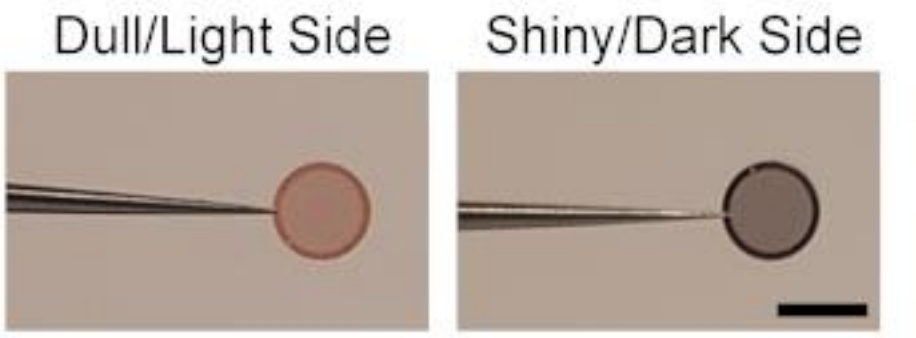
Nanoscale Biomedical Imaging Facility
(CMEM sub-core)
Standard Operating Procedure

Negative Staining

1. Prepare proper dilution of your sample in water, buffer or other solvents.
2. Prepare a strip of clean parafilm on the bench top.
3. Glow discharge your carbon grids to clean the carbon and ensure optimum sample dispersion.
4. Use fine-tipped forceps to pick up a formvar/carbon coated or mesh copper transmission electron microscopy grid. Be sure to only pick up the grid by the edge, If the forceps touch the film, the formvar/carbon coated film will be damaged, making imaging in that area of the grid impossible.
5. You can either use inversion method (drop of sample on the parafilm first and then the grid floats on top) or drop method (put the grid face on the parafilm and then deposit sample drop over the grid). Either case, make sure the carbon coated side (shiny side) contacts the drop of sample



6. Time your sample incubation with the grid. Pick up the grid,
7. Float the grid as above on the second drop of ddH2O for 1 min and wick away the ddH2O. You may consider skipping this step.
8. Float the grid on the drop of 2% uranyl acetate for 1 min and wick away the excess with filter paper.
9. Float the grid as above on the third drop of ddH2O briefly and wick away the ddH2O. You may consider skipping this step.

NOTE: Grids should dry for at least 5 min before imaging.