APPLICATION NOTE

Carbon thin film preparation for ambient and cryo-TEM grids using the Q150V Plus

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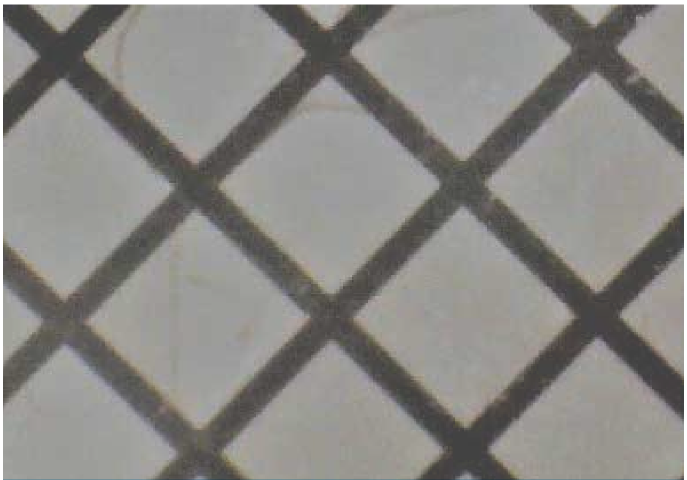
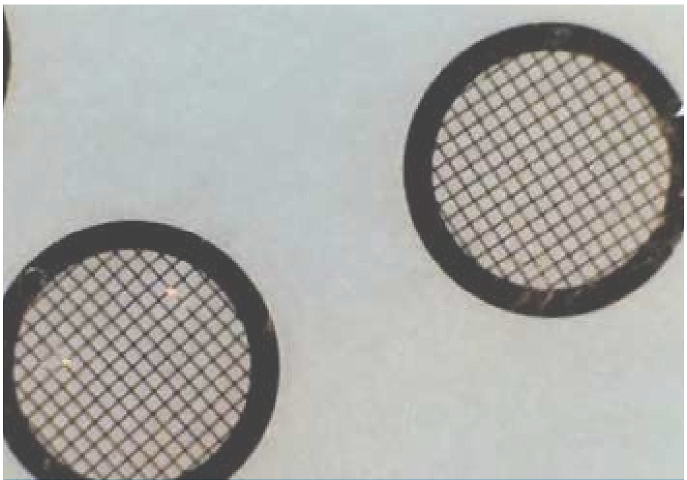
The 2017 Nobel Prize in cryo-Transmitted Electron Microscopy highlighted the significance of direct observation of molecular world of the cell. Understanding the structure of biomolecules as well as their changes over cell processes plays an important role in curing diseases and in drug discovery. Both ambient and cryo-TEM require correct and precise sample preparation, in which the quality of carbon support film for sample application is essential.

CARBON EVAPORATION ON GLASS SLIDES - REPRESENTATION SHOWING CONSECUTIVE INCREASE OF LAYER THICKNESS



5NM CARBON LAYER SET ON 200MESH TEM GRID



FLOATING CARBON FILM FROM MICA SHEET

Why use carbon film?

Carbon films must be transparent to an electron beam, conductive and easy to produce, free from contamination, smooth and strong, and most of all, they should be prepared thin enough to not attenuate the contrast when imaging specimen structural elements.

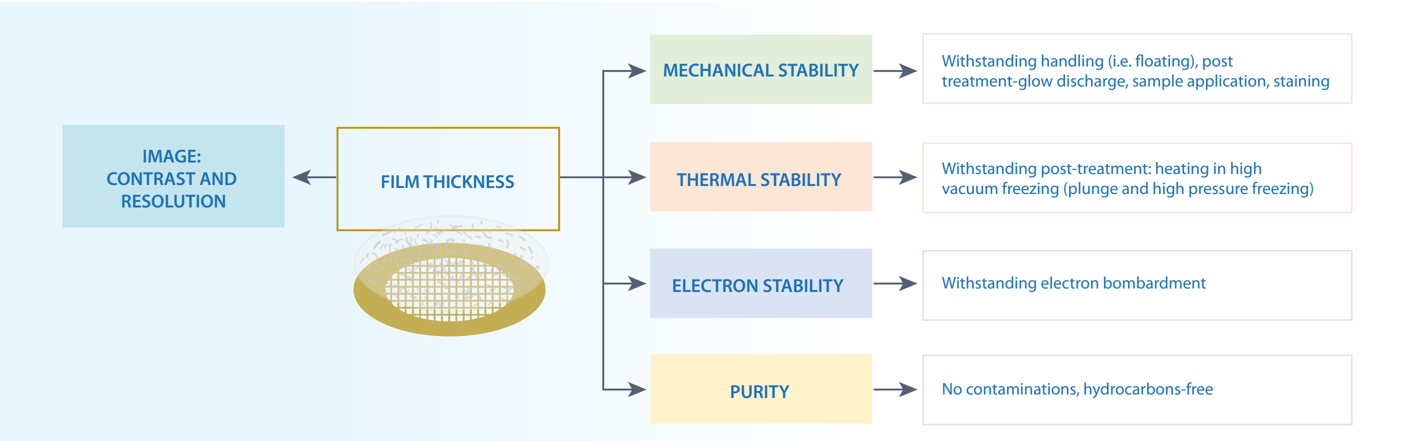
Commercially available TEM grids come in different versions- they can be neat (made of metal: Cu, Ni, Au), covered with polymer film and/or holey(lacey) carbon. In TEM imaging the support layer has to be as thin as possible due to the fact that the thickness and density of its material influences image resolution and contrast. Another advantage of using carbon is that, its surface properties can be altered in processes like glow discharge, UV irradiation or chemical treatment. This gives a way to overcome problems caused by different affinities of molecules to carbon. Sample preparation for TEM imaging consists of a few steps, and in each of them care has to be taken to achieve desired result.

The choice of carbon film thickness, the grid itself, the method of transferring and post-treatment for achieving desired surface properties and finally application of the sample makes the whole process very prone to failure. From one side, the correct execution at each step is curtail and from the other, the quality of the carbon film used for the preparation has tremendous influence on the final imaging.

The aspects which need to be considered when preparing TEM grids with backing carbon film.

1 Choosing the correct thickness

Choosing the correct thickness for the application influences contrast in imaging. Usually for structural biology samples 5nm carbon layer is applied, although there are molecules which need a thinner carbon layer applying for better imaging contrast. In such cases there is a trade-off between contrast and carbon layer stability.



THE TRADE- OFF BETWEEN CARBON FILM THICKNESS AND STABILITY

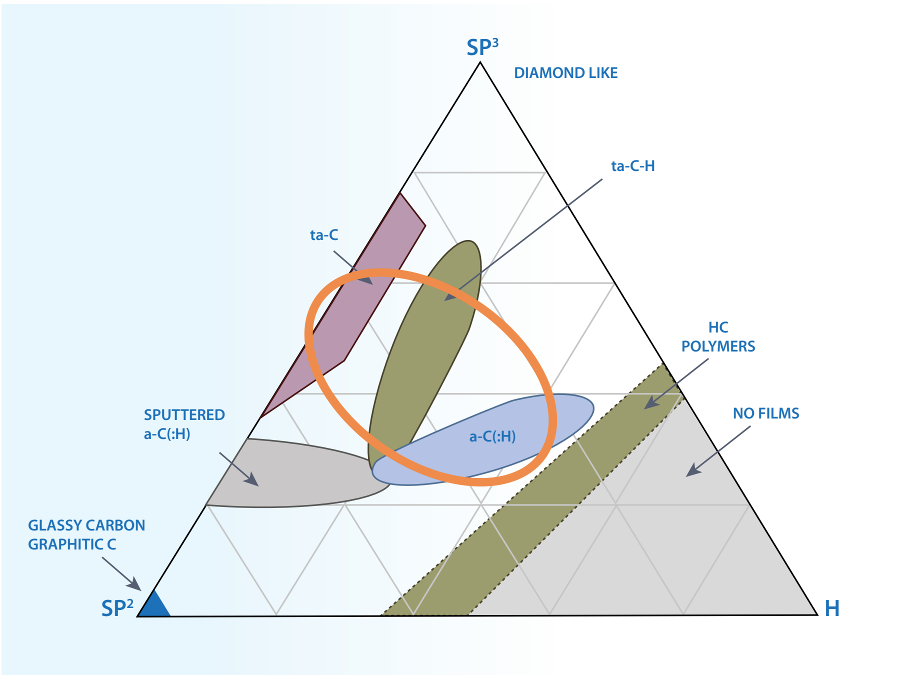
2 Choosing the correct method and parameters for carbon film preparation

The carbon layers used for TEM imaging have to be of a high quality. They need to be dense, free from inclusions and texture, pure, stable and amorphous. This is typically achieved by using hydrogen free carbon sources. Such layers consist of different types of carbon, usually in various proportions, depending on the method and parameters used for their preparation.

Carbon layers for TEM applications must have a minimal fraction of hydrocarbons, as their presence spoils imaging in a considerable way. Such films could be only produced in a high vacuum ($1x10^{-6}$ mbar or better) with the use of hydrocarbon free sources.

The hydrogen content, as well as sp^2 to sp^3 ratio, determines the structure and properties of the Diamond Like Carbon (DLC) films obtained during such deposition. It also depends on the parameters of the deposition method employed.

The Bradley method, thermal carbon rod evaporation, utilized by the Quorum Q150V Plus coater can be used to produce thin layers of amorphous carbon, which possess the quality to be employed in variety EM applications.



TERNARY PHASE DIAGRAM OF BONDING IN AMORPHOUS CARBONHYDROGEN ALLOYS, DIAMOND-LIKE AMORPHOUS CARBON, MATERIALS SCIENCE AND ENG R 37 9202 129-281

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Carbon thermal evaporation head

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Shaped carbon rod for thermal evaporation

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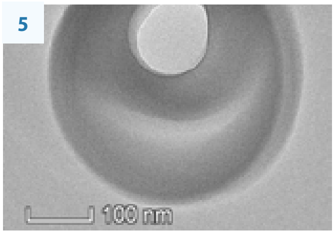

TEM image of burn-out caused by the beam in 10nm carbon film (floated off mica) prepared in a vacuum of 4E-05 mbar

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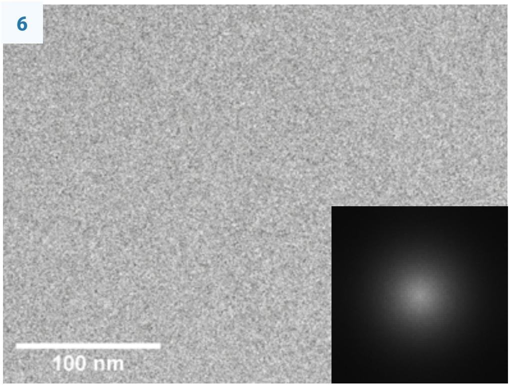

TEM image of 200 mesh grid with no carbon layer

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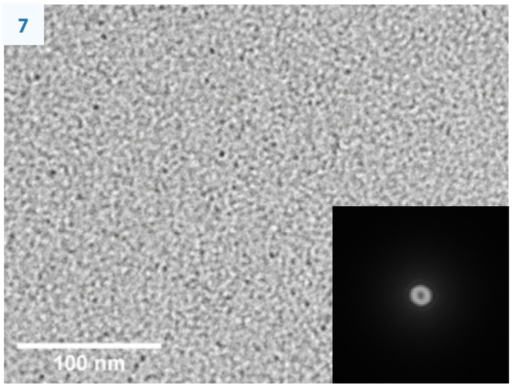

TEM image of 5nm C floated onto TEM grid produced in a vacuum of 7E-7 mbar (Q150V plus)

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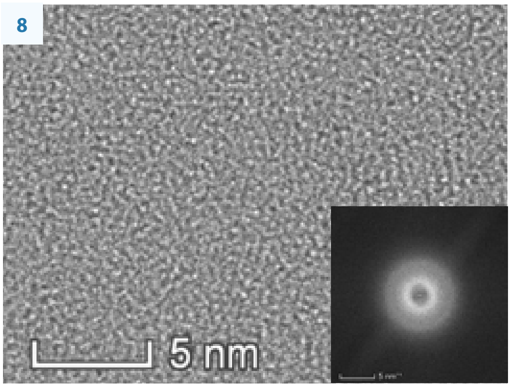

TEM image of 10nm C produced in a vacuum of 8 E-07 mbar, floated from micacarbon layer

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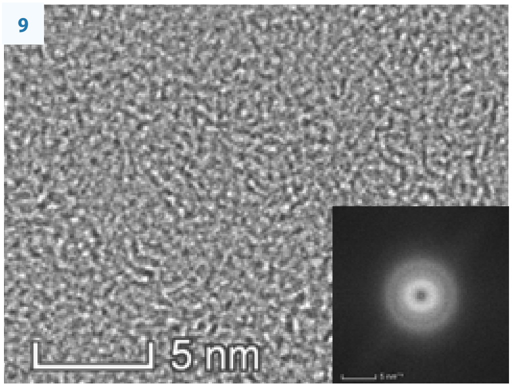

TEM image of 10nm C film produced in a vacuum of 4 E-05 mbar, floated from mica

The most common and simplest method of testing the quality of produced layers is high resolution TEM imaging with FFT analysis that points out amorphicity, density and structure of obtained films. The vacuum level plays an important role in the deposition process; the better vacuum the denser and more sturdy amorphous carbon layer with less amount of impurities. Thin carbon layers produced with the use of a lower vacuum will not possess as greater stability in the electron beam as depicted in figure 5.

3 Correct way of transferring carbon layers onto TEM grids

The most popular method for transferring carbon thin films onto TEM grids is floating them from mica. Despite the fact its principle is very simple, only the appropriate execution leads to the success. A mica sheet with a thin carbon layer inserted slowly into water under an angle of ~30° results in the carbon layer detaching from the support and floating on the water surface. This way the floating carbon layer can be easily deposited onto the TEM grids by either raising the TEM grids to the water surface bearing the carbon layer or slowly draining water and allowing carbon layer to set onto the TEM grids (depicted in figure 10). The vacuum used for the carbon layer preparation influences its mechanical stability. Films produced with the use of a low vacuum tend to break during floating, creating cracks across the grids. Such flaws might result in a disturbance of the sample preparation and imaging.

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Floating carbon film from mica sheet: TEM grids are placed on a floating paper, moved under the layer of floated carbon to have it set during slow draining of water.

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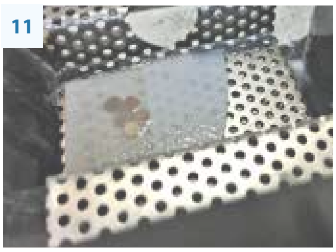

5nm carbon layer set on TEM grids

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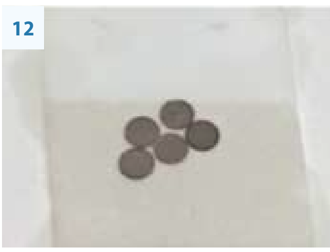

TEM grids covered with carbon floated from mica (5nm C deposited in vacuum of 7E-07mbar)

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TEM grids covered with 10nm C prepared in vacuum 4 E-05 mbar, visible cracks in carbon layers

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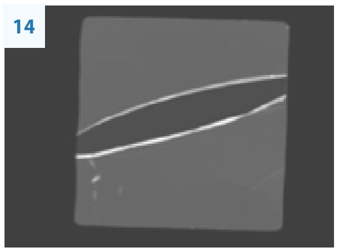

TEM image of 200 TEM grid mesh with 10nm C layer prepared with use of 4 E-05 mbar, visible crack across

4 Post-treatment vs mechanical and thermal stability

After the successful transfer of a carbon thin film onto the TEM grid, in the majority of cases, the post treatment of the carbon film is needed. Whilst glow discharge or UV treatment might not cause big damages to the carbon film, the thermal treatment – heating and/ or freezing tend to be challenging to the carbon film stability. This is especially the case when freezing and preparing samples for cryo-TEM, which tend to cause the greatest damage. It is good practice to check the coverage of the TEM grids before applying samples onto them. Such screening prevents using grids with flaws.

A wrinkled carbon layer or overlapped carbon layer is not ideal, as it causes uneven spread of the sample and influences the imaging contrast. The quality of produced carbon thin film for ambient and cryo-TEM is greatly influenced by the sample preparation steps taken. Dense, sturdy and pure films made with the Q150V Plus coater will ensure sample preparation is consistent and simple throughout the process - from transferring carbon onto TEM grids, post treatment, sample application and the final imaging steps.

Carbon thin film prepared under high vacuum using our Q150V Plus are dense, sturdy and do not crack when subjected to floating. Reproducible, even and good quality layers are a great help in sample preparation for cryo-TEM imaging. Fully covered grids with no flaws enable the end user to image precious structural biology samples without worrying about backing film quality.

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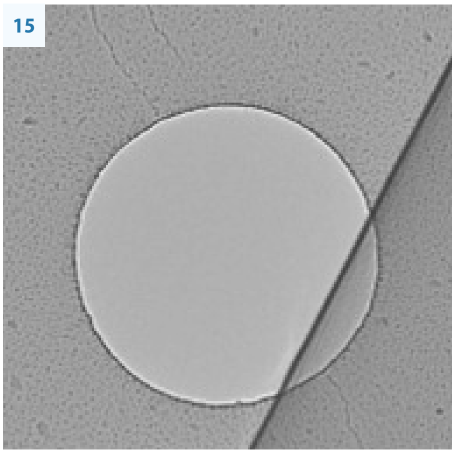

TEM grid covered with carbon 10nm C prepared with the use of a 4E-05 mbar vacuum. Visible crack in the layer and an overlapping.

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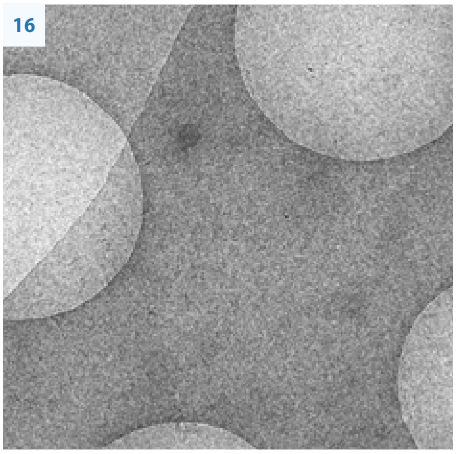

cryo- TEM image of DNA replication complex, TEM grid with holey carbon and 5nm carbon floated from mica, preparation vacuum of 7E-07mbar

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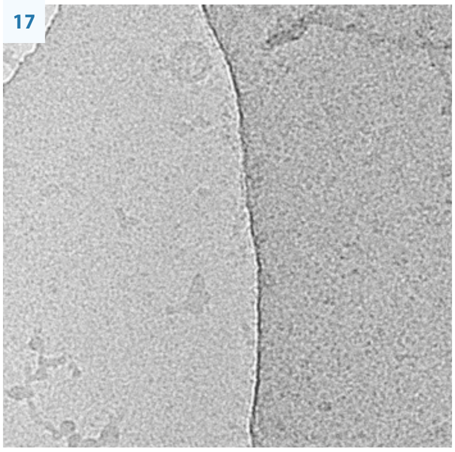

200m-in of figure 17, mag x 92k

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

The Q150V Plus is available in three configurations:

Q150V S PLUS

An automatic sputter coater for non-oxidising metals

Available sputtering targets including gold, gold-palladium and platinum.

Q150V E PLUS

An automatic carbon coater (rod/cord) for SEM applications

(E.g. EDS and WDS). Metal evaporation/aperture cleaning option available.

Q150V ES PLUS

A combined system with both sputtering and carbon coating

The deposition head inserts can be swapped in seconds. Metal evaporation/aperture cleaning option available.

The Q150V Plus is part of Quorum Technology's internationally acclaimed Q series of coaters, used by more than 1000 customers worldwide. Designed to provide high-quality coating solutions for SEM, TEM and thin-film applications, the Q series is versatile, affordable and easy to use.

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