

## **Uranyl formate preparation for negative staining (0.7% w/v)**

- a) Weigh 37.5mg of uranyl formate in a beaker and cover it to prevent exposure to light.
- b) Bring 5ml H<sub>2</sub>O to boil in small flask.
- c) Add the boiling H<sub>2</sub>O to the uranyl formate in the beaker. Stir for 5 minutes.
- d) Add 5μl NaOH (5M). A color change to a darker yellow should occur. Stir for 5 minutes.
- e) Filter and store in container protected from light exposure.
- f) Mark the tube with your name and date.

## **Glow discharge grids**

- a) Before applying the sample to the grid you should glow discharge the grid to recover the hydrophilic properties of the carbon.
- b) Put the grid on a glass slide wrapped in parafilm in the glow discharger.
- c) Close the lid and make sure it aligns well.
- d) Push the start button and wait until the process will finishes (you should see purple glow).
- e) Take your grids and close the lid.

## **Preparation of negative stain grid**

- a) Before starting, prepare parafilm, filter paper, H<sub>2</sub>O and stain.
- b) Grab a glow discharged grid on the edge with forceps with the dark side (carbon) up.
- c) Pipette two drops of H<sub>2</sub>O and two drop of stain (30-40μl each) onto the parafilm.
- d) Apply the sample (~3-5μl) and wait 30-60s.
- e) Blot the excess sample on the filter paper.
- f) Wash the grid briefly (5-10s) in H<sub>2</sub>O by touching the drop and blot it on the filter paper X2.
- g) Wash the grid once with the stain drop and blot on the filter paper.
- h) Put the grid on the second stain drop for 1-1.5 min then blot the stain and dry the grid with vacuum.