

**Instrumental technique presentation**

# **Electron Microscopy (EM) Grid**

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# What I will talk about.....

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- Some basic topics about EM grid
- Home-made grid preparation
- Grid cleaning
- Carbon coating and carbon film preparation

# Some basic topics about EM grid

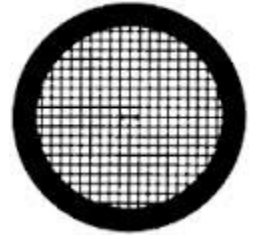
## What is EM grid?

- The EM grid is to the TEM what the glass slide is to the light microscope. It provides a way for supporting the specimen and delivering the specimen into the TEM column for observation and data collection.
- The EM grid is a 3.05 mm diameter, thin-foil mesh, and it can be made from a number of different metals (copper, gold, nickel, molybdenum, stainless steel, tungsten, aluminum, titanium etc.), and have various spacing patterns.
- The diameter of grids is standard, as 3.05 mm. The Thickness is variable based on the materials of the grid. The thickness range is from 10  $\mu\text{m}$  to 25  $\mu\text{m}$ . The mesh of the grid is defined by the number of holes within 1 inch. For example, the 200 mesh grid has 20 holes along diameter direction, 400 mesh grid has 40 holes in diameter.
- Since the diameter of grid is a standard length, 3.05 mm, each hole size is less than 200  $\mu\text{m}$  in 100 mesh grid, 100  $\mu\text{m}$  in 200 mesh grid, 75  $\mu\text{m}$  in 300 mesh grid and 50  $\mu\text{m}$  in 400 mesh grid.

# What we need to do with EM grid before we can use it?

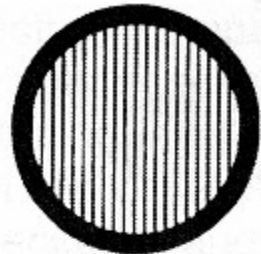
- Often an electron lucent film is deposited upon the grid to aid in specimen support. The TEM techniques that are often used to investigate macromolecular structures require films made of a thin layer of carbon which may or may not have numerous small perforations. The macromolecules are then examined while adhered to the carbon or while suspended in the holes.
- The grid have to be cleaned up for any dirty or chemicals left over from manufacture or process of depositing electron transparent film before putting specimen on it.
  - ✓ Eliminate any possibility for alternating or damaging your specimen.
  - ✓ Eliminate or reduce charging problem to get quality data
  - ✓ Keep pole piece and column clean.

# How to choose right grid- some preliminary considerations



- The type of grids and films used are usually determined by the desired specimen preparation technique and sample characteristics.
- Copper grids are usually used because they are conductive, stable in the beam and inexpensive. But when treating the sample on the grid with some substances such as acids, bases, some salts, etc. more expensive non-reactive grids such as gold or platinum may be necessary.
- Both holey films and continuous thin carbon films are mounted on grids with appropriate mesh value for stability in the beam at higher magnifications.
- Vitrified samples are best examined when suspended in the holes of a holey carbon film, while negative stained samples are examined on continuous carbon films.

# Specialized grids



200P



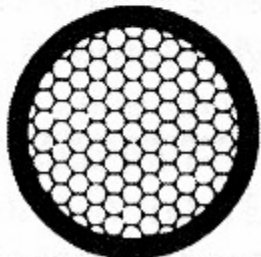
50/100



100/100



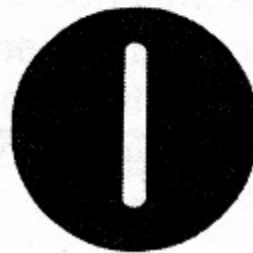
100PB



100 Hexagonal



1x2 mm



0.4x2 mm

Specialized grids include:

- Bar grids
- Mixed bar grids
- Folding grids
- Slot grids
- Hexagonal grids

# Home-made grid preparation

## 1) Pre-clean copper grids.

- a) Copper grids should be pre-cleaned by sonicating for 10 sec. in Acetone, followed by 10 sec. sonication in ethyl alcohol.
- b) Allow grids to dry on filter paper in a dust-free environment before use.



## 2) Prepare Formvar solution.

- a) Add 0.12 g of Formvar powder to 50 ml of ethylene dichloride and mix well on a magnetic stirrer until dissolved.
- b) Pour the solution into a clean Coplin jar. This solution is hygroscopic but will store well for several months if kept in the dark, tightly sealed, and refrigerated.



# Home-made grid preparation

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- 3) Clean a glass slide with water and detergent.
  - a) To prevent contamination with oil from the skin do not touch the slides with bare hands (use gloves).
  - b) Scrub the glass slide with lint-free paper, put them into the slide holder and soak in 2% RBS for at least one day. (RBS: mixtures of anionic and non-ionic surfactants, <http://www.chromspec.com/Catalogue/Misc/CleaningSolutions.html>)
  - c) Rinse the slides thoroughly by running de-ionized water over the slides.
  - d) Dry the slides by air drying but keep them dust free or dry them in an oven at relatively low temperature. After drying the slides can be stored in a slides box.
  
- 4) Coat the clean slide with Formvar.
  - a) Dip the cleaned slide into the Formvar solution and touch edge to filter paper to drain off the excess fluid. The film will be somewhat milky when wet but it will clear as it dries.
  - b) Dry upright in a dust-free environment (this requires 5 to 10 min).





# Home-made grid preparation

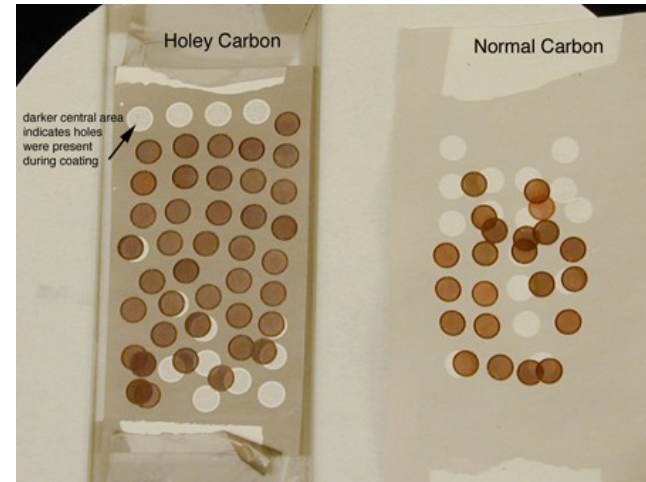
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- 5) Remove Formvar film from slide and add grids.
  - a) Fill the black dish with de-ionized water until a meniscus is formed on top of the dish. Then drag the lucite rod across the surface of the water to remove any floating debris.
  - b) Cut the edges of the Formvar film with an acetone-cleaned razor blade.
  - c) Dip the slide slowly in water and Formvar film will be floating on the water.
  - d) Add the grids



# Home-made grid preparation

6) Carbon coat film to desired thickness.



7. Remove Formvar film.

- a) Place the paper with grids onto a piece of filter paper saturated with ethylene dichloride in a covered Petri dish. The level of ethylene dichloride should be sufficient to completely soak the backing paper without submersing the tops of the grids. One half hour should be sufficient time to dissolve the Formvar film and not damage the carbon support.
- b) Remove paper with the grids and let dry in a dust-free area.

# Grids Cleaning

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Why need to clean grid before use?

- Good for your specimen
- Good for imaging
- Good for microscope

## Organic Solvents used for grid cleaning

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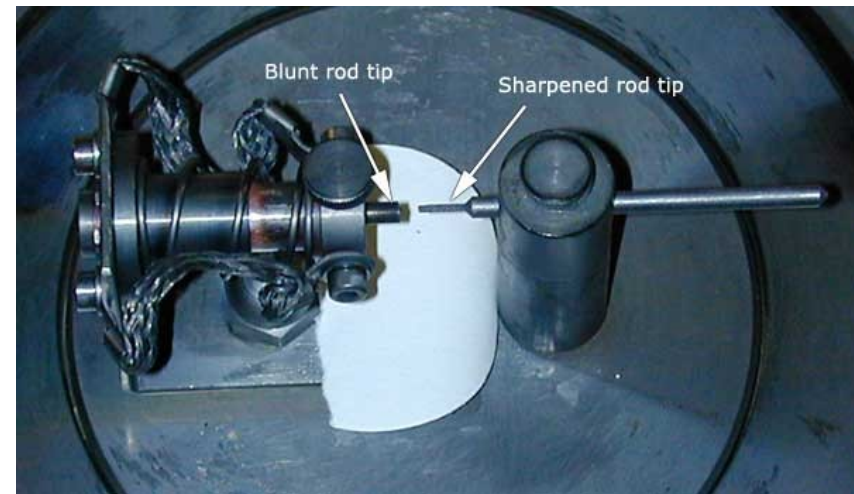
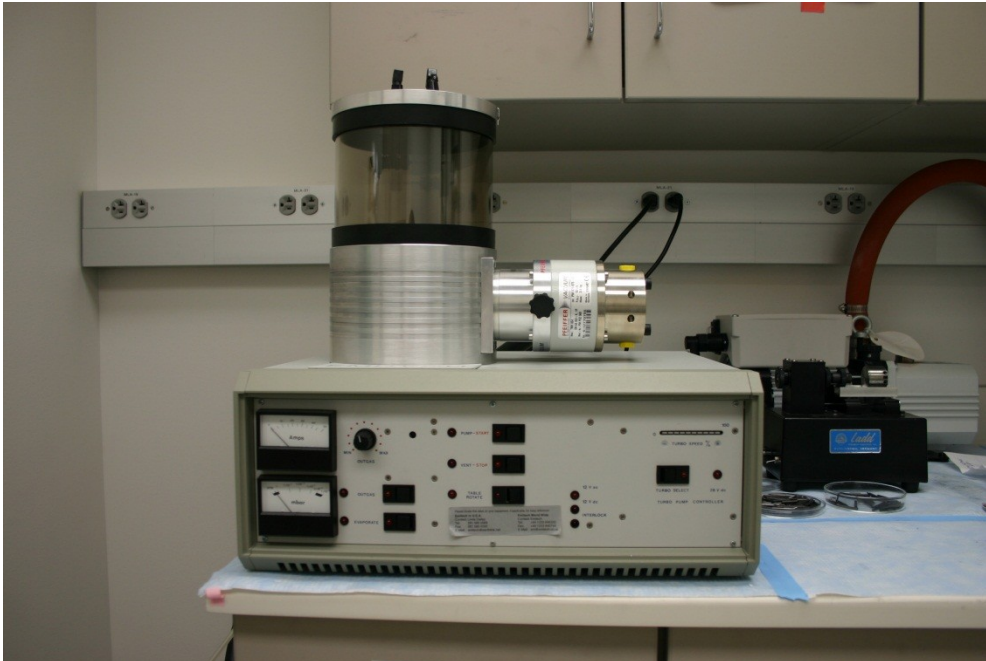
Two organic solvents have been used in this lab,

- Acetone ( $(\text{CH}_3)_2\text{CO}$  )
- ethyl acetate ( $\text{CH}_3\text{COOC}_2\text{H}_5$  )

People in other places also use chloroform ( $\text{CHCl}_3$ ) and ethylene dichloride ( $\text{C}_2\text{H}_4\text{Cl}_2$ )

# Carbon coating and carbon film preparation

- Evaporate a uniform carbon layer over plastic films or freshly cleaved mica to be used as a stand alone support film
- Produce very thin support films ( $\sim 250\text{\AA}$ ) for use in testing EM and negative staining



**Thank You**