Protocol for Electroformation of Giant Unilamellar Vesicles (GUVs)
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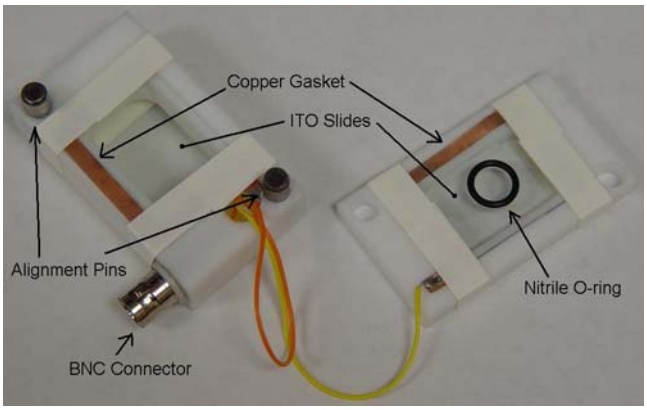

Materials:

- vacuum grease
- DI water
- Ethanol
- Kim Wipes & Que-tips
- 10 μl glass syringe
- 20-200 μl pipette (with wide mouth tips)
- one mini-centrifuge tube
- nitrile O-ring (9.25 mm ID x 1.78 mm radius)
- vacuum desiccator
- appropriate buffer; usually DDI H_2O(no higher than 30mM concentration of anything)
- desired lipid; concentration such that there are ~6 bilayers for a typical droplet (for DOPC 2mg/ml seems to work well)

Preparation:

- Make sure the ITO slides in the chamber are firmly in place and the copper conductor gasket is in contact at the top and bottom of each slide, check the resistance from BNC to slide, it should be no more than 40 Ohms
- Clean the chamber with ethanol and DI water using Que-tips and Kimwipes alternating the solvents at least twice to make sure any grease or sugars are removed from the slides
- Using the 10 μl glass syringe apply a 2-3 μl droplet of the lipid/chloroform solution in the center of the ITO slide on the shorter side of the chamber. The layer should appear blue/greenish under white light reflection.
- Quickly (within a few minutes) place the whole chamber in the vacuum desiccator, desiccate for approximately 1 hour
- Meanwhile, clean the nitrile O-ring using a Kimwipe and ethanol. Using a Que-tip, apply a thin layer of grease to one side of the O-ring.
- Remove the chamber from the desiccator and place the O-ring approximately in the center of the short side of the chamber, greased face down.
- Using the 200 μl pipette, place 140 μl of buffer solution in the O-ring.
- Put the top half of the chamber on using alignment pins, and ensure there are no bubbles in the wet part of the chamber.
- Carefully bring chamber over to the microscope.

Procedure:

- Place the chamber on the microscope stage, lipid coated side down.
- Attach the BNC connector to the chamber.
- Set the voltage amplitude between 1V and 5V, never exceed 6V as this will damage the ITO coating. Set the driving frequency to ~10Hz. One can vary the amplitude and frequency to suit the lipid/buffer composition (vesicles have been formed with frequencies as low as 3Hz and as high as 20hz and 1-5V)
- Wait and watch as vesicles begin to form over the course of about 2 hours. Blistering of the lipid layer should be noticeable immediately.
- Turn equipment off, and remove chamber from microscope, pipette out 100 μl of the solution from the chamber into the mini-centrifuge tube already filled with ~1 ml DI water. Mix this solution in the centrifuge tube with a wide-mouth pipette. The vesicles are now ready! They are good for about 48 hours.