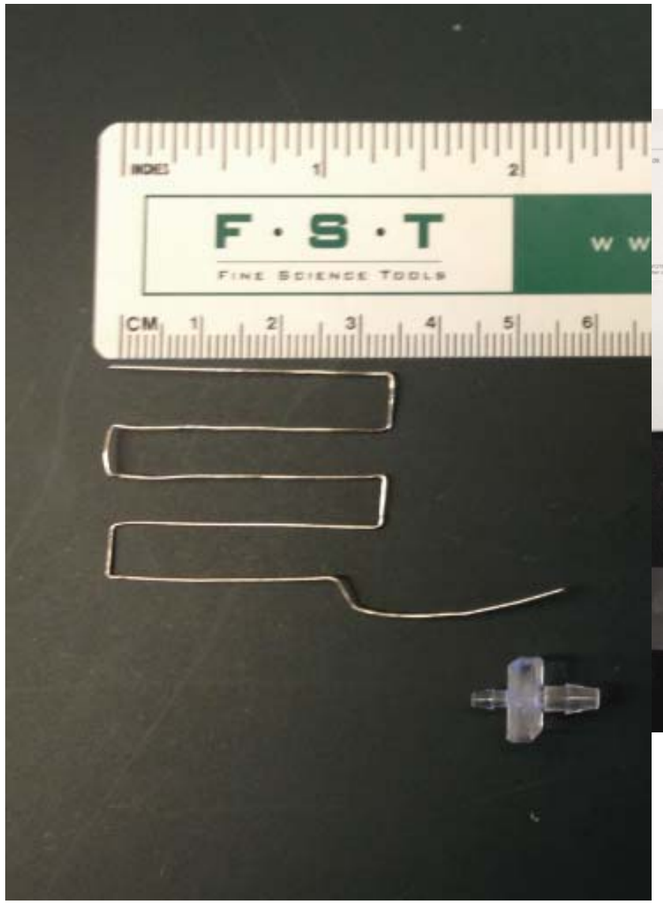


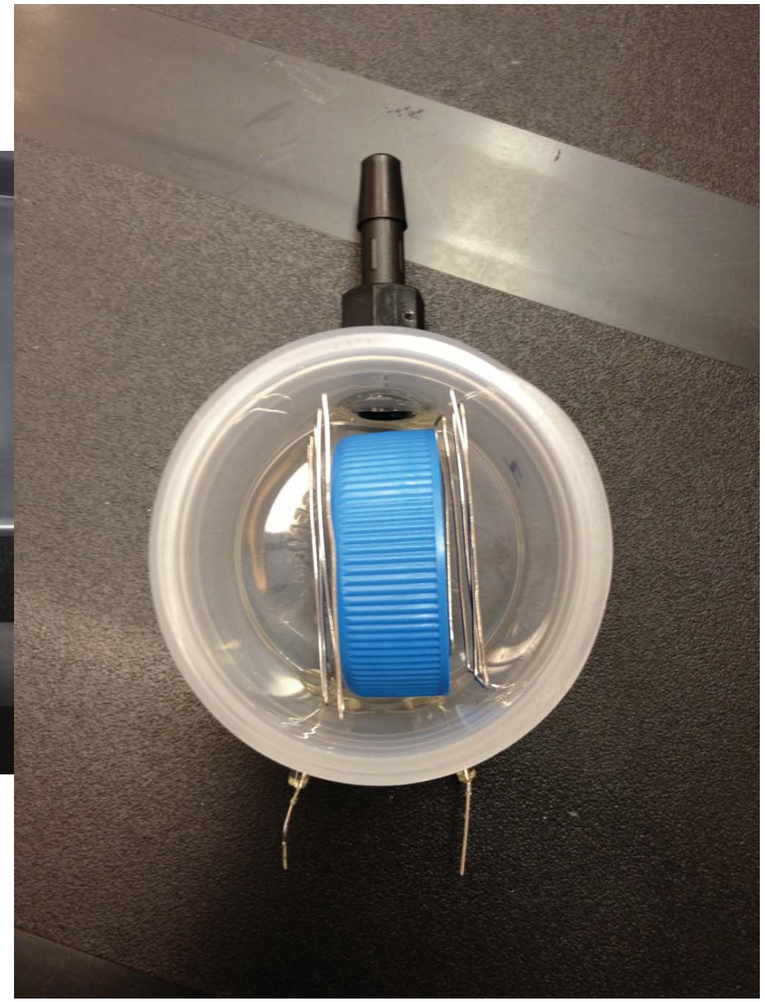
Solder holes for electrode inserts. Insert the smaller end into bottle while plastic is melted. Epoxy only the outside squares of fittings to bottle, making sure to leave the fitting interior clear for electrode insertion. Allow to dry overnight.





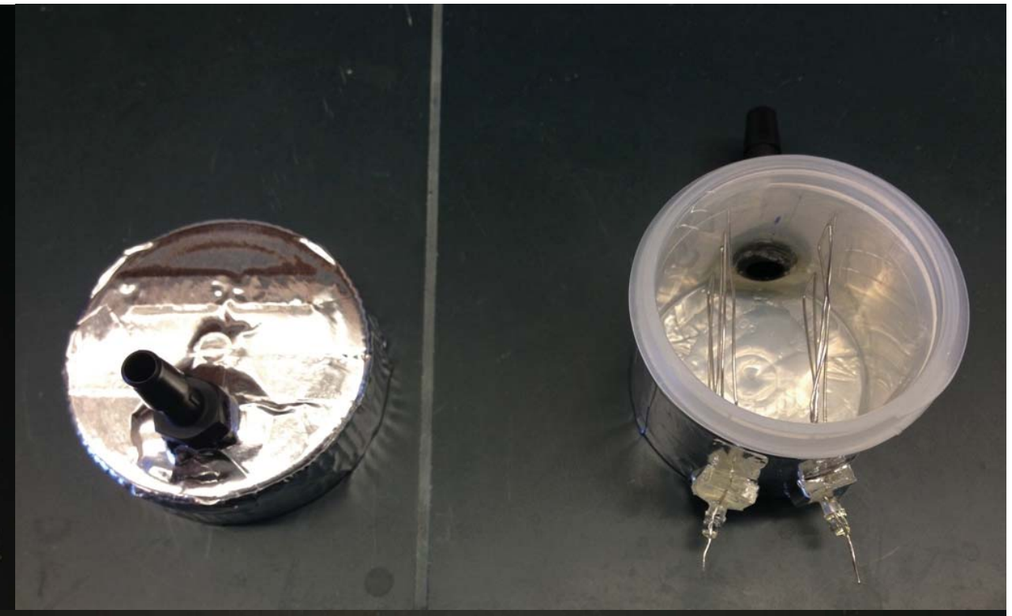
Fabricate electrodes out of .5mm Pt wire and insert into electrode fittings delicately. Ensure that the electrodes are spaced to fit the sample cup snuggly (the same size as a 50mL conical tube top). Fill dead space in electrode fittings with epoxy. Allow to dry overnight.

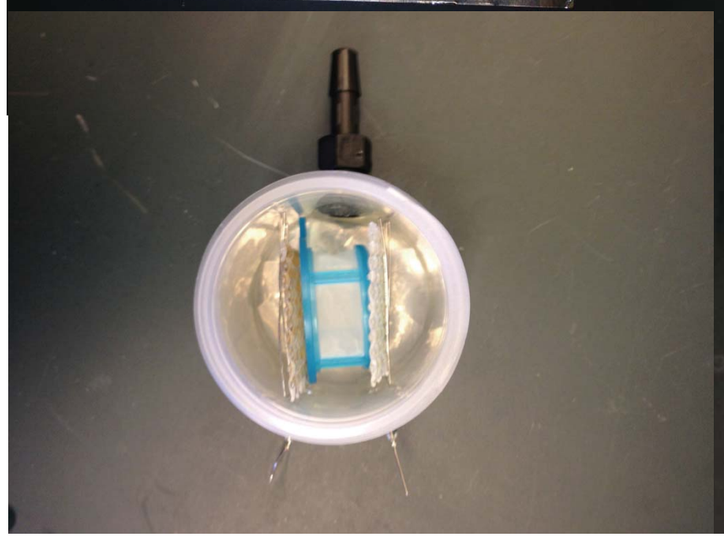


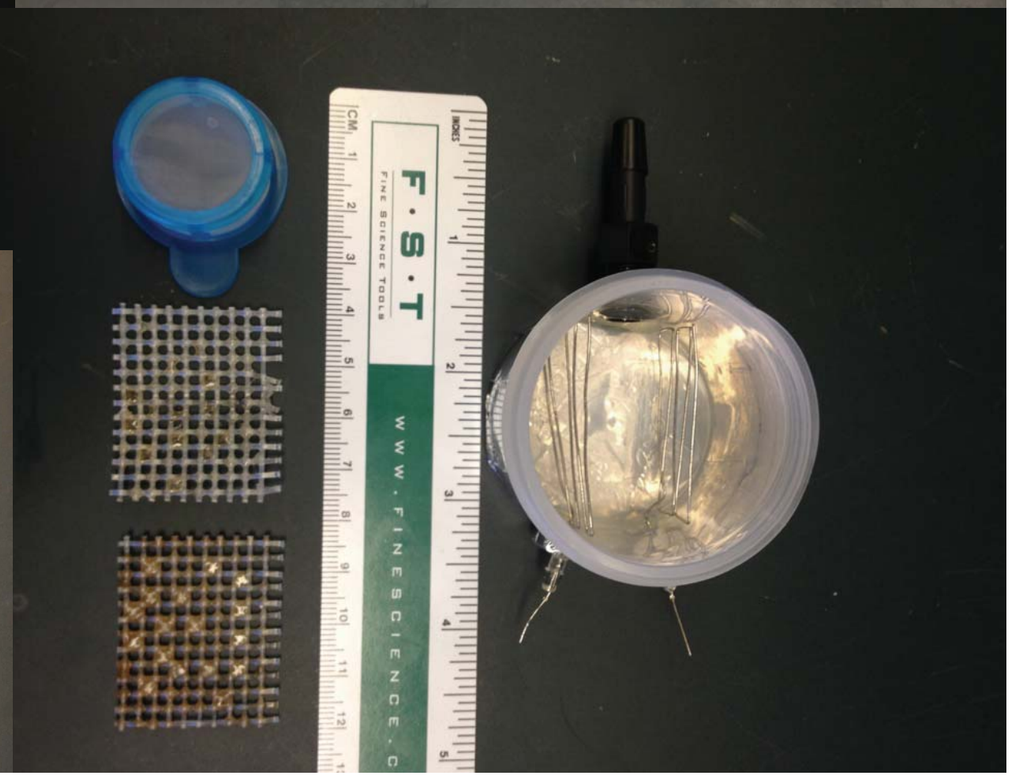


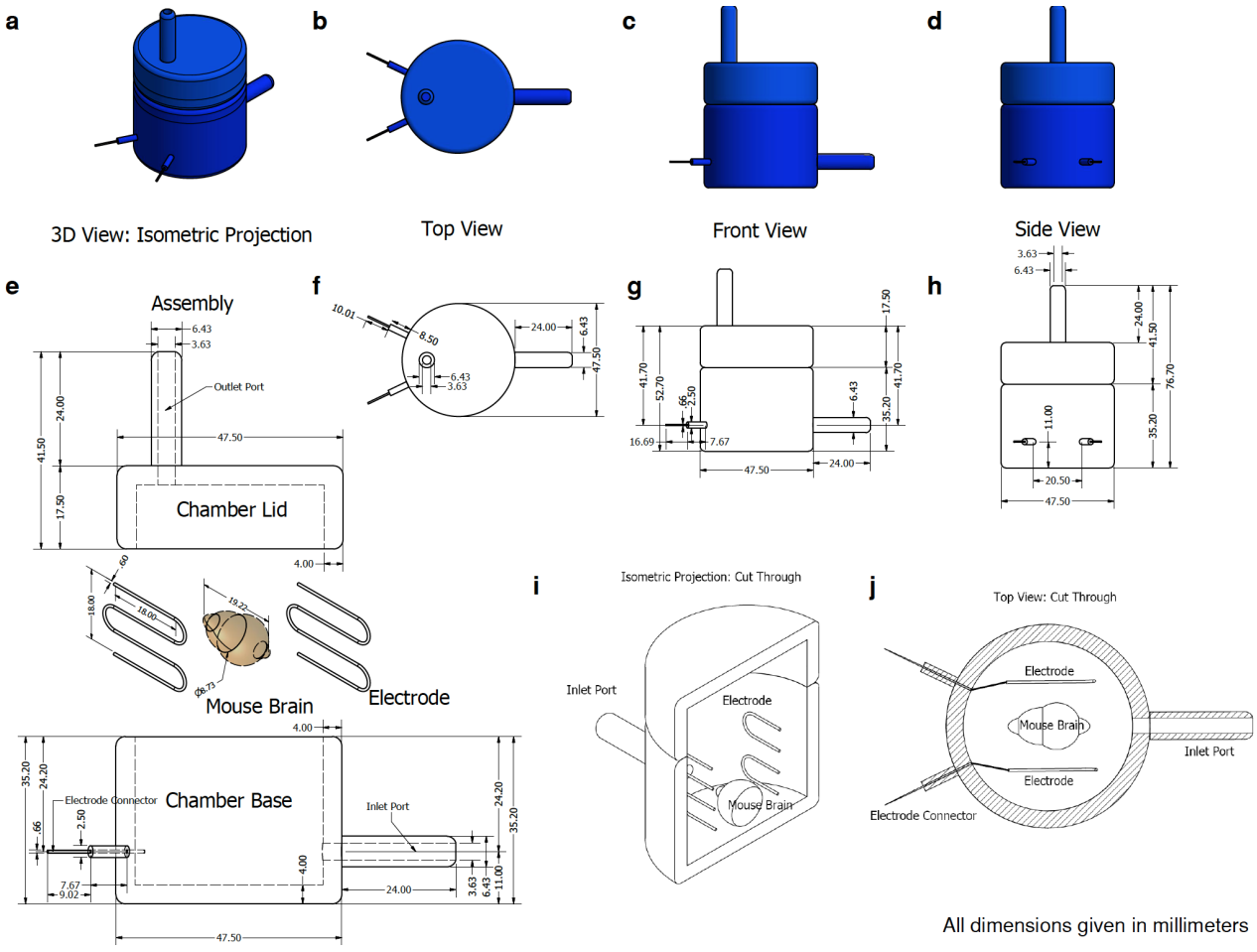
If you are not dealing with samples containing fluorophores, your chamber is ready for use as is... Place your sample into a BD falcon “cell strainer” flanked with mesh between electrodes prior to running in ETC setup

If you are clearing samples with fluorophores, lightproof your chamber prior to use with aluminum foil ‘tape’. Place sample in BD “cell strainer” between two mesh squares prior to running ETC setup









ETC chamber design. (a-d) Various views of the custom device showing the cylindrical plastic housing, the inlet / outlet ports (Cat. No. 5463K245, McMaster, Robbinsville, NJ) and the two platinum electrodes (Cat. No. 267201, Sigma, St. Louis, MO). The buffer inlet and outlet are located such that buffer flow through the chamber effectively removes air bubbles generated by electrolysis of the buffer solution. (e-h) Components of the assembly and dimensions of the device. All dimensions given in millimeters. (i-j) Sections through the device in (i) isotropic and (j) top views indicate component positions in assembled chamber. The hydrogel-embedded tissue is placed in the sample holder (Cell Strainer, BD Biosciences, Durham, NC) located in the middle of the chamber between the two electrodes. The single end of each electrode that is exposed outside the chamber is connected to a power supply.