Carbon thin films preparation for ambient and cryo-TEM grids

Application Note

Dr. Anna E Walkiewicz (2018)

The 2017 Nobel Prize in cryo-transmitted electron microscopy (TEM) highlighted the significance of direct observation of the molecular world of a 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 the carbon support film for sample application is essential.

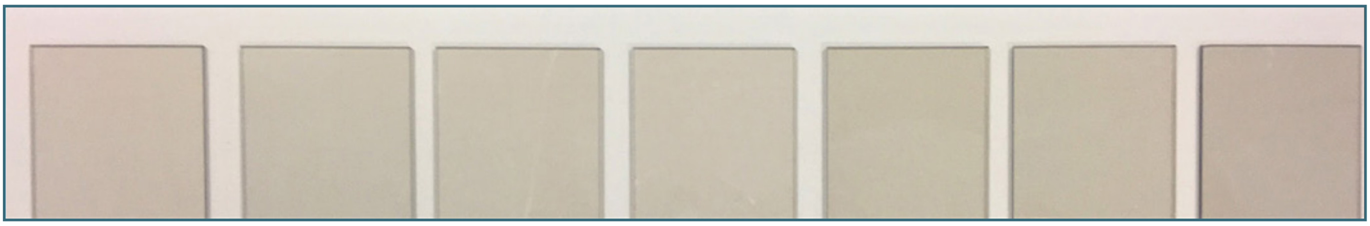

Fig. 1: Carbon evaporation on glass slides – representation of consecutive increase of layer thickness

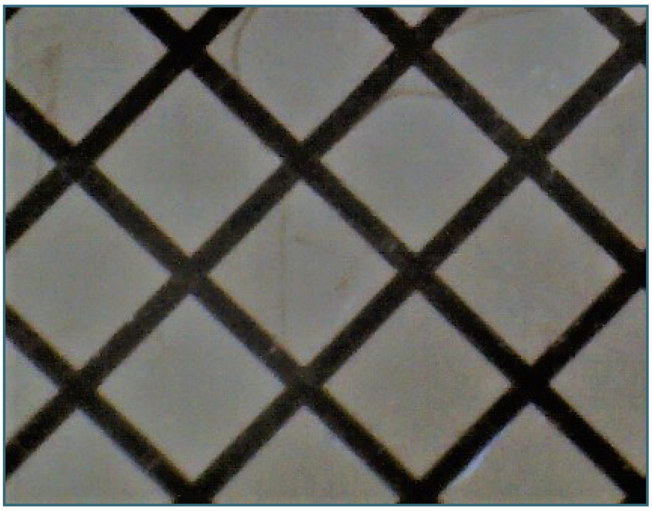

Fig. 2: 5 nm carbon layer set on 200 mesh TEM grid

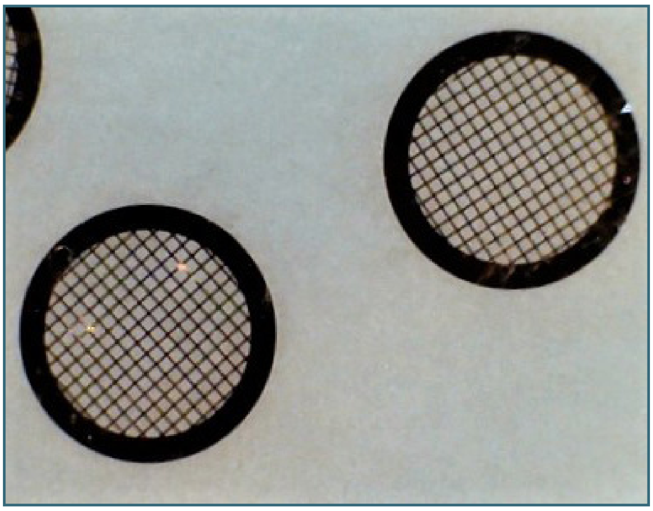

Fig. 3: TEM mesh grids with 5 nm carbon layer deposited by floating from mica

1. Why use carbon films?

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 variants – 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 the 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 prone to failure. The correct execution at each step is crucial, especially since the quality of the carbon film used for the preparation has tremendous influence on the final TEM image.

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Processes for consideration when preparing TEM grids with backing carbon films

2. Choosing the correct thickness

Choosing the correct thickness for the application influences contrast in imaging. Usually for structural biology samples a 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.

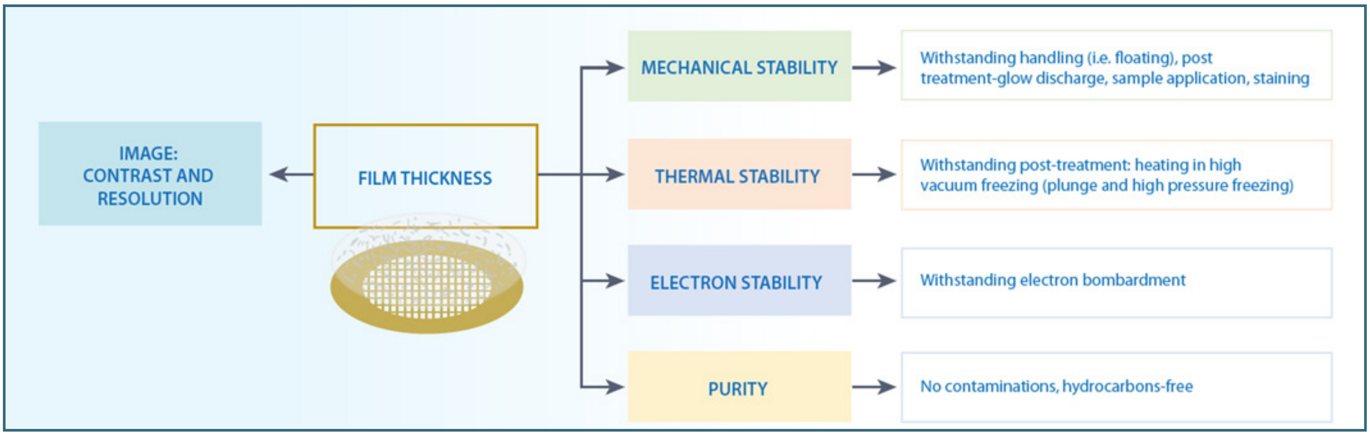

Fig. 4: The trade-off between carbon film thickness and stability

3. 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. They are 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 very small fraction of hydrocarbons, as their presence spoils imaging considerably. Such films can only be produced in high vacuum (10^{-6} mbar or better) with 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 and it 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 that possess excellent quality for use in a wide variety of EM applications.

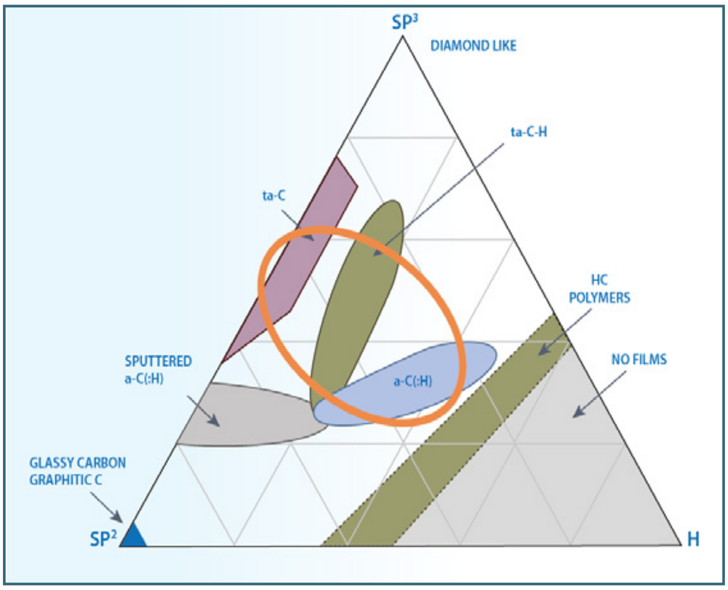

Fig. 5: Ternary phase diagram of bonding in amorphous carbon-hydrogen alloys. Diamond-like amorphous carbon. Circled constituents of carbon film produced in carbon thermal evaporation.

Robertson, J. (2002) Diamond-like carbon. Materials scienece and Engineering: R: Reports, 37, 129-281

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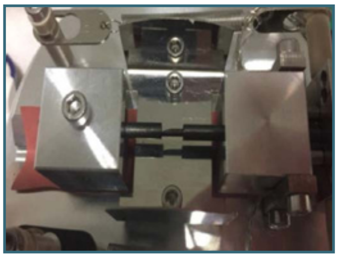

Fig. 6: Carbon thermal evaporation head

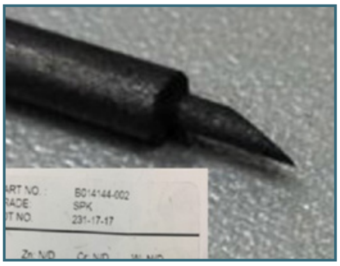

Fig. 7: Shaped carbon rod for thermal evaporation

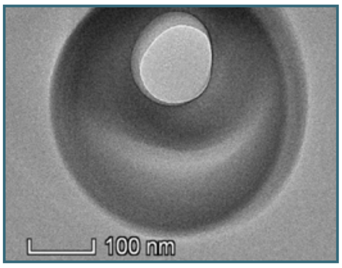

Fig. 8: TEM image of 200 mesh grid with no carbon layer

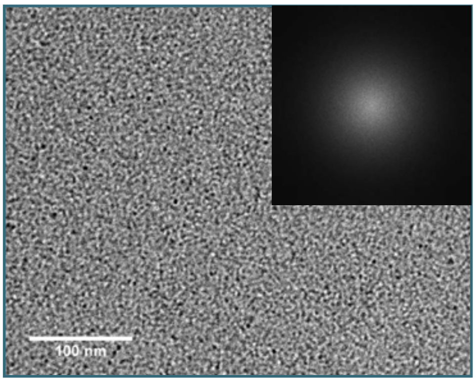

Fig. 9: TEM image of 5 nm C floated onto TEM grid produced invacuum $7x10^{-7}$ mbar (Q150V plus)

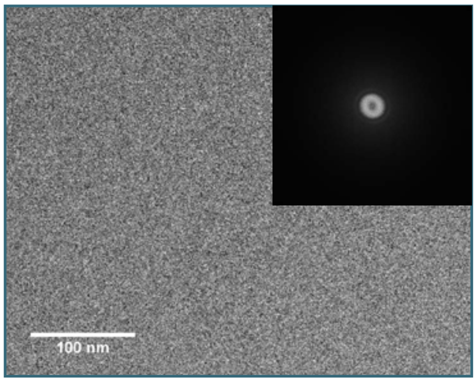

Fig. 10: TEM image of burn-out caused by the beam in 10 nm carbon layer (floated from mica) prepared in vacuum $4x10^{-5}$ mbar

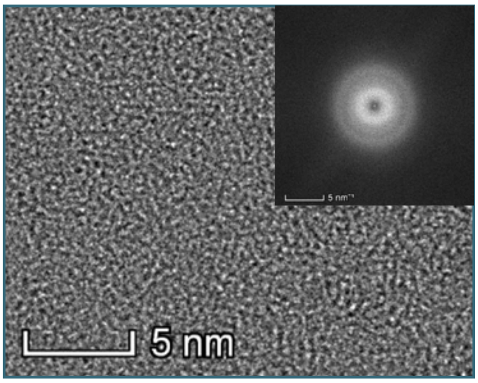

Fig. 11: TEM image of 10 nm C produced in vacuum of $8x10^{-7}$ mbar, floated from micacarbon layer

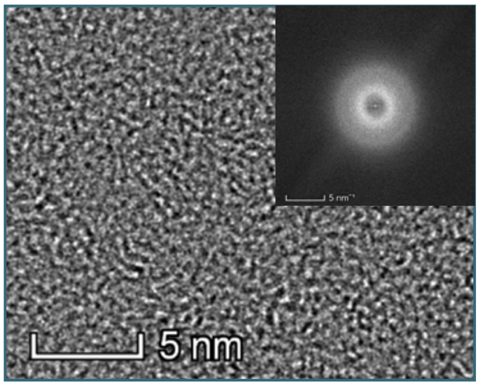

Fig. 12: TEM image of 10 nm C film produced in vacuum of $4x10^{-5}$ mbar, floated from mica

The most common and easiest 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 the vacuum, the denser and more sturdy the amorphous carbon layer will be with the least amount of impurities. Thin carbon layers produced with use of lower vacuum will not possess as great stability in the electron beam as depicted in figure 9.

4. 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 success.
A mica sheet with thin carbon layer inserted slowly into water under an angle of ~30° results in a carbon layer detaching from the support and floating on the water's surface. This way the floating carbon layer can be easily deposited onto TEM grids by either raising TEM grids to the water surface bearing carbon layer or slowly draining water and allowing the carbon layer to set onto TEM grids (as it is depicted in figure 13). The vacuum used for carbon layer preparation influences its mechanical stability. Films produced with the use of a low vacuum tend to brake during floating, creating cracks across the grids. Such flaws might result in a disturbance of the sample application and imaging.

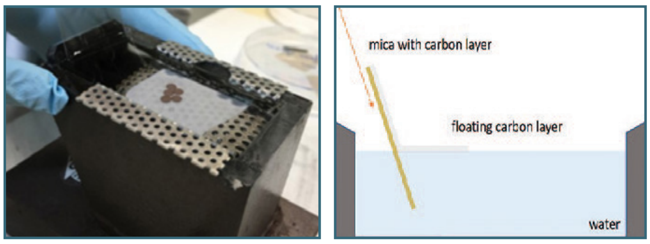

Fig. 13 a and b: Floating carbon film from mica sheet: TEM grids are placed on a bloating paper, moved under the layer of floated carbon to have it set during the slow draining of water

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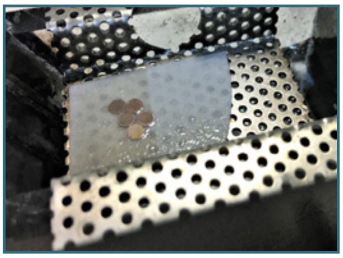

Fig. 14:5 nm carbon layer set on TEM grids

Fig. 15: TEM grids covered with carbon floated from mica (5 nm C deposited in a vacuum of $7x10^{-7}$mbar)

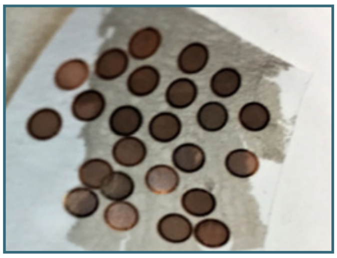

Fig. 16: TEM grids covered with 10 nm C prepared in vacuum $4x10^{-5}$ mbar, visible cracks in carbon layers

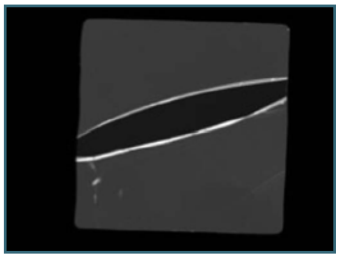

Fig. 17: TEM image of 200 TEM grid mesh with 10 nm C layer prepared with use of $4x10^{-5}$ mbar, visible crack across a grid box

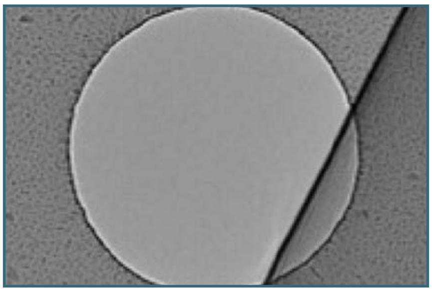

Fig. 18: TEM grid covered with carbon 10 nm C prepared with use of $4x10^{-5}$ mbar vacuum, visible crack in the layer and an overlapping

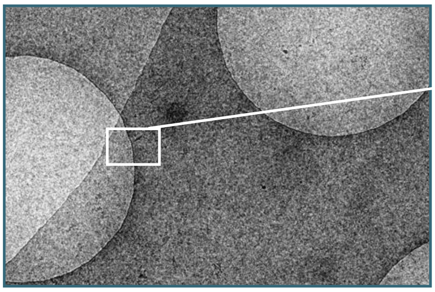

Fig. 19: Cryo-TEM image of DNA replication complex, TEM grid with holey carbon and 5 nm carbon floated from mica, preparation vacuum $7x10^{-7}$mbar

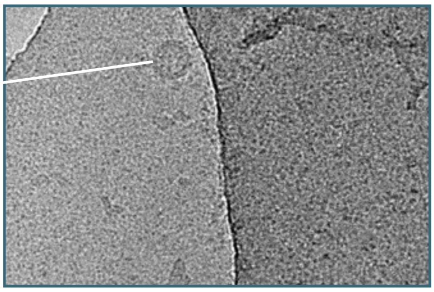

Fig. 20: Zoom in of Fig. 16

5. Post-treatment vs mechanical and thermal stability

After successful transfer of carbon thin film onto a 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 damage 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 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 films 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 make sample preparation much easier giving certainty and confidence along the process, from transferring carbon onto TEM grids, post treatment, sample application and finally imaging.

Carbon thin films prepared under high vacuum (Q150V Plus) are dense and 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.

6. 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.

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