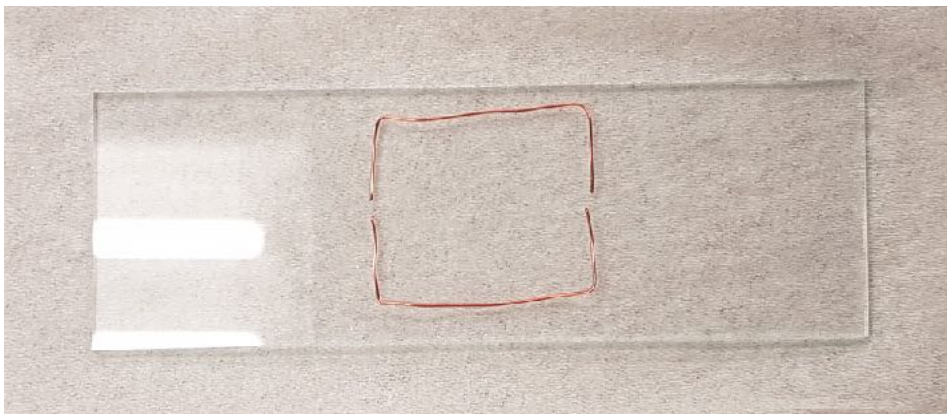
Protocol: Making Sample Chambers with vacuum grease

General Instructions:
The following instructions assume you are using vacuum grease to seal your sample. There are experiment-specific instructions at the end of this document.

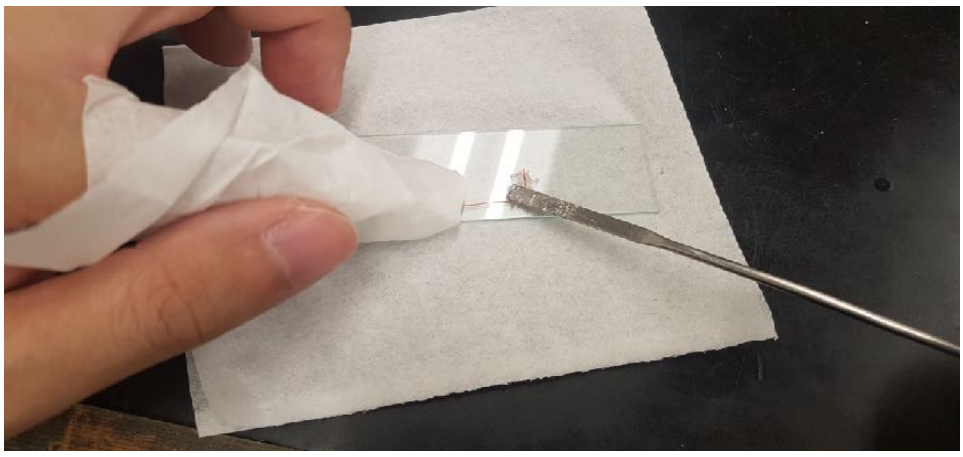
1. Take a glass slide and a Number 1 thickness cover glass (18mm x 18mm, or 22mm x 22mm) from the respective boxes. Use a thin solid wire (e.g., the 0.28mm diameter wire in the sample preparation) as a spacer. We will usually want relatively thin samples (< 0.5 mm), so that it is possible for the microscope to focus all the way through the sample to the other side.

2. Cut two wires (**best length:** each ≈30mm long for 18mm x 18mm cover glass, and ≈38mm long for 22mm x 22mm cover glass) with a cutting pliers and bend them into U shape (see figure below). Note that there won't be too much room for air gaps on both sides if the wires are too long, and the sample cannot be sealed completely if the wires are too short.

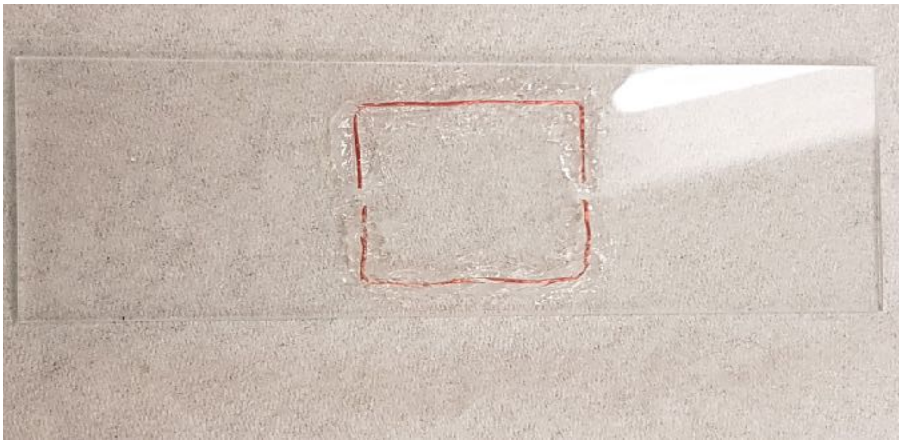


3. Now we can position the wires with vacuum grease. First wrap your finger with tissue paper (Kimwipes), and hold one end of a wire. This will keep the glass slide surface clean and prevent any unwanted movement of the wire when applying the vacuum grease.

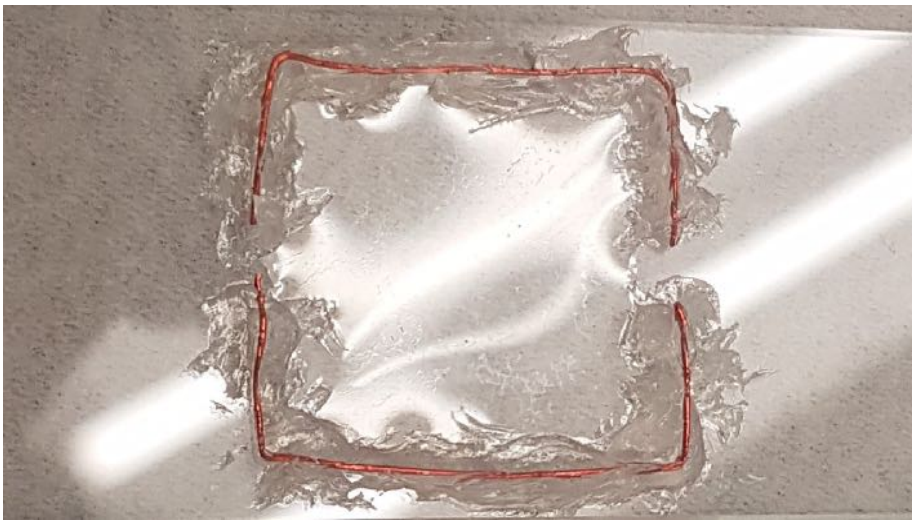
Put a small amount of grease on a spatula, and use it on both sides (inside and outside the chamber!) of the wire. **Do not** seal the air gaps. (The tricky part is to control the amount of the grease applied: too much grease will make a mess; too little might not seal it completely).



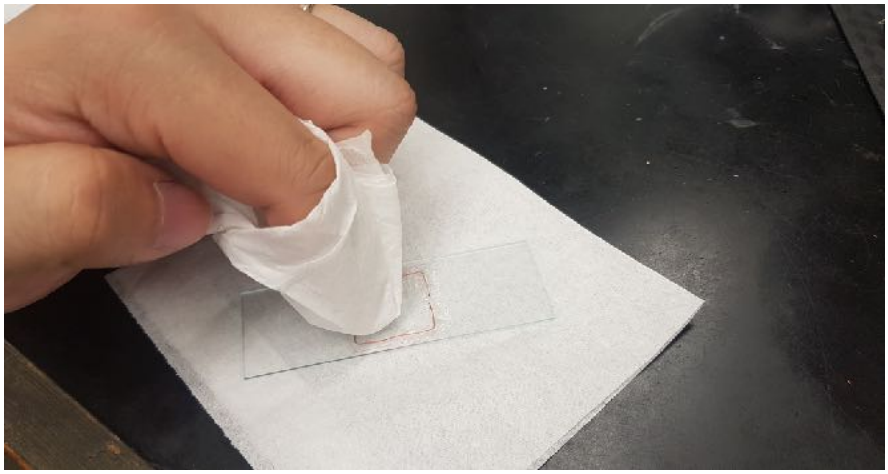
4. Do the same to the other wire and your work should look like the image below. Note how there are two small gaps between the U-shaped wires. In the image below, there is no coverslip.



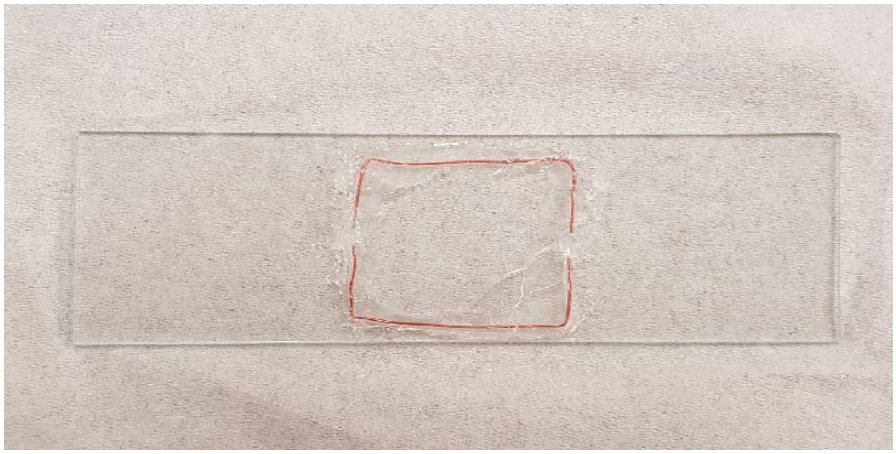
5. Use a pipette to put drops of bead-water solution at all four corners. This will reduce the number and size of any eventual air bubbles in the sample chamber.



6. Place the cover glass on the wire frame. Again, wrap your finger with tissue (Kimwipe) and apply a small pressure to the cover glass and along the wire. (Applying the pressure with a spatula is a good choice, too; the key is to **keep your cover glass clean.**) The solution might “leak out” through the side gaps. This is fine; use a Kimwipe to clean it after pressing down gently.



7. Seal off the side air gaps with a **small amount** of vacuum grease. Use a thicker wire to apply the grease.



Note 1: It is totally fine if there are air bubbles inside the sample chamber, as long as they are small. You can always adjust the chamber’s position to avoid the bubbles when mounting the sample in your experiment. The important point is to make sure you are observing the sample and not the air bubbles.

Note 2: Keep the centre of the cover glass clean. If it is too greasy, the microscope will give a bad (fuzzy) image of the bead.

Note 3: Handle your sample chamber with care! Don’t drop it, crack it, scratch it, or you will have to make a new one! Note that focussing the microscope objective “too close” is one way to crack the coverslip.

Experiment-specific Instructions:

It's a good idea to inspect your sample quickly before you start to use it. For those who are doing the **microscopy lab**, you already have a working microscope. For those doing the FCS or either of the **optical trapping** labs (bio & instrumentation focus), the home-built microscope is harder to use (and may not be in perfect working order). So, to help in such cases, we are providing an **inspection microscope** at the front of Room 8444. You can use it to get an idea of the concentration of beads in your sample. At lower magnification, you can inspect for leaks.

For experiments on the **FCS instrument**, you can make two chambers on one slide by mounting two cover-glasses side-by-side on one slide. Enough space should be left between the two cover glasses that each chamber can be filled and sealed independently.

For the **microscopy lab**, thinner samples can be helpful. To reduce the thickness, pipette a small volume (try 5 µl) of bead (or *E. coli*) solution and lightly drop a glass coverslip on top of the microscope slide, without any spacer wire. The fluid should spread by capillary action to the sides of the coverslip. Then seal the cell with vacuum grease all around the edges of the coverslip.

To mount a small piece of onion membrane, add a drop of water to the centre of the slide, lay the onion membrane on the drop (the drop of water will help flatten the membrane) and place a coverslip on top. Then seal the cell with vacuum grease all around the edges of the coverslip.

For the **biophysics optical trapping lab**, position the sample chamber towards one end of the glass slide for ease of use with the instrument.

For **both optical trapping lab**, after you have injected the bead solution into the trapping chamber, you can try balancing the chamber upside down for a couple of minutes, so that beads settle due to gravity and stick to the cover glass, which will be the front surface of your chamber. This will make it easy to determine when you are focused near the front chamber. (You might not need this step; try it if you are having trouble finding the front water-glass interface of the sample.)

Please clean up the sample preparation area after you are finished or your sample preparation privileges may be revoked.