

- wrapped in parafilm
- placed in 60°C oven for 10 min



After Vortex

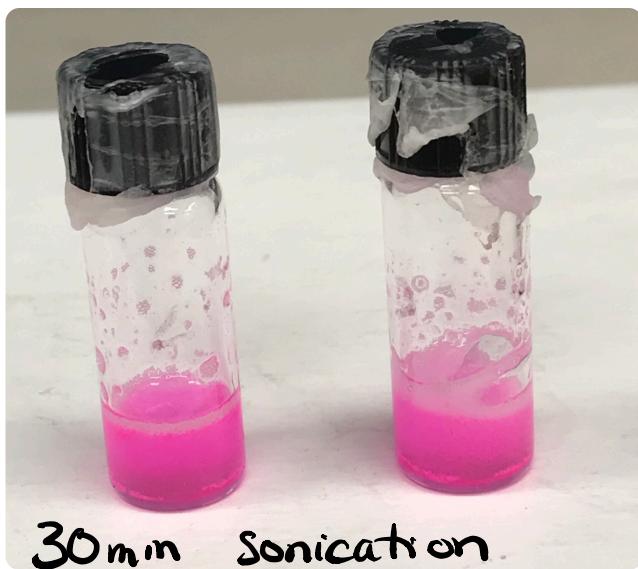
- Using Vortex
- Holding vials vertically for 10 min
- Orderless

Place in oven for 3 hours in 60°C



After Heating

Place in 60°C sonicator for 30 min



30 min sonication

⇒  
+30 min  
-not fully  
resuspended



60 min sonication

Samples split and run simultaneously using posted and adapted protocol

Posted:

Step 2: Self-assembly of the Liposomes by centrifugation

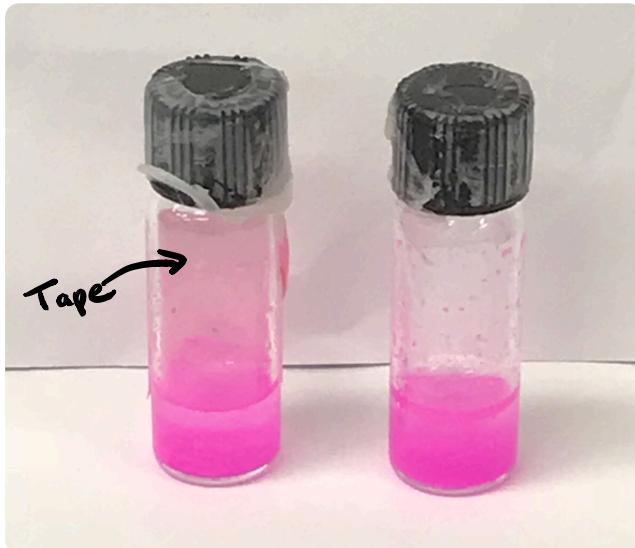
1. Place 225  $\mu$ L of centrifuge buffer (100 mM HEPES + 200 mM glucose, pH 8) into a labeled eppendorf tube.
  - a. Note: When TX-TL systems are used, the outer solution should also contain the small molecular weight components of the TX-TL system.
2. Add in 30  $\mu$ L of inner solution (100 mM HEPES, 200 mM sucrose pH 8, and, if needed, 2  $\mu$ M of a water-soluble fluorescent dye like HPTS or calcine.) to 500  $\mu$ L of suspended lipid in oil from Step 1.
  - i. Vortex for 30 s. Equilibrate for 10 min at 4 degC.
  - o. Note: When TX-TL systems are used, buffer is omitted. TX-TL systems come with their own buffers.
3. Add the emulsion on top of the 225  $\mu$ L centrifuge buffer by pipetting against the wall of the tube, wait at least 1 minute for the interface to stabilize and flatten between the emulsion and buffer
4. Centrifuge at 18000 rcf at 4 degC for 15 min
  - o. Note: If the top phase (oil) is clear, that suggests that droplets have passed into the bottom buffer, becoming vesicles.
5. Carefully remove as much mineral oil as possible with a gel-loading-pipette-tip from the top.
  - o. \*\*Note: \*\*The goal is to remove as much oil up to the interface without disturbing the buffer below.
6. Place 225  $\mu$ L of wash buffer (100 mM HEPES + 250 mM glucose, pH 8) into a labeled eppendorf tube.
7. Add the centrifuge buffer (where the vesicles should have formed) to eppendorf tubes with wash buffer. Make sure to use a new tip to avoid contamination.
  - o. Note: An alternative method is to open the eppendorf tube and use a 21-gauge needle to punch a hole at the bottom of the eppendorf tube. Remove the needle and close the lid to allow the buffer solution to drip out.
8. Centrifuge at 12000 rcf at 4 degC for 5 min
9. Transfer 225  $\mu$ L from the bottom of the eppendorf tubes with wash buffer into a new labeled eppendorf tube.

Adapted:

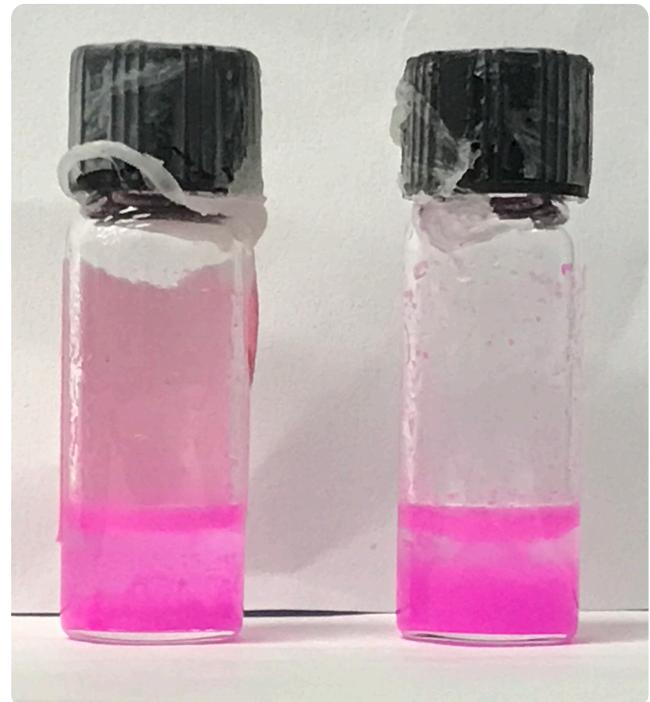
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8. Centrifuge at 12000 rcf at 4 degC for 2 min
9. Transfer 225  $\mu$ L from the bottom of the eppendorf tubes with wash buffer into a new labeled eppendorf tube.

Internal solution and Lipid-oil emulsion

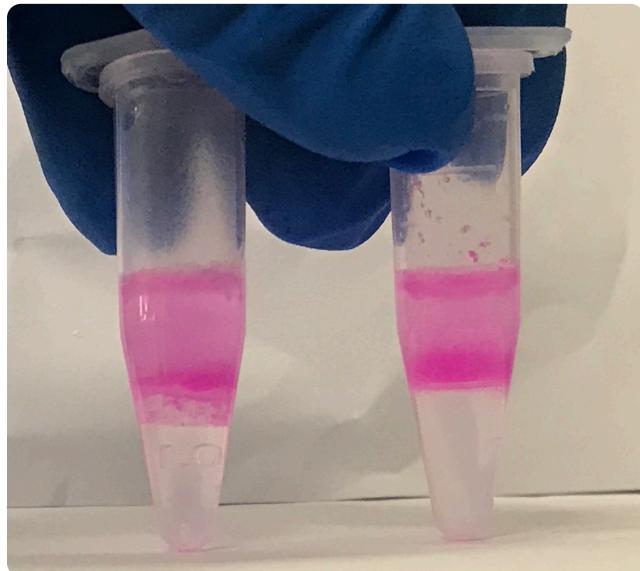


After 30s vortex



After Equilibrate for 10 min

Moving Lipid + internal solution to tubes

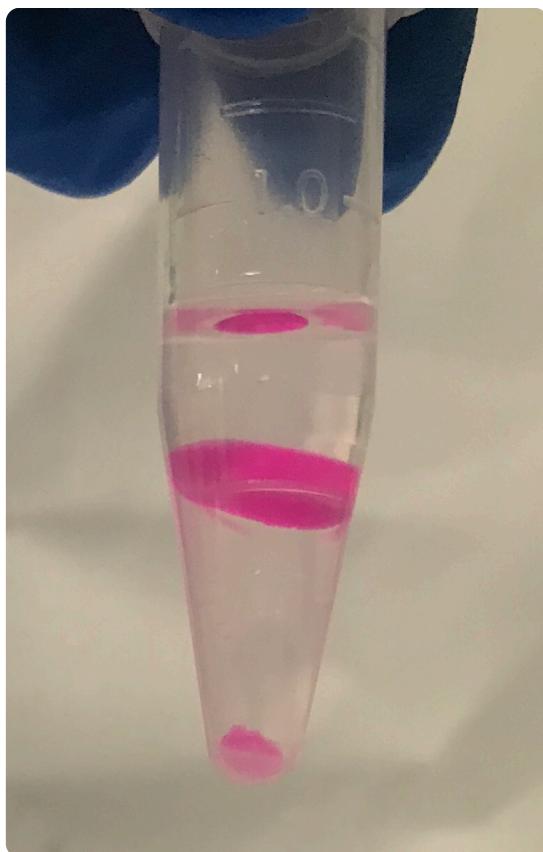


Emulsion over centrifuge  
buffer

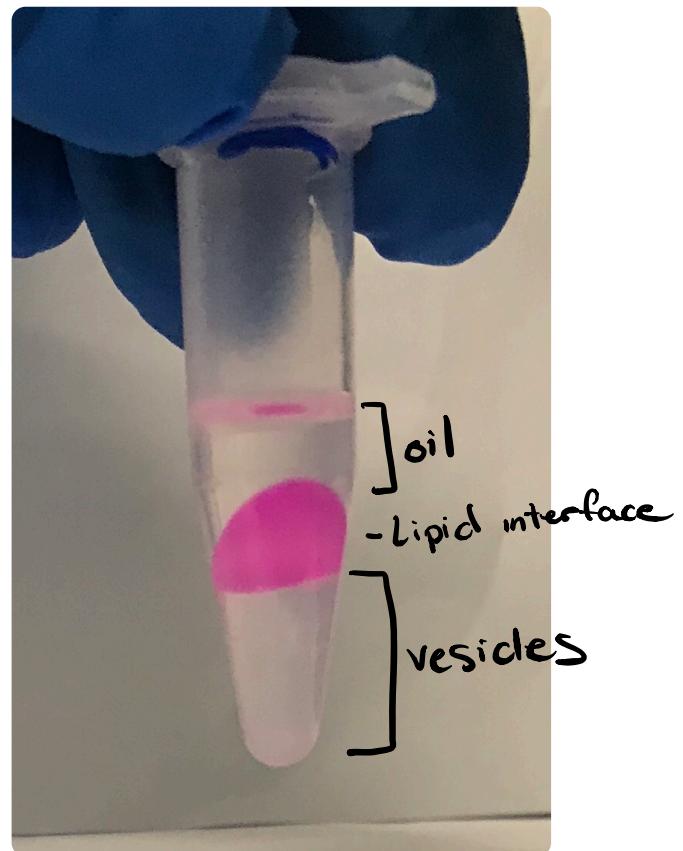


Vials, after Emulsion removed

Posted

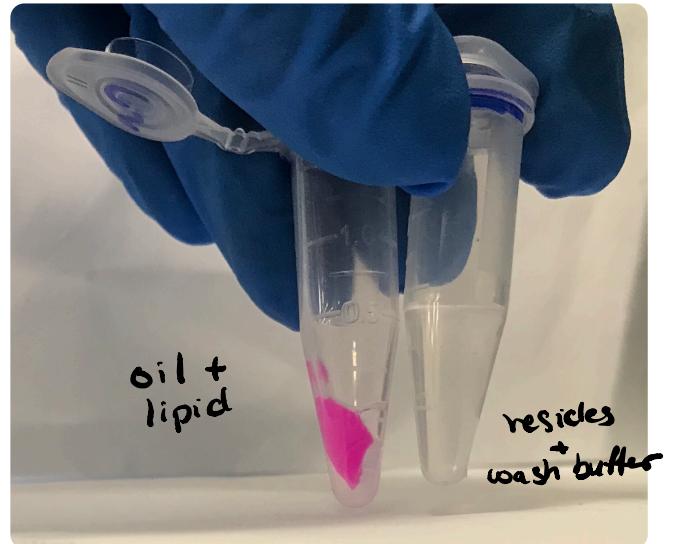
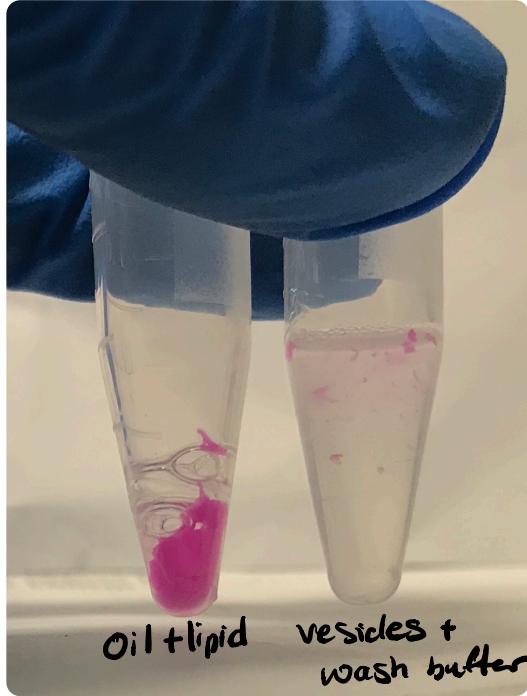


Adapted

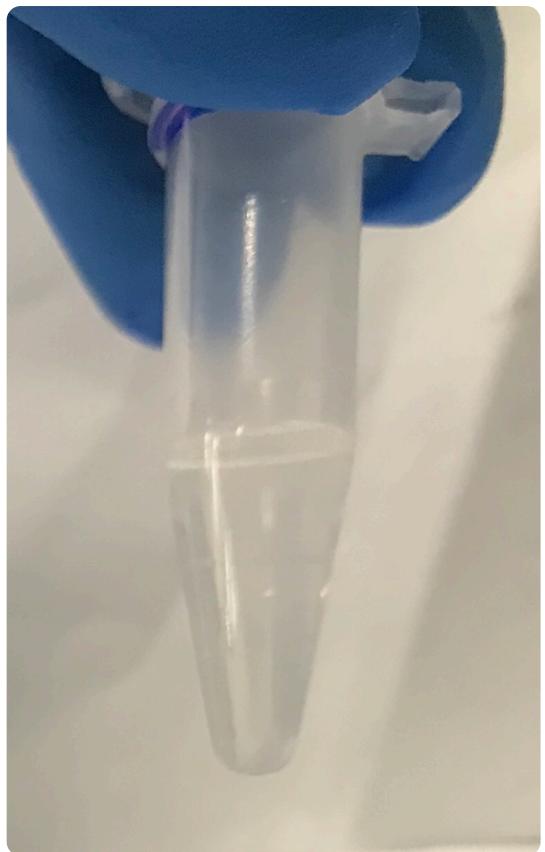
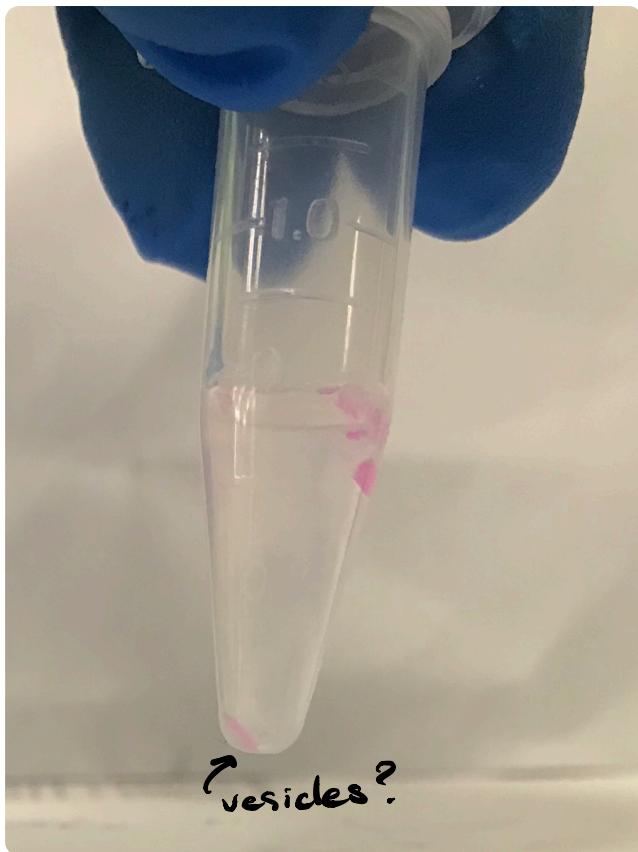


# Bottom Phase Removed: moved to wash step

-using 250 $\mu$ L tip, with plunger down, all solution below lipid raft was moved to wash step

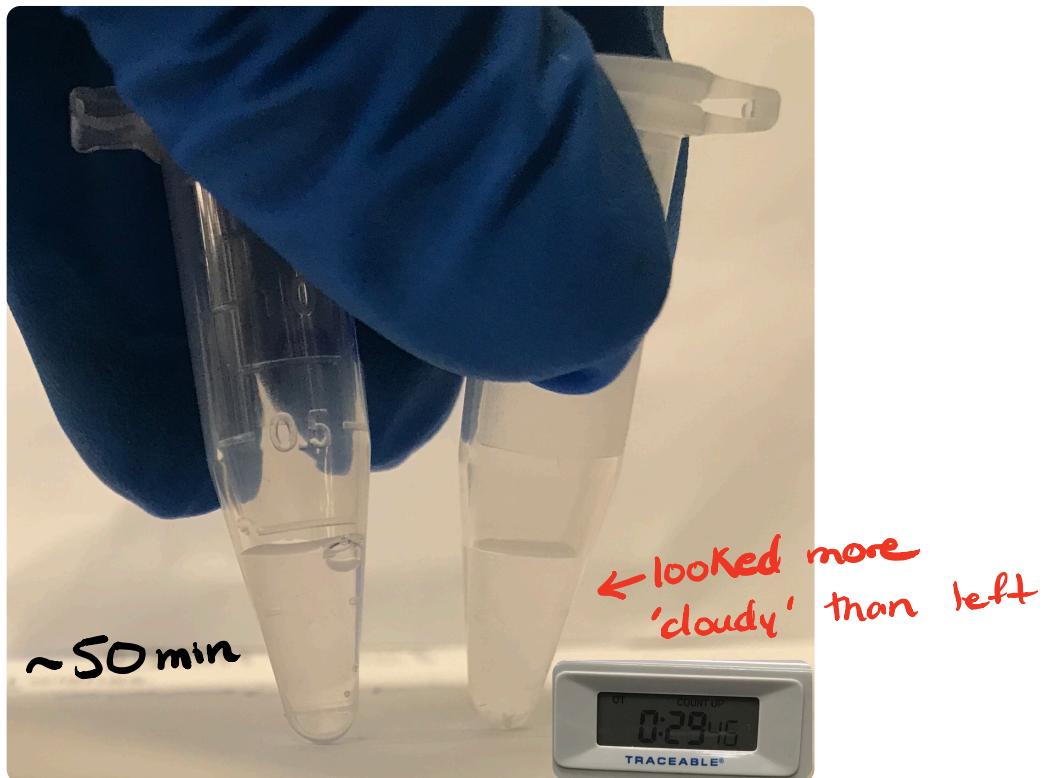


## After Wash



225 $\mu$ L removed from bottom of tube, and  
10 $\mu$ L was used from microscopy

# Final residues:



## Step 3: Microscopy Visualization

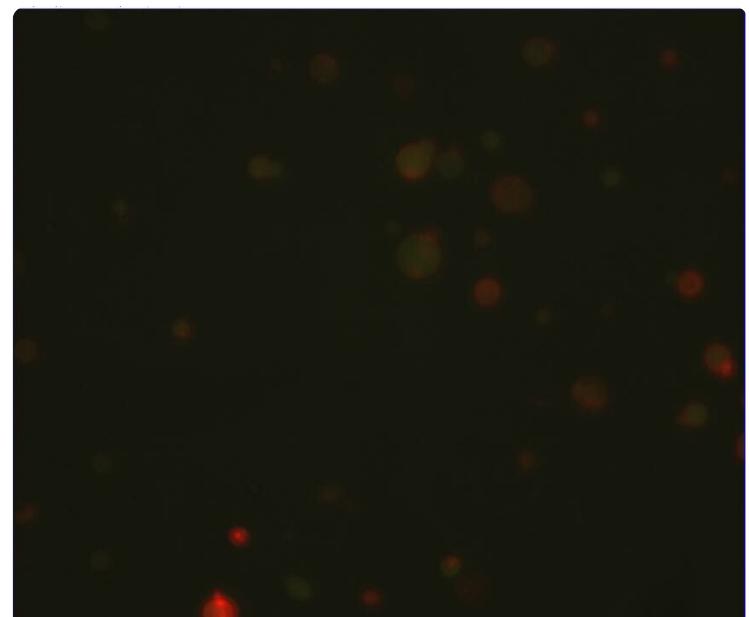
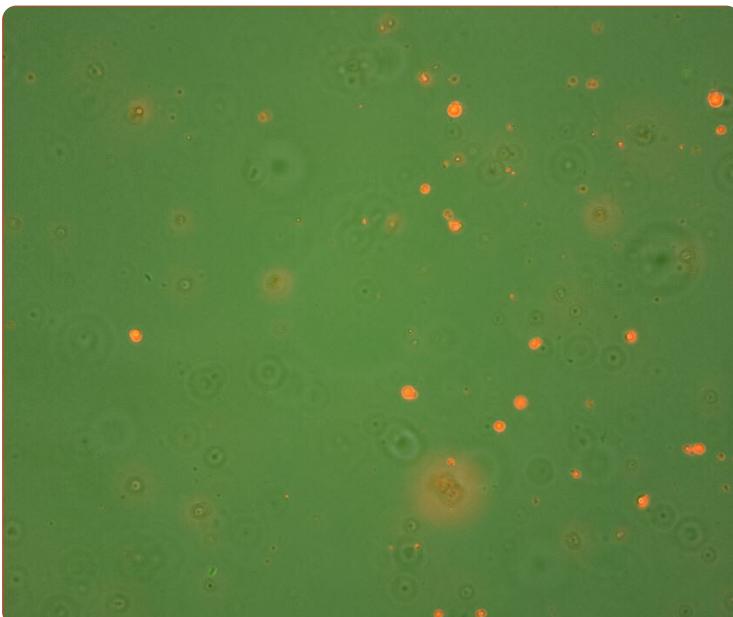
1. Use FrameSeal or Spacer (20 mm D x 0.12 mm depth) to make a small chamber on a microscope slide.
2. Add 10  $\mu$ L of the final solution to the chamber and seal with cover slip
3. Wait 5 minutes before imaging (since the liposomes will float everywhere and can be tricky to catch them).
4. Observe on an inverted microscope. [Sample images] of what you should see:

Images done  
@ 40 X mag

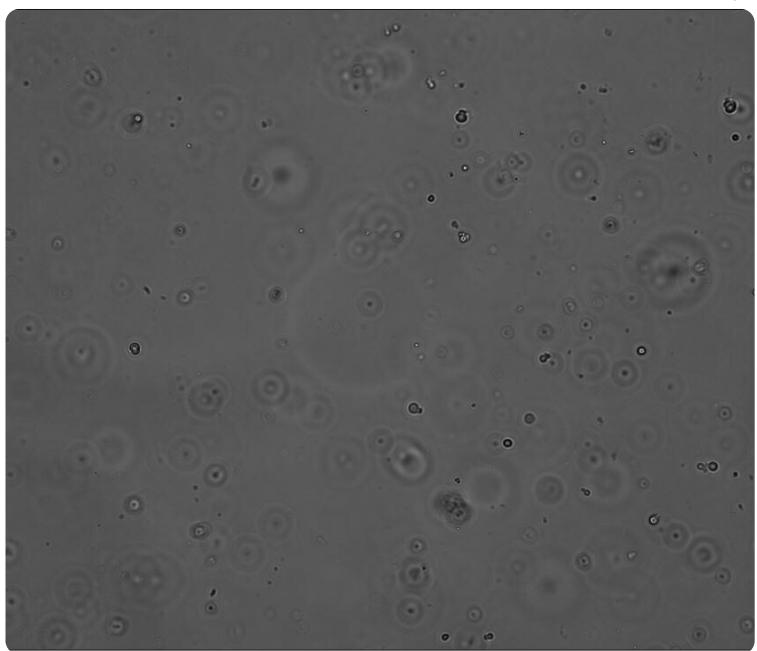
### Alternative method (neha):

- Use a glass bottom chamber: <https://www.thermofisher.com/order/catalog/product/154453>
- Block glass by adding 0.5 mL of a 1mg/mL BSA in PBS solution for 10 minutes (alternatively block by adding 0.5 mL of SuperBlock for 5 min). The blocking step is important because phospholipid vesicles will rupture to some degree on glass.
- Rinse with PBS 3X. Add 0.5 mL of final buffer from above to chamber. Add 10  $\mu$ L of vesicle sample to chamber, let settle

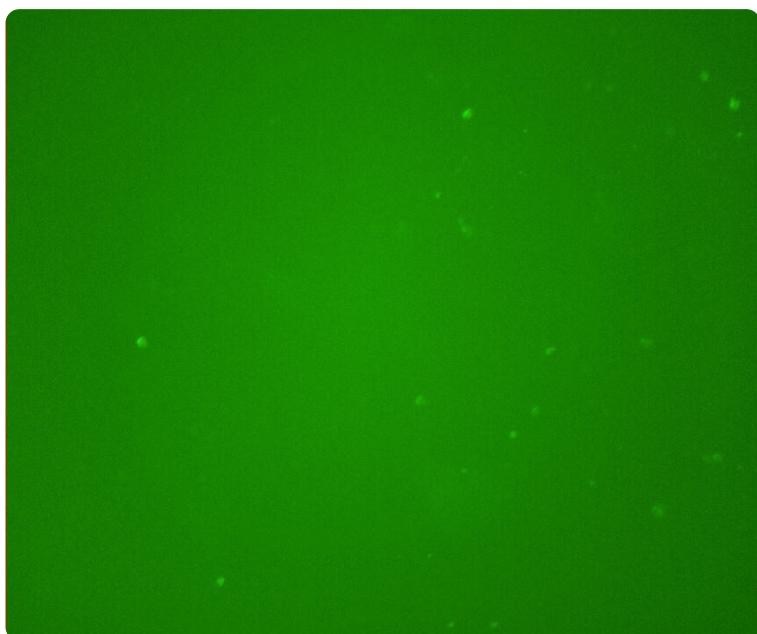
## Overlay:



*BF*



*GFP*



*RFP*

