EM RESOLUTIONS
Supporting Electron Microscopy

Graphene Oxide (GO) support films

Technical information

Graphene Oxide (GO) offers distinct benefits as a thin support film for TEM. The background contrast due to a monolayer of GO is less than half that of even ultra-thin (3nm) amorphous carbon supports, opening up new applications and increased resolution for TEM.

Graphene has been heralded as the latest wonder material and its potential as thin support films for TEM along with Graphene Oxide (GO) has been known for some time. However, whilst Graphene grown by Chemical Vapour Deposition (CVD) offers the possibility of a continuous monolayer film it has not gained wide acceptance as a TEM support film. The methods of production are technically complex and expensive for large scale manufacture of coated TEM grids.

GO offers a cost effective alternative to Graphene for routine TEM applications. GO monolayers are typically <1nm and ideal for viewing nanoparticles by TEM when a continuous support is necessary. GO films are almost transparent under the electron beam and not easily visible under an optical microscope. The differences in thickness bewteen GO and the adjacent carbon film can clearly be seen in Figure 1.

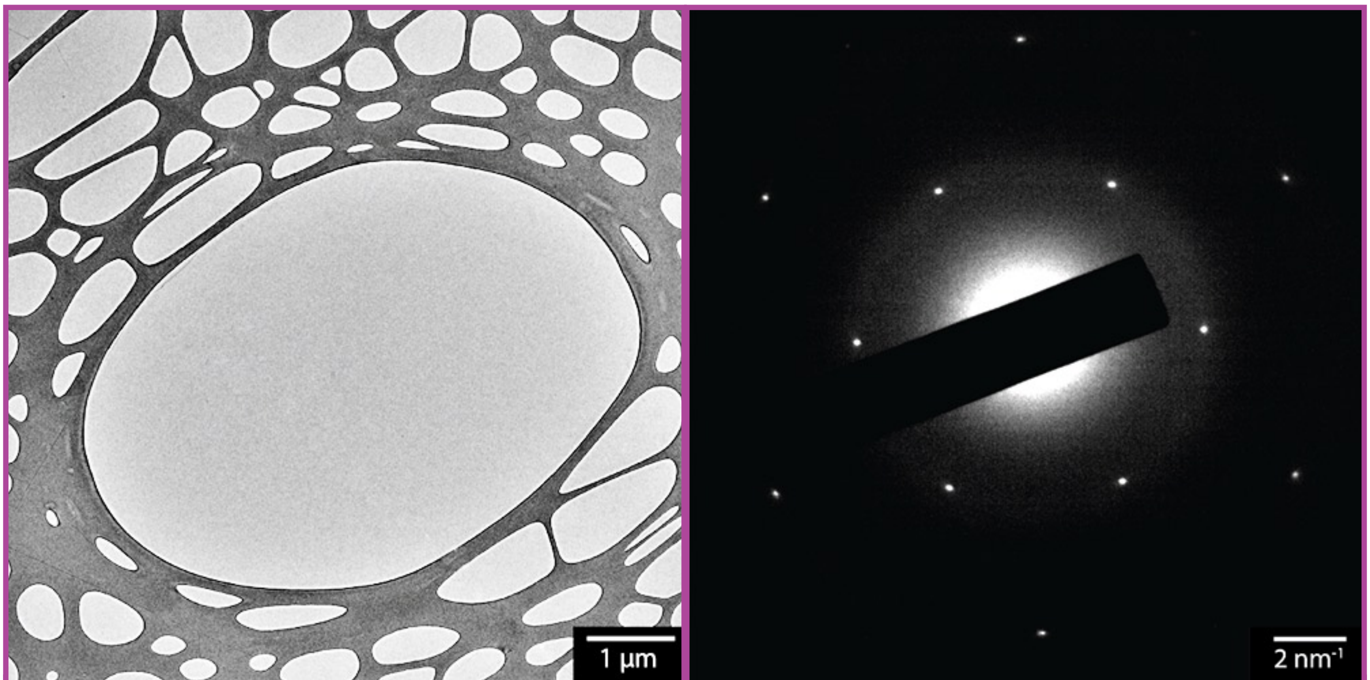


Figure 1. Single layer Graphene oxide is almost electron transparent and can prove difficult to 'see' in the TEM. The characteristic hexagonal diffraction pattern (here taken at the centre of the larger 'hole'), shows it is present across this holey carbon film.

Structure and composition of graphene oxide:

Graphene oxide is composed of a graphene like sheet randomly decorated with oxygen functional groups such as epoxy and hydroxyl. Due to its graphene-like carbon backbone, apparent in the characteristic diffraction pattern shown in figure 1, graphene oxide, even when only a monolayer thick, is strong enough to span the holes in a lacey carbon support (specially made for hole size) or Quantifoil.

Graphene oxide is synthesized from graphite powder using a modified Hummers' method. As produced, graphene oxide support films are hydrophilic. However, this can readily be changed by heating in air if a hydrophobic surface is required [1].

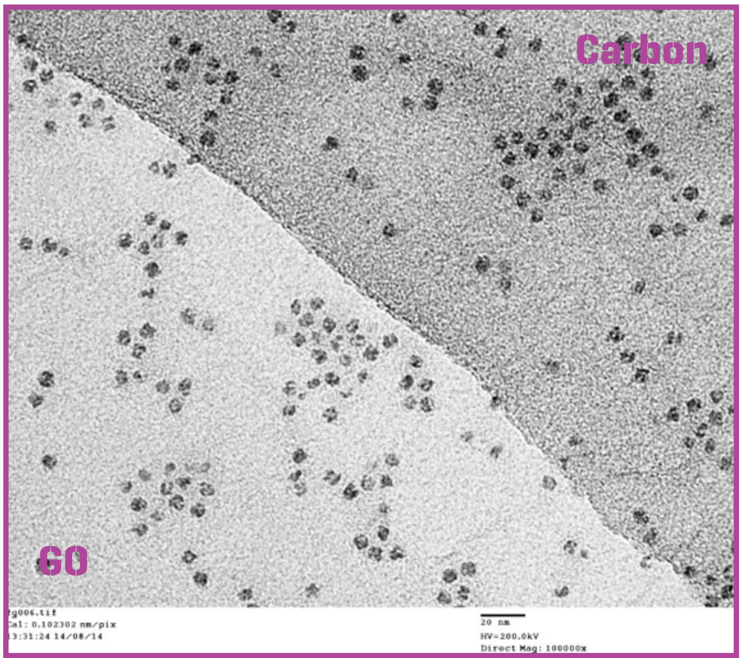


Figure 2. shows ferritin suspended on graphene oxide, a typical example of their use as a support film. The ferritin was deposited from aqueous solution by placing a drop on the graphene oxide coated TEM grid and allowing it to dry. (Courtesy Dr.J Skepper, University of Cambridge)

TEM applications for GO:

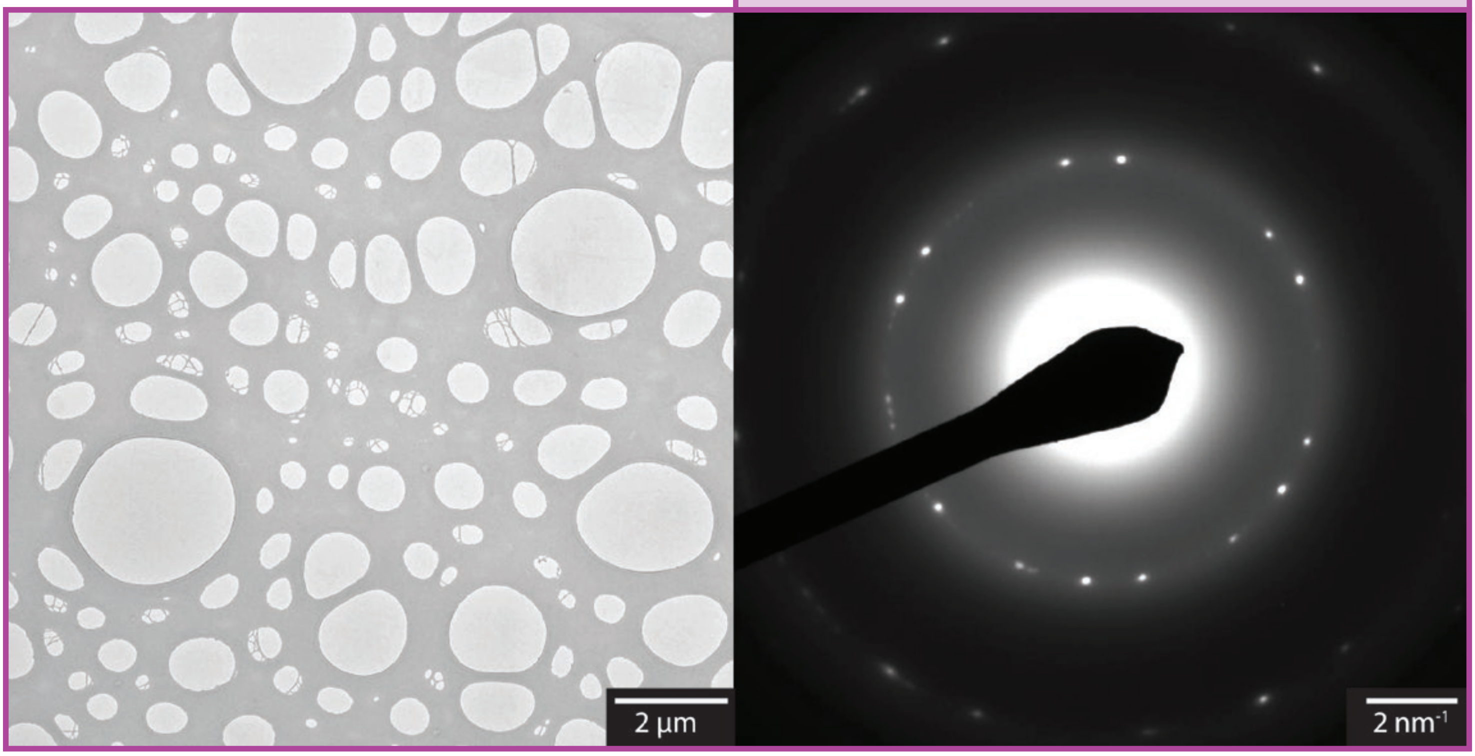
Graphene oxide (GO) membranes are robust, conductive and almost electron transparent when used as support films for TEM. The low atomic number and thin-layer thickness results in significantly lower background contrast than conventional supports. This ultra-low contrast makes them particularly useful for imaging small nanoparticles or nanowires whose structure is not readily resolvable on conventional carbon supports [2].

Being inherently hydrophilic to which macromolecules readily attach, GO support films can also be used for imaging and analysis of polymeric, macromolecular and biological samples without the need for heavy metal staining [1].

GO support films are particularly useful for the preparation of unstained, vitrified biological macromolecules analysed by Cryo-TEM[3] providing higher contrast and hence higher resolution for structural determination.

The crystalline nature of GO gives a characteristic diffraction pattern which can be used as a convenient calibration for the analysis of other samples either by high resolution TEM or electron diffraction [1,2].

Figure 3. Double layer GO provides a very thin support film for many applications. The diffraction shows a double hexagonal pattern indicating 2-layer GO with complete coverage of this area of the Holey carbon support



GO grid morphology:

Monolayer GO sheets need a sufficiently fine support so they can span the gaps. GO can be applied to various types of TEM support such as specially produced Holey or Lacey carbon films and Quantifoils (Figure 4). The latter is particularly useful for automated microscopy applications in structural biology.

The GO sheets are distributed across the EM grids such that some holes in the lacey carbon support are uncovered, some covered by a single monolayer of GO, and some by two or more layers of GO (Figure 5). On average, roughly 50-75% of the lacey carbon holes are spanned by graphene oxide and of those roughly 50% are monolayer.

GO support films are available from stock on Holey and Lacey carbon supports on 300 mesh hexagonal mesh copper grids. Other grid types such as Nickel and Gold and other mesh sizes are available on request.

GO is also available on R1.2/1.3, R2/2 and R2/4 Quantifoils on 200, 300 and 400 mesh copper grids. Other Quantifoil grid types such as Nickel and Gold are available on request.

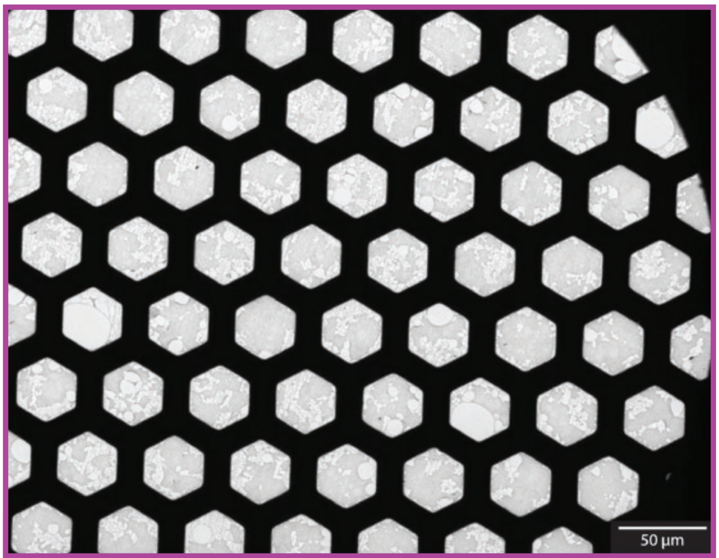


Figure 5. At large defocus, contrast of GO is increased. Here darker areas indicate the expanse of GO over the lacey carbon support film on a hexagonal mesh grid.

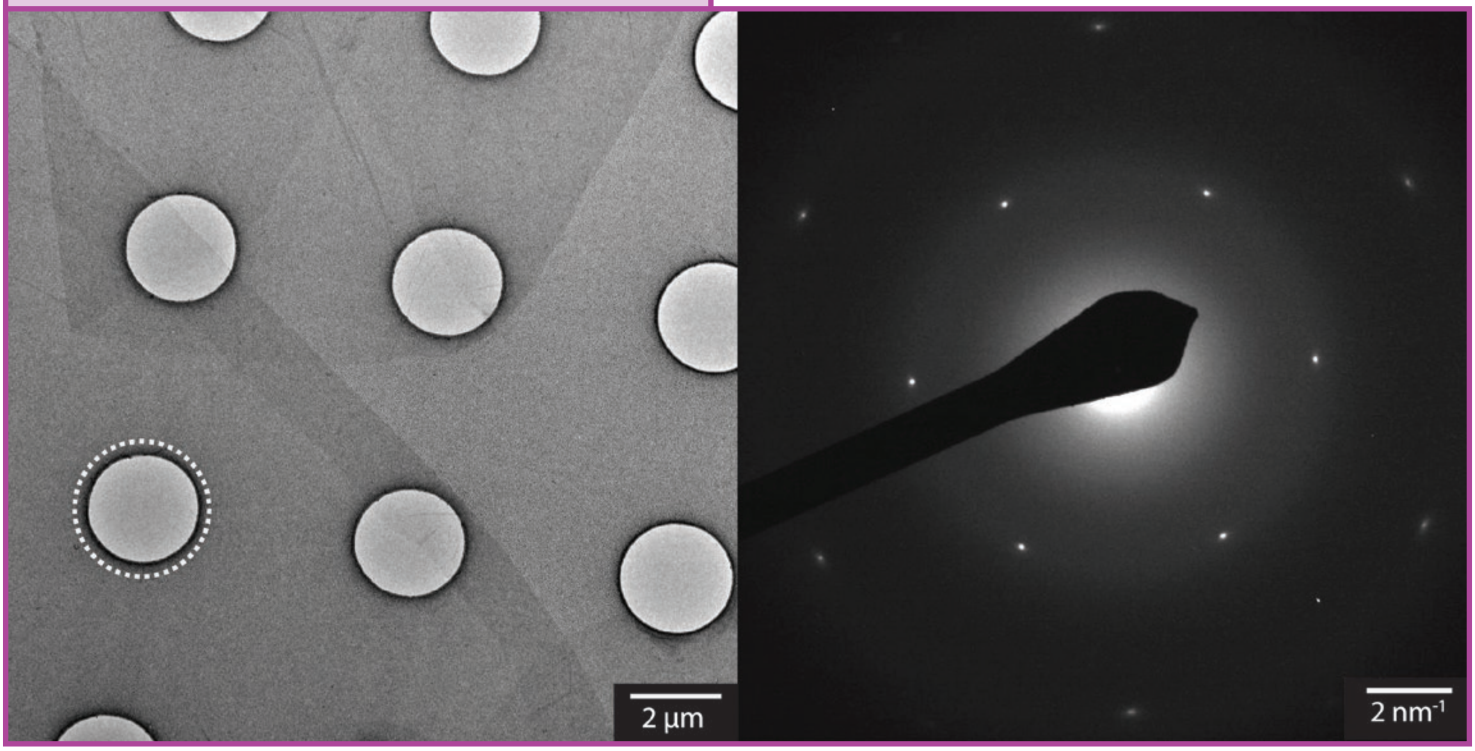
Assuring high quality GO support films:

The production of high quality GO support films requires regular batch checking using TEM, to ensure the correct coverage of monolayers.

Monolayer thick GO is so thin as to be almost transparent under the electron beam and is often only apparent through small wrinkles or creases in the film. Overlapping sheets are also readily visible. The most effective way of determining the distribution of monolayer thick regions is from the diffraction pattern: a single layer has a hexagonal diffraction pattern similar to graphene, whilst multiple layers, that are usually in different orientations, give a multiple rotationally misaligned hexagonal pattern [1,4]. It should be noted that sometimes this can also be caused by creases in a single sheet so some care must be taken with this interpretation.

During manufacture each batch is checked for the correct thickness using TEM and diffraction. Before packing a further quality check is made where each grid is checked with an optical microscope before being placed in a specially selected white grid box.

Figure 4. Monolayer GO covering a R2/4 Quantifoil as shown by the diffraction pattern. NB. One of the sheets has folded slightly.



EM Resolutions manufactures coated grids with different thicknesses of GO, on request, to best suit your application. When deciding which is best for you one needs to consider a compromise between grid coverage vs the number of layers (thickness of GO).

As part of our quality process we check each batch using TEM imaging and diffraction to characterise the grids.

We ensure that the GO covers a given % of holes in the support film using three key metrics:
(1) % of the area of the grid holes covered (coverage)
(2) the average number of GO layers across each hole
(3) what % of the covered holes are monolayer.

For good 'monolayer' coverage we would expect ~ 50-70% coverage of the grid area, of which the majority is monolayer (i.e. a total of >30% monolayer across the grid area). Depending on your application you need to decide if it is more important to have high coverage or a high monolayer fraction.

For good overall coverage of >80% coverage we would expect typically 2-3 layers of GO with < 20% as monolayer GO. These films are ideal for carbonaceous particles where carbon supports are not desirable.

EM Resolutions offers GO support films on a range of carefully selected coated grid types. We use specially made holey and lacey support films on hexagonal grids to ensure the most even coverage of GO that is suitable for your application.

References

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[4] Pandey, Priyanka A. (2012) Structure and applications of chemically modified graphene. PhD thesis, University of Warwick. http://webcat.warwick.ac.uk/record=b2654517~S1

Acknowledgements

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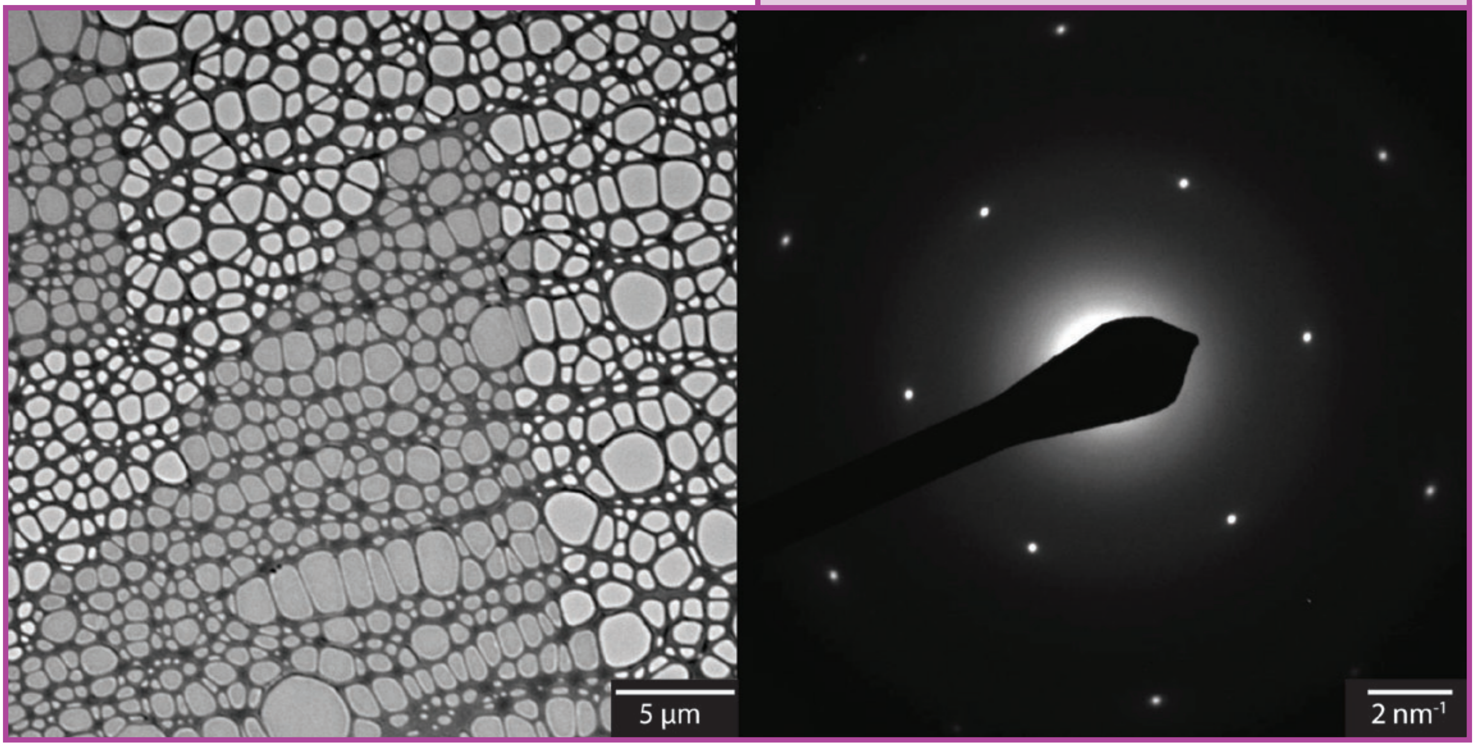
Figure 6. At large defocus, contrast of GO is increased. Here the darker area indicates monolayer sheets of GO over the lacey carbon.



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