Producing superior quality carbon films for EM sample preparation with the Q150V Plus

TEM grids, EDS analysis and replicas

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In carbon thin film applications the quality of carbon film production is key for successfully conducting complex experiments. Commonly, carbon coatings are used in sample preparation for TEM (ambient and cryo) imaging, EDS analysis and replicas. The advantage of using a carbon film is that carbon is transparent to the electron beam. The thin carbon films are conductive and easy to produce, they are free from contamination, smooth and strong, and most of all they can be prepared thin enough to not attenuate the contrast when imaging specimen structural elements.

For example, in the most demanding of high quality carbon film applications, TEM imaging, the support layer has to be as thin as possible due to the fact that the thickness and density of the material influences image resolution and contrast. On the other hand, the layers have to be dense and sturdy to withstand a complex sample preparation processes. Another advantage of using carbon is that its surface properties can be altered in processes like; glow discharge, UV irradiation or chemical treatment. This provides an excellent solution to issues caused by different affinities of molecules for carbon.

Required characteristics of Carbon Thin Film:

- To be as amorphous as possible without contamination and minimal hydrocarbons
- To be sturdy and not to break in the floating or replica preparation processes
- Create a thin film that can be easily floated from mica sheets and placed on to TEM grids
- Withstand freezing when used as a support for cryo-TEM samples

Carbon film quality:

The typical factors that affect the quality of produced carbon films are: the base vacuum during carbon deposition and the purity of the carbon source. To achieve superior carbon film quality the base vacuum of 1 x10^-6 mbar or better is necessary, as well as the use of an ultra-pure carbon source.

The Q150V E Plus coater, using a carbon thermal rod evaporation head (Bradley method) with thickness monitor, is successfully used in preparation of carbon films exhibiting the quality required for a variety of EM applications. The use of high purity carbon rods allows for the precise deposition of a set thickness and overcomes the source out-gassing problem, which usually influences the purity of the formed layers.

The influence of the deposition base pressure is depicted in figures 1 and 2. The simplest and the most straight-forward test for carbon film mechanical stability is floating the layer from a mica surface and setting it onto TEM grids. This method is used as one of the steps in sample preparation in TEM imaging. The quality of carbon layers has a direct impact on the final process result.

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Base pressure influence on carbon stability:

### Influence of poor base vacuum on the mechanical and electron stability

3. 5nm carbon layer produced with base pressure $4 \times 10^{-5}$ mbar floated from mica onto TEM grid - visible cracks and flaws of the carbon layer.

4. 5nm carbon layer produced with base pressure $4 \times 10^{-5}$ mbar floated from mica onto 200 mesh TEM grids - visible cracks and wrinkles, and the carbon is not covering the whole grid.

5. HR TEM image of 5nm C film produced with base pressure $4 \times 10^{-5}$ mbar, floated from mica onto TEM 200 mesh grid - breaks in the carbon layer and an overlap of the layer caused by poor mechanical stability.

6. HR TEM image of 5nm C film produced with base pressure $4 \times 10^{-5}$ mbar, floated from mica onto TEM 200 mesh grid - the amount of hydrocarbons influences the layer stability under the beam, showing visible burn out.

### Influence of high base vacuum on the mechanical and electron stability

7. 5nm carbon layer produced with base pressure $8 \times 10^{-7}$ mbar floated from mica onto TEM grids - neat, stumpy and dense layer.

8. 5nm carbon layer produced with base pressure $8 \times 10^{-7}$ mbar floated from mica onto 200 mesh with holey carbon TEM grids - neat, stumpy and dense layer covering the whole grid.

9. HRTEM image of 5nm C film produced with base pressure $8 \times 10^{-7}$ mbar, floated from mica onto TEM 200 mesh grid - sturdy and even layer.

### Conclusion:

The quality of carbon thin film for ambient and cryo-TEM significantly influences sample preparation. Dense, sturdy and pure films made using the Q150V E Plus coater will make sample preparation much easier, giving certainty and confidence about the process - from transferring carbon onto TEM grids, post treatment, sample application and finally during imaging.

10. HR TEM image not covered with carbon TEM 200 mesh grid.

11. HR TEM image and corresponding FFT of 5nm C film produced with base pressure $8 \times 10^{-7}$ mbar, floated from mica onto TEM 200 mesh grid showing the film quality.

12. cryo-HR TEM image of DNA replication complex placed onto 200 TEM mesh grid with holey carbon and 5nm additional carbon layer (produced with use of $8 \times 10^{-7}$ mbar, floated from mica); image showing no flaws of floated carbon film.

13. Zoom-in of cryo-HR-TEM image of DNA replication complex, mag x 924

TEM images - courtesy of Dr Julia Locke, Macromolecular Machines Laboratory, The Francis Crick Institute, London, UK.