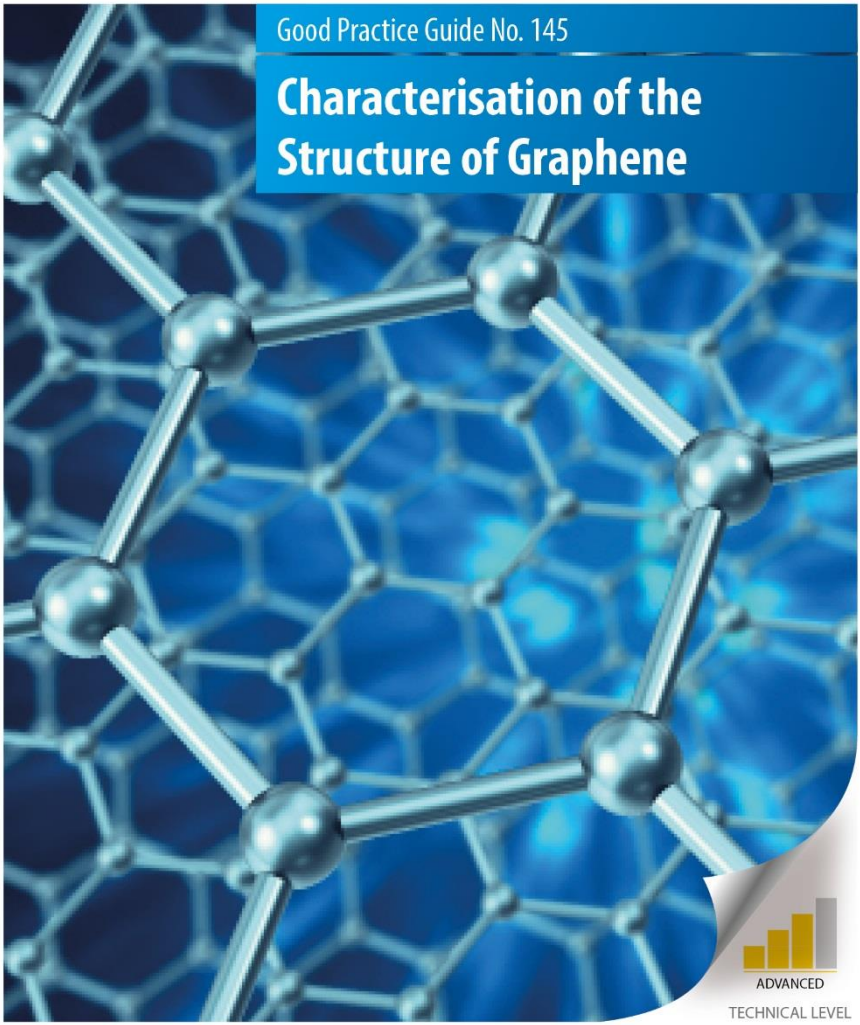
NPL

National Physical Laboratory

Good Practice Guide No. 145

Characterisation of the Structure of Graphene



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The National Graphene Institute (NGI) at the University of Manchester

The £61m National Graphene Institute (NGI) is the world's leading centre of graphene research and commercialisation. Opened in March 2015, it is not only home to graphene scientists from The University of Manchester but also from across the UK, partnering with leading commercial organisations interested in producing the applications of the future. Currently the NGI has more than 40 industrial partners, working on a range of applications across a wide variety of disciplines. With 7,800 square metres of collaborative research facilities and 1,500 square metres of cleanroom space, the NGI is the largest academic space of its kind in the world for dedicated graphene research. The NGI is funded by £38m from the Engineering and Physical Sciences Research Council (EPSRC) and £23m from the European Regional Development Fund.

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Guide information

What is it about?

This guide provides protocols for determining the structural properties of graphene in two different forms, either a CVD-grown graphene sheet on a substrate, or graphene flakes present in either a liquid dispersion or powder form. It describes how to assess what measurements are required initially, depending on the type of sample, and includes decision trees and flow diagrams to aid the user. This guide describes a select range of advanced techniques for the accurate determination of i) the substrate coverage of a CVD-grown graphene sheet, ii) the number of layers/thickness of the graphene, iii) the lateral dimensions of flakes, iv) layer alignment and v) the level of disorder. Details on how to prepare the samples, measurement issues, sources of uncertainty and how to analyse data are included.

Who is it for?

The guide is for producers and users of graphene who need to understand how to measure the structural properties of graphene. Such information is essential in a host of technology application areas where graphene may be used.

What is its purpose?

This guide provides a detailed description of how to determine the key structural properties of graphene, so that the graphene community can adopt a common, metrological approach that allows the comparison of commercially available graphene materials. This guide brings together the accepted metrology in this area. It describes the high-accuracy and precision required for verification of material properties and enables the development of other faster quality control techniques in the future. The guide is intended to form a bedrock for future interlaboratory comparisons and international standards.

What is the pre-required knowledge?

The guide is for users in research and industry who have experience with and access to the advanced techniques described herein. It is targeted at analytical scientists and professionals who have a Bachelor’s degree in science.

Foreword

There are many exciting applications using graphene that I get to see being developed every day at the University of Manchester and from around the world, both in academia and industry. This may be light-emitting diodes that use the thermal properties of graphene to make extremely energy efficient light bulbs, cheap water purification membranes utilising films of graphene oxide or advanced nanocomposites containing graphene in the panels of high-performance cars. Graphene is set to improve the quality of life of many people in different ways, across the globe.

However, at the same time I also see that there are barriers to commercialisation that are impeding the progress of graphene-enabled products, which need to be overcome. One of these crucial barriers is answering the question “What is my material?”. I have heard many stories of companies trying to use graphene, to find what they have received is not really graphene at all. Similarly, we cannot develop innovative products when we do not know why different initial materials are leading to either positive or negative outcomes. Therefore, the actual material properties of the graphene supplied must be well-characterised. Furthermore, without a standardised way of measuring the properties of graphene that the whole industry can follow, end-users cannot reliably compare material data sheets for the numerous types of ‘graphene’ material that are now commercially available.

To this end, this good practice guide has been developed by the NGI and NPL teams to allow the nascent graphene industry to perform accurate, reproducible and comparable measurements of commercially supplied graphene. This will address this important commercialisation barrier by providing users with a consistent approach to the structural characterisation of graphene whilst international measurement standards are being developed. People will ultimately be able to say “These are the properties of my material” and better understand how to create value from commercialisation of any new product or application.

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Chapter 1: Overview of graphene

- Introduction
- Scope
- Key principles of measurement
- Measurement and terminology standardisation
- Determination of material type

1.1 Introduction

Due to the many superlative properties of graphene and related 2D materials, there are many application areas where these exciting nanomaterials may be disruptive, areas such as flexible electronics, nanocomposites, sensing, filtration membranes and energy storage. Applying metrology to graphene is now vital to enable the emerging global graphene industry to flourish and bridge the gap between academia and industry. A major barrier is the inability to accurately measure or describe the material properties of the many different real-world materials now available commercially. Without this ability, the potential of graphene can never be realised. End-users of these materials must be able to rely on the advertised properties of the commercial graphene on the market, instilling trust and allowing worldwide trade in this fledgling industry. Ultimately, the performance of graphene-enabled products cannot be improved if the material properties of the graphene used are not first understood. Reliable and repeatable measurement protocols are required to address this challenge.

1.2 Scope

This guide describes the best practice for characterising the structural properties of graphene using a series of measurement techniques. Measurement protocols are detailed for both CVD-grown graphene on a substrate and graphene flakes present in a dispersion or powder form. Flowcharts illustrating the measurement technique combinations and the decision-making process are also included. The different sample preparation protocols necessary before the measurements are undertaken are described, as well as the process of data analysis. The physical properties such as the number of layers/thickness, lateral flake size, the level of disorder, layer alignment, as well as the distribution and relationship of these properties, are determined through these protocols. The method of reporting these important properties is also described. It should be noted that this guide only describes CVD-grown graphene provided on a copper substrate or a silicon substrate with an oxide layer (Si/SiO_2). It does not cover graphene provided on other types of substrate, such as nickel or silicon carbide. The chemical characterisation of graphene is not included in this guide and therefore the protocols are intended for material that primarily contains sp^2-hybridised carbon with no significant chemical functionalisation.

1.3 Key principles of measurement

Before undertaking extensive characterisation of materials such as graphene, it should be noted that without an understanding of the uncertainty associated with a measurement, the measurement cannot be truly understood or compared. As the comparison of commercial sources of graphene is a key objective of this guide, to instil confidence in end-users of

graphene who will develop real-world products in the future, the uncertainty associated with any measurement must be addressed.

To this end, the fundamentals of evaluating uncertainty can be found in detail in the NPL Good Practice Guide No. 11 'A Beginner's Guide to Uncertainty in Measurement' [1], and should be referred to for users not confident in this type of analysis.

1.4 Measurement and terminology standardisation

There are many international ISO (International Organization for Standardization) standards published in the area of measurement and characterisation of nanomaterials, particularly referring to some of the techniques detailed here. For further information the ISO/TC 229 'Nanotechnologies', ISO/TC24/SC4 'Particle characterization' and ISO/TC 201 'Surface Chemical Analysis' websites should be examined, which list both the published and under-development ISO standards in this area.

The terms and definitions from 'ISO TS 80004-13: Nanotechnologies -- Vocabulary -- Part 13: Graphene and related two-dimensional (2D) materials' apply here and should be referred to [2]. Several of these terms and definitions are shown below:

Graphene; graphene layer; single layer graphene; monolayer graphene

Single layer of carbon atoms with each atom bound to three neighbours in a honeycomb structure

Note 1 to entry: It is an important building block of many carbon nano-objects.

Note 2 to entry: As graphene is a single layer, it is also sometimes called monolayer graphene or single layer graphene and abbreviated as 1LG to distinguish it from bilayer graphene (2LG) and few-layered graphene (FLG).

Note 3 to entry: Graphene has edges and can have defects and grain boundaries where the bonding is disrupted.

Bilayer graphene (2LG)

Two-dimensional material consisting of two well-defined stacked graphene layers

Note 1 to entry: If the stacking registry is known, it can be specified separately, for example, as "Bernal stacked bilayer graphene".

Few-layer graphene (FLG)

Two-dimensional material consisting of three to ten well-defined stacked graphene layers

Hence, graphene, bilayer graphene and few-layer graphene are the materials that this guide will focus on. However, it is well understood that for commercial powder and dispersion samples that contain graphene, there may also be thicker flakes of graphite, with nanoscale dimensions, present. In fact this is one of the key reasons why protocols for characterising the properties of real-world 'graphene' samples is required.

1.5 Determination of material type

There are many different types of 'graphene' commercially available and it is therefore important to firstly describe the type of material. Figure 1 shows that commercial material can be broadly separated into at least two classes, 'CVD-grown graphene' and 'graphene flakes'. The structural characterisation of these two classes are addressed within this guide in Chapters 2 and 3 respectively.

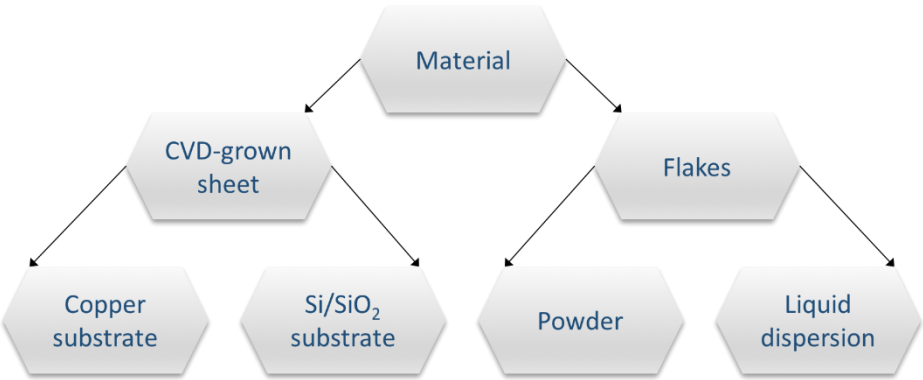

Figure 1. *Different classes of commercial material described as 'graphene' that can be divided into two classes and further separated into sub-classes.*

A chemical vapour deposition (CVD) grown graphene sheet is assumed to be one continuous, typically polycrystalline, sheet of graphene (1LG), bilayer graphene (2LG) or few-layer graphene (FLG) on a substrate. Meanwhile, powder or liquid dispersions that contain graphene flakes typically also contain flakes of different thicknesses and lateral sizes, depending on the process conditions used for exfoliation from graphite, or other processes.

Other classes or sub-classes exist for graphene sheets that are not detailed here, such as growth of a graphene sheet on a silicon carbide substrate, known as 'epitaxial graphene' [2]. Similarly, although CVD-grown graphene is described in this guide, only the most common case of CVD-grown graphene on copper is included. There are many different metal catalysts that can be used to produce CVD-grown graphene, and this guide can still be used to characterise

CVD-grown graphene not grown on copper, but has been transferred to a Si/SiO_2 substrate or a transmission electron microscopy (TEM) support grid.

Note, that this guide does not address the chemical characterisation of graphene, therefore functionalised graphene, graphene oxide derivatives and similar materials will require separate chemical characterisation as well as structural characterisation. Furthermore, the protocols detailed in this guide for techniques such as Raman spectroscopy and TEM will not provide accurate values for functionalised graphene material. For more details, please refer to Chapter 3.

Graphene, as a single layer, has every atom at the surface, therefore the storage conditions of these materials is particularly important to avoid changes due to damage or contamination. Samples that require characterisation must be stored in a controlled environment to avoid changes in the material and thus their properties. In each case, the conditions and time period of storage between production and characterisation should be recorded.

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Chapter 2: CVD-grown graphene on a substrate

- Sample transfer
- Optical characterisation of graphene coverage
- Raman spectroscopy of CVD-grown graphene
- TEM imaging of CVD-grown graphene

The flow diagram in Figure 2 should be followed to identify the techniques required to measure the structural properties of a CVD-grown graphene sheet, on a copper or Si/SiO_2 substrate, and the order in which analysis should be performed.

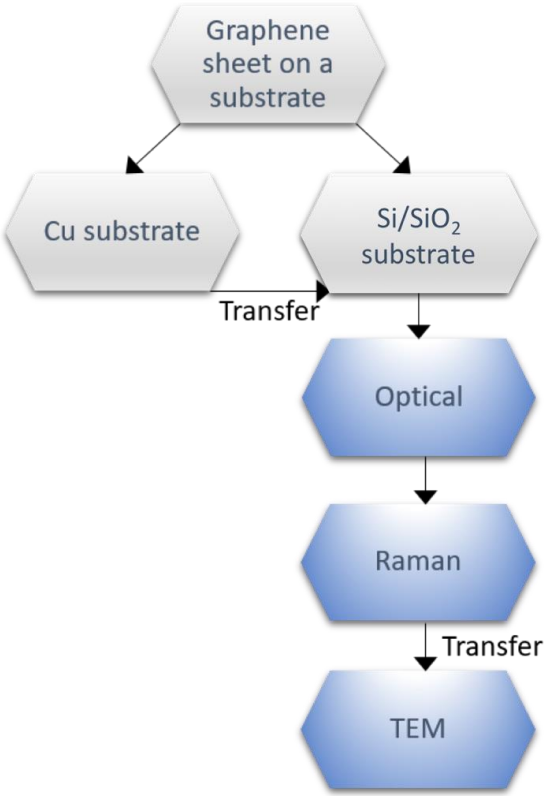

Figure 2. *Order and process of measurement techniques used to determine the structural properties of a CVD-grown graphene sheet on a substrate.*

The flow diagram in Figure 2 should be followed to identify the techniques required to measure the structural properties of a CVD-grown graphene sheet, on a copper or Si/SiO_2 substrate, and the order in which analysis should be performed. Firstly, the type of substrate must be determined, that is, whether it is a CVD-grown graphene sheet still on the copper catalyst, or whether the sheet has been transferred to a Si/SiO_2 substrate. The substrate that the graphene sheet is on will determine the path of sample preparation.

If the graphene sheet is on a copper substrate it should be removed from the copper (Section 2.1.1) and transferred to a Si/SiO_2 substrate, as detailed in Sections 2.1.3 and 2.1.4, for optical

characterisation of the coverage of the sheet, as described in Section 2.2. If the graphene sheet is already on a Si/SiO_2 substrate, this transfer step is not required. Then the sheet should be characterised with Raman spectroscopy on the Si/SiO_2 substrate (Section 2.3). This provides a microscale determination of the coverage of the substrate, that is, if there are microscale gaps in coverage, the percentage of coverage of single layer graphene, and the level of disorder. Next, a sample of the sheet should be transferred to a TEM grid (Sections 2.1.2 and 2.1.4) for further measurements to confirm the number of layers and level of disorder observed using Raman spectroscopy, as well as determine the layer alignment. This is described in Section 2.4.

2.1 Sample transfer

2.1.1 Removal from Cu substrate

In the case of CVD-grown graphene on Cu, the graphene growth takes place on both sides of the metal substrate. It is therefore necessary to remove the graphene from one side, so that only one CVD-grown graphene sheet is transferred to another substrate.

1. The side being prepared for transfer should be covered with a ~300 nm layer of poly-methyl-methacrylate (PMMA) using a spin coating system.
    a. This is in order to protect and support the underlying graphene during the transfer process.
    b. As an example, a ~300 nm layer of PMMA can be produced at 3000 rpm for 1 minute when using 950,000 molecular weight PMMA dissolved in Anisole at 4 % dilution.
2. The other side should be exposed to ~2.7 Pa (20 mTorr) of oxygen plasma at 50 W for 3 mins.
3. To minimize the contamination left on the graphene, a 0.1 mol L-1 ammonium persulphate ($(NH_4)_2S_2O_8$) solution should be used as an etchant to remove the copper foil, through a slow redox reaction over ~1 hour. The separation of the graphene film from the copper substrate is achieved through completely dissolving the copper, at which point the graphene layer attached to the PMMA film (PMMA/graphene) will be floating at the surface of the solution, with the graphene layer on the underside of the PMMA.
4. After the etching procedure, the PMMA/graphene sample should be immersed in deionised (DI) water for 1 hour or more, followed by transfer to fresh DI water for further immersion for 1 hour or more, to remove any further contaminants.

2.1.2 Removal from Si/SiO_2 substrate

For TEM imaging the CVD-grown graphene sheet will typically have to be transferred from a Si/SiO_2 substrate to a TEM grid.

1. The side being prepared for transfer should be covered with a ~300 nm layer of poly-methyl-methacrylate (PMMA) using a spin coating system.
    a. This is in order to protect the underlying graphene and to ease the transfer process.
    b. As an example, a ~300 nm layer of PMMA can be produced at 3000 rpm for 1 minute when using 950,000 molecular weight PMMA dissolved in Anisole at 4 % dilution.
2. The sample should then be baked at 130 °C for 5 mins.
3. The topmost layer of the Si/SiO_2 substrate should then be etched away using a 3 % potassium hydroxide (KOH) solution for ~6 hours at room temperature, so the PMMA/graphene film can be detached from the substrate in Step 4.
4. Before the PMMA/graphene film has fully detached from the substrate, the Si/SiO_2 wafer should be immersed into a DI water bath, to fully detach the PMMA/graphene and to remove chemical residues from the etchant and of possible contamination from the Si/SiO_2 wafer. The PMMA/graphene film will then float in the DI water.
5. The PMMA/graphene film should then be immersed in deionised (DI) water for 1 hour or more, followed by transfer to fresh DI water for further immersion for 1 hour or more, to ensure the best sample cleanliness.

2.1.3 Si/SiO_2 substrate preparation

1. Cut a substrate from a Si/SiO_2 wafer (90 nm or 290 nm oxide thickness [3]) as required in terms of lateral size, using a diamond scribe.
    a. These particular thicknesses of oxide are used due to the favourable optical conditions making graphene easily visible optically.
2. Sonicate the substrate in acetone for 5 mins, followed by a sonication step in DI water for 5 mins and finally in isopropanol (IPA) for 5 mins, without drying the substrate between solvents.
3. Dry the substrate using a pressurised source of N_2 or Ar.
4. Inspect the substrate using optical microscopy to determine if any particles (≥500 nm) or solvent residue (observed as a different colour than the substrate) is present.

a. If contamination is present then repeat the cleaning process in Steps 2 and 3.

2.1.4 Transfer to a Si/SiO_2 substrate or a TEM grid

After the PMMA/graphene has been removed from the copper or Si/SiO_2 substrate, it can then be transferred onto either a Si/SiO_2 substrate for optical microscopy (i.e. graphene was removed from copper) or a holey carbon TEM support grid (i.e. graphene was removed from Si/SiO_2), for analysis.

1. The PMMA/graphene layer should be removed from the deionised (DI) water bath by scooping it out with the desired substrate, held with tweezers.
    a. TEM support grids consist of holes in an amorphous carbon film supported on a metal (Cu or Au) mesh, typically with an overall diameter of 3 mm. TEM grids should be cleaned carefully prior to contact with graphene, by washing in IPA and vacuum baking the empty grids at ~120 °C for at least 30 minutes.
    b. Preparation of the Si/SiO_2 substrate is described in Section 2.1.3.
    c. The graphene should be on the underside of the PMMA film unless the vertical orientation of the film has changed during the washing steps described in Sections 2.1.1 or 2.1.2.
    d. The carbon side of the TEM support grid should be brought into contact with the graphene side of the PMMA/graphene to improve the likelihood of adhesion.
        i. The field of view in the TEM is limited so it is important to ensure that the graphene region of interest is transferred within the central region of the support grid, typically >1 mm from the edge, as shown in Figure 3.
    e. Care should be taken to prevent the graphene layer folding during transfer.
2. The sample should be dried at 50 °C for 10 mins.
3. The sample should then be baked for 10 mins at 120 °C in clean room conditions.
    a. This will improve the adhesion of the sheet on the substrate/grid, remove the remaining water content, and importantly, flatten (anneal) possible wrinkles, which may have been created during the transfer.
4. The PMMA protective layer should then be dissolved by immersing the sample into acetone for 15 mins.
5. After removing the PMMA the sample should be dried in a critical point drier (CPD).

a. This is to protect the delicate, CVD-grown graphene against any rupture, damage or deformation due to surface tension. For the case of a TEM grid, a CPD prevents the volume change on evaporation from damaging the graphene membrane.

b. A CO_2 medium should be used for the drying process due to its low critical temperature of only 31 °C and low pressure 7.39 MPa, compared to 374 °C and 22.064 MPa for water.

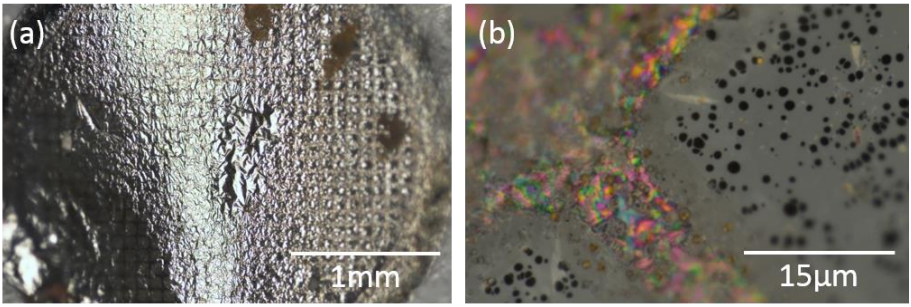

Figure 3: *Optical microscopy images of CVD-grown graphene transferred on to a holey carbon TEM support grid.*

2.2 Optical characterisation of graphene coverage

Optical microscopy is a widely used and simple technique for the structural characterisation of graphene and related 2D materials. This technique is the initial post-transfer material characterisation step, as shown in Figure 2, as the optical images produced are important for understanding the substrate coverage of the CVD-grown graphene sheet, through direct visual observation.

The presence of graphene can be identified on certain substrates because graphene is sufficiently transparent to add to an optical path, which changes its interference colour with respect to a clean wafer [3]. For a certain thickness of Si/SiO_2, even single layer graphene has been found to give sufficient, albeit slight, contrast to allow identification of the graphene coverage of the substrate. To maximise the contrast observed through optical microscopy a silicon wafer with a ~90 nm or ~290 nm silicon oxide layer (Si/SiO_2) [3] should be used.

Note that for CVD-grown graphene with more than one layer thickness, the layers typically have turbostratic alignment and thus the contrast observed may vary between different areas of >1 layer thickness. This is due to overlapping between the Dirac cones, which generates Van Hove singularities in the density of states [4]. However, it is still possible to determine the coverage of the graphene sheet on the substrate versus the percentage of substrate that does not have any graphene present.

2.2.1 Measurement protocol

1. Place the sample on the optical microscope stage.
2. An objective lens with a magnification of 20× should be used to determine the coverage of the graphene layer.
3. The number of measurements and measurement positions should be selected as described in Figure 4 or Figure 5 depending on whether the substrate is circular or square/rectangular in nature and the size of the substrate.
    a. For the purposes of determining the coverage of the substrate with graphene, measurement positions are relative to the substrate, rather than the graphene sheet itself.
    b. This allows a good representative set of measurements, whilst also minimising the number of measurements.
    c. For samples where $x/4 < 12$ mm, where x is the size of the sample, the number of measurement positions must be reduced from those in Figure 4 or Figure 5, so that the distance between measurements is at least 6 mm in each case.
    d. For samples where $x < 12$ mm, one measurement should be taken at the centre of the sample and one measurement should be taken near the edge of the sample.
    e. Measurement positions should be recorded.
4. The optical images should be captured at each of the positions.
5. The data should then be analysed to determine the percentage coverage of graphene for each image.

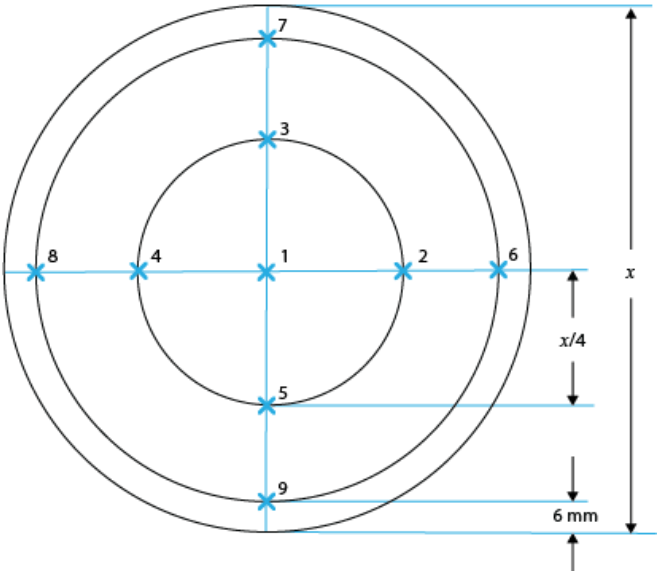

Figure 4: *Schematic of measurement positions for a circular substrate.*

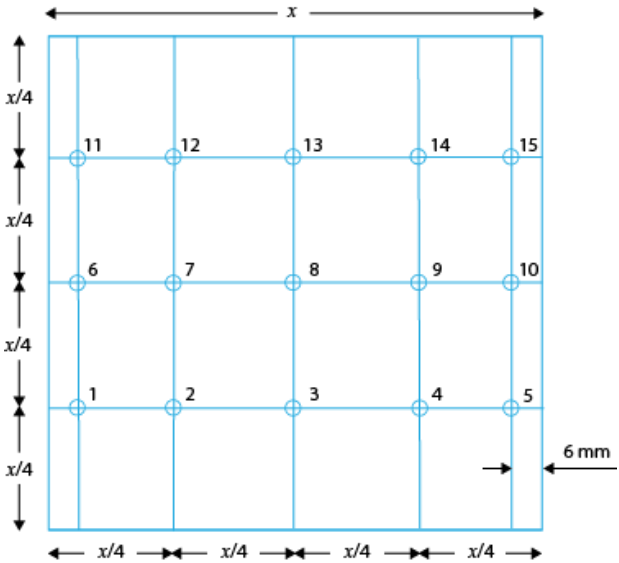

Figure 5: *Schematic of measurement positions for a square substrate.*

2.2.2 Data analysis

Using the variation of contrast observed in the optical images taken, the percentage of the substrate surface should be calculated for each of the three conditions below:

- No graphene coverage.
- Area of single layer graphene coverage.
- Area of more than 1 layer of graphene coverage.

Note that if there are areas where the graphene coverage cannot be determined, such as for areas with polymer residue present, these areas should be ignored for the percentage coverage calculations. If the graphene coverage is discontinuous then it should be specified whether the graphene constitutes islands or contains holes (see Figure 6) or a mixture of both, following the example in Figure 7:

- If >90 % of graphene domains are joined to other domains the film contains holes.
- If 50-90 % of graphene domains are joined to other domains the film contains a mixture of holes and islands.
- If < 50 % of graphene domains are joined to other domains the graphene is formed of islands.

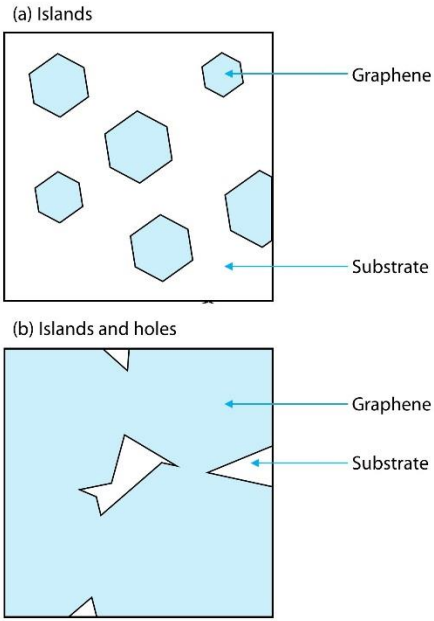

Figure 6: *Schematic of (a) islands vs. (b) hole coverage for CVD-grown graphene on a substrate.*

Number of graphene layers	Area (%)
1 layer	
>1 layer	
No graphene present	
Type of coverage	
Islands or holes?	

Figure 7: *Example reporting scheme for characterisation of CVD-grown graphene on a Si/SiO_2 substrate.*

2.3 Raman spectroscopy of CVD-grown graphene

In Raman spectroscopy and microscopy, a laser is used to induce Raman scattering within a material so that a spectrometer collects the scattered light and can determine the subsequent Raman shift (cm^{-1}). This technique is particularly powerful for studying carbon nanostructures such as graphene. For CVD-grown graphene, the coverage of graphene, number of layers and level of disorder can be determined.

Raman spectroscopy should be performed in a 180° backscattering geometry to provide the best signal-to-noise ratio, with a 100× objective lens (NA = 0.9). A full spectral calibration of the system should be regularly performed using a neon lamp (or another gas lamp with traceable features), and an auto-calibration should be performed daily before use, using for example the 1st order Si peak, for each laser excitation wavelength and grating. All raw data must be corrected in terms of spectral position accordingly. All measurements should be taken at a room temperature of 21 ± 2° C and the humidity should be recorded.

A green laser with a wavelength of 532 nm (2.33 eV) should be used for graphene on a Si/SiO_2 substrate. It should be noted that Raman spectroscopy measurements using different laser wavelengths cannot be directly compared for graphene [5-7] and so the wavelength of the laser must be recorded along with the Raman spectra.

To avoid damaging the graphene material itself through localised heating effects [8, 9] a total laser spot power (incident on the sample) of less than 1 mW must be used for a laser spot diameter of 500 nm or greater and acquisition times of less than 30 seconds for each measurement position. The laser spot power should be measured using a calibrated power

meter and the laser spot diameter should be measured using the edge of a graphene flake/sheet [10] or ideally a carbon nanotube sample [11].

The spectral range should be chosen such that the relevant Raman lines (D-line (~1350 cm^{-1}), G-line (~1580 cm^{-1}), 2D-line (~2700 cm^{-1})) and associated widths are included, either in the same spectrum or in two separate spectra. Note that the 2D-peak may also be referred to as the G'-peak elsewhere in the scientific literature. A suitable grating must be used that achieves a spectral resolution of ≤3 cm^{-1}. Typically a Raman spectrum is obtained for the range 1150-3100 cm^{-1}, in particular when measuring on a silicon substrate, as this does not include the second order silicon peak at ~1000 cm^{-1}. However, note that in the most sensitive spectrometers, the third order of the silicon peak at ~1450 cm^{-1} can be observed. This should not be confused with any carbon feature associated to graphene.

Several Raman spectroscopy maps should be performed for different areas of the substrate to understand the local variation across the sample, as detailed in Section 2.3.1.

2.3.1 Measurement protocol

1. Secure the sample so that it is horizontal with respect to the excitation laser beam and will not be displaced with respect to the stage, when the stage is moved during sample scanning.

2. As is the case for optical microscopy, depending on whether the substrate is circular or square/rectangular in nature, and the size of the substrate, the measurement positions should be selected as described in Figure 4 or Figure 5.

    a. For samples where $x/4 < 12$ mm, the number of measurement positions must be reduced from those in Figure 4 or Figure 5, so that the distance between measurements is at least 6 mm in each case.

    b. For samples where $x < 12$ mm, there should be one measurement position at the centre of the sample and one position near the edge of the sample.

    c. Measurement positions should be recorded.

3. After locating the measurement area with optical microscopy, set the Z-focus position using Raman spectroscopy measurements at different Z-axis positions to determine the position of highest Raman scattering intensity.

    a. Approximate the focus position using optical microscopy, such that the substrate surface/graphene layer is in focus.

    b. Set the instrument to measure the Raman spectra for at least 11 positions in the Z-axis, over a total travel area of 2 µm, which is 1 µm below the optical focus position to 1 µm above, using the same ≤1 mW laser power over ≥500 nm diameter laser spot.

c. A measurement time of 5 seconds should be used, but if this does not provide a signal-to-noise ratio of at least 10 for the G- and 2D-peak, a longer measurement time should be used to achieve this ratio.

4. To determine the local variations for different parts of the sample, microscale mapping is performed, for each sampling position an image of 10 x 10 μm^2 is obtained consisting of 121 (11 × 11) Raman spectra, with 1 µm steps between positions.

    a. The same laser power as in Step 3b should be used, with a 5 second exposure and 2 accumulations, to achieve a signal-to-noise ratio of at least 20 for the G- and 2D-peak. If the signal-to-noise ratio is less than 20, use a longer exposure time as required.

5. Repeat Step 3 and 4 at each measurement position as determined in Step 2.

6. After the maps have been acquired, the data should be analysed to determine the coverage of graphene, percentage of single layer graphene and level of disorder, as described in Section 2.3.2.

2.3.2 Data analysis

Firstly, the D- and G-peak area of interest (~1350 cm^{-1} and ~1580 cm^{-1} spectral positions respectively) and the 2D-peak (~2700 cm^{-1}) should be extracted for separate analysis. A baseline should be determined and subtracted for each spectrum, before peak fitting is performed, using a Lorentzian function for the D-, G- and 2D-peaks. If the 2D-peak is not symmetric, due to a combination of multiple peaks, or for the D- and G-peak due to the signals of other carbonaceous materials being present, a Voigt function can be used instead to fit the data. For defective graphene, the D'-peak at ~1620 cm^{-1} can be observed and will need to be fitted separately from the G-peak. These Raman peaks for an example single layer graphene sample with varying levels of defect density are shown in Figure 8.

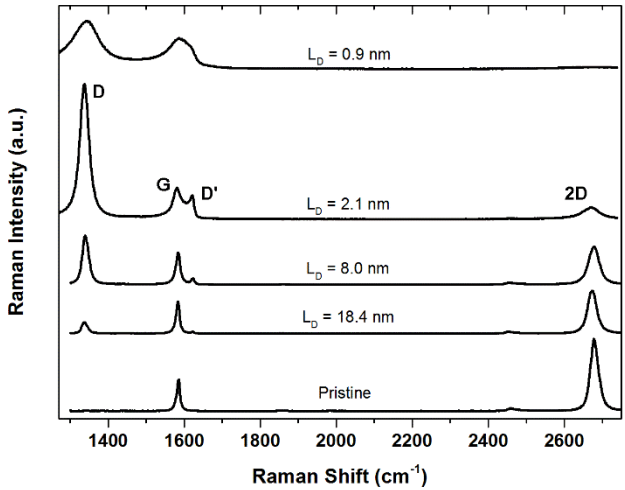

Figure 8: *Raman spectra for single layer graphene samples with different inter-defect distances (L_D) with labelled Raman peaks, from Ref [8].*

Four parameters that should be calculated for each spectra and displayed as histograms and two-dimensional maps for each measurement area of the substrate (as determined using Figure 4 and Figure 5) are:

- Peak area for the G-peak, A_G.
- Peak intensity ratio for the G- and 2D-peaks, I_{2D}/I_G.
- Peak intensity ratio for the D- and G-peaks, I_D/I_G.
- Full-width at half-maximum (FWHM) of the 2D-peak (FWHM[2D]).

The peak intensity is the maximum value of intensity for a Raman peak, after a baseline has been determined/subtracted and peak fitting has been performed. The peak area is the area under the same peak after the peak fitting has been performed.

Maps of the above parameters can produce artefacts for samples that are not completely covered in graphene, or have other materials present that cause significant undesired variations in intensity due to background or other Raman scattering peaks. These artefacts should be removed and their locations recorded.

Although, the *area* under a peak is preferentially used in Raman spectroscopy to determine the strength of the Raman band, the ratios of the peak intensities are also used in specific cases, as described above, and the ratios of peak intensities are predominantly used in this guide. This is because the peak area measurement can be less reliable for spectra with overlapping bands present (due to defective graphene or other carbonaceous materials) and the peak intensity ratios allow a less distorted representation of the sample.

Note that spectra from different substrates and using different laser excitation wavelengths cannot be directly compared [12], and thus the laser wavelength should be recorded alongside any of the individual spectra, histograms or 2D maps.

The FWHM[2D] is used to determine whether the graphene is 1LG or greater than one layer and thus the percentage of single layer graphene present in the sample. Turbostratic stacking of the graphene layers is assumed for more than one layer of graphene in this section, however, if the graphene layers are Bernal-stacked, the 2D-peak will not be a single Lorentzian peak when there is more than one layer of graphene. This is discussed in more detail in Section 3.3.4 and illustrated in Figure 28.

For 1LG on a Si/SiO_2 substrate, the 2D-peak must be a single Lorentzian peak with FWHM[2D] of ≤35 cm^{-1}, where I_D/I_G ≤ 0.2. The variation of these parameters, indicates areas where there are more than one layer of graphene. However, the FWHM[2D] is affected by other factors such as the roughness of the substrate and therefore TEM imaging (described in Section 2.4) is required to confirm the number of layers determined through Raman spectroscopy.

For example, if the 2D-peak is found to be a single Lorentzian peak with a FWHM of 45-50 cm^{-1} across the surface, the CVD-grown graphene sheet may be predominantly thicker than one layer, or the increase in the FWHM[2D] may be primarily due to lattice strain in a corrugated single layer of graphene. Through TEM imaging of the sheet, this can be determined.

Note that for certain alignments of the graphene layers in turbostratic bilayer or few-layer graphene, the Raman spectra recorded may also show a single Lorentzian 2D-peak with FWHM[2D] of ≤35 cm^{-1}, even though there is more than 1 layer present, due to superlattice formation, which strongly affects the Raman spectrum [13]. In the case of 2 layers, when their mismatch angle is low (<5°), the presence of additional peaks at either 1370-1470 cm^{-1} or 1540-1620 cm^{-1} must first be assessed. If these peaks are not observed, the sheet could be single layer or twisted-bilayer with a higher (>5°) mismatch angle between the two layers. Therefore, TEM imaging (see Section 2.4) is required to confirm if these areas classed as single layer through Raman spectroscopy are indeed single layer graphene.

It should be noted that the peak intensity ratio I_{2D}/I_G can also be an indication of the number of graphene layers, as for a pristine single layer of graphene this value is typically >2. However, this ratio can be reduced by other factors such as the level of disorder and doping and as such it cannot be explicitly used as an indication of single layer graphene.

The values of I_D/I_G should be recorded as the measurands for the level of disorder of the graphene. For low and moderate defect density, I_D/I_G increases with an increasing defects density, however, at high defect density I_D/I_G decreases with increasing defect density [6-8]. Therefore, a second parameter, FWHM[2D], also needs to be investigated. FWHM[2D] slightly increases at low and moderate defect density, whilst a strong broadening is observed at high

defect density, eventually leading to the disappearance of the 2D-peak [6]. The level of disorder for the sample should be expressed as the average of I_D/I_G for each spectra acquired for single layer/bilayer/few-layer graphene.

Alongside the two-dimensional maps detailed above, the percentage of the substrate covered by single layer/bilayer/few-layer graphene should be calculated through the number of measurement points that revealed a G-peak (shown by the G-peak area), to determine the microscale variations in coverage of graphene. The two-dimensional maps allow direct visualisation of any parts of the substrate where graphene is not present and any microscale variations in coverage. A percentage of coverage can be calculated using the pixels of the maps obtained as individual measurements and used in conjunction with the coverage determined by optical methods, providing an understanding of the coverage on the microscale. Variations found in the coverage of graphene (i.e. microscale holes in graphene) should therefore be used to amend the results recorded with optical microscopy in Figure 7. For example, if 10 % of the pixels of the map are found not to have any graphene coverage for an area that was classed as 100 % single layer graphene coverage using optical microscopy, the new values of coverage would be 90 % '1 layer' and 10 % 'no graphene present'. The percentage of Raman spectroscopy measurements of graphene that were found to be single layer graphene versus bilayer/few-layer graphene should be used similarly to amend the data recorded in Figure 7 for '>1 layer'.

Due to the limitations in determining the number of layers of graphene with Raman spectroscopy alone, TEM measurements should be performed on a sample of the CVD-grown graphene, as detailed in Section 2.4, to confirm the conclusions from the analysis of Raman spectra are correct. Figure 7 should then be modified according to the Raman spectroscopy and TEM results. For example, if single layer graphene is observed with Raman spectroscopy and TEM, when bilayer coverage was estimated using optical microscopy, this should be changed to reflect the conclusions of the Raman spectroscopy and TEM results. Similarly, if optical microscopy results led to the conclusion of 100 % coverage of single layer graphene, yet bilayer graphene was observed using Raman spectroscopy and TEM, the percentage of bilayer graphene determined with Raman spectroscopy should be recorded in Figure 7.

2.4 TEM imaging of CVD-grown graphene

In a transmission electron microscope, a high energy beam of electrons is passed through a thin electron-transparent sample in a high vacuum environment. Electromagnetic lenses located between the electron source and specimen are used to focus the electron beam on the sample and produce plane wave illumination. Electromagnetic lenses positioned after the specimen are used to produce a magnified image of the intensity of the electron wave at the exit surface of the sample. Alternatively a diffraction pattern can be obtained by imaging the back focal plane after the specimen. A selected area aperture is inserted in an image plane

after the sample in order to choose the area of the image from which the diffraction pattern originates. In this way, crystallographic information can be obtained from nanoscale volumes just 100 nm in diameter. If greater spatial resolution is required, nanobeam electron diffraction or convergent beam electron diffraction should be employed. Diffraction contrast imaging can be used to determine which areas of the sample are responsible for which features of a diffraction pattern, as well as to investigate crystal defects. This is achieved by inserting a small objective aperture in a back focal plane after the sample in order to select only certain diffracted beams from which to form the TEM image. TEM imaging can provide high resolution structural data for a wide range of materials. However, care should be taken when interpreting TEM images as the contrast is highly dependent on the precise imaging and diffraction conditions. Details of TEM image formation and electron diffraction are beyond the scope of this guide and for further information, Ref. [14] should be consulted.

The measurement protocol necessary to use TEM technique to determine the number of layers of a graphene sheet, layer alignment and the level of disorder of the transferred CVD-grown graphene sheet are described herein. The use of diffraction contrast TEM imaging, lattice resolution imaging and selected area electron diffraction (SAED) are detailed as these are achievable with most modern TEM instruments.

High resolution TEM imaging or scanning transmission electron microscope (STEM) imaging of graphene can also be used to reveal the atomic structure of point defects, edges, grain boundaries defects and dopant impurities, for examples see Ref. [15, 16]. This type of atomic resolution imaging requires a high performance TEM or STEM instrument, equipped with aberration corrected lenses and with a Scherzer imaging resolution better than ~0.14 nm at 80 kV. Atomic resolution imaging of CVD-grown graphene is often limited by the presence of surface contamination either remaining from transfer residues or having migrated from the TEM grid or the surrounding environment. H, C, O, Si and Cu are common contaminants found associated with CVD-grown graphene [17]. Complementary chemical information on bonding and elemental distribution is achievable using a TEM equipped with high efficiency energy dispersive X-ray spectroscopy or electron energy loss spectroscopy [18]. However, the information gained from atomic resolution TEM or STEM imaging and spectroscopy is beyond what is necessary for routine characterisation of graphene and the details of these techniques are beyond the scope of this guide. The interested reader can find a deeper understanding of analytical aberration corrected STEM and TEM in Ref. [19].

2.4.1 Measurement protocol

1. If the graphene does not cover the whole of the TEM grid the user should obtain optical microscope images of the transferred sheet on the TEM grid, ideally after the graphene sample has been loaded into the appropriate TEM specimen holder, to aid in locating the graphene in the TEM.

    a. If this is not possible, record the orientation of the grid and sheet in the holder before inserting into the microscope. Single tilt specimen holders are acceptable as it is rarely necessary to tilt in order to orientate the graphene film in the microscope.

2. To reduce sources of contamination the sample should be baked at ~150 °C for ~8 hours in a moderate vacuum immediately before loading the grid into the microscope.

3. The TEM should be operated using an accelerating voltage of 80 kV or below in order to reduce the likelihood of knock-on damage degrading the sample during imaging.

    a. Note that although 80 kV is below the knock-on damage threshold for pristine graphene, defects and edges will have a lower threshold and have found to be susceptible to damage even at 80 kV.

    b. The quality of the vacuum in the microscope may also affect the damage susceptibility of graphene (with UHV instruments seeing lower damage rates).

    c. The presence of metal contamination has been found to increase damage locally [17] while the presence of carbon contamination can actually facilitate healing of damage in the graphene [20].

4. If the graphene is not visible immediately, at the lowest magnification in bright field (BF) TEM imaging mode, find the centre of the TEM grid and use the previously obtained optical image to navigate to the position of the transferred graphene sheet.

5. Increase the magnification and acquire representative images of the morphology of the sheet.

    a. Use a small objective aperture to increase the contrast of the graphene sheet if necessary.

6. To acquire a SAED pattern, choose an area of the sheet that is flat, at least 200 nm from any tears, does not contain visible wrinkles and is entirely suspended over vacuum. In TEM mode, insert the smallest selected area aperture available and switch to diffraction mode.

    a. SAED measurements under these conditions will allow for a diffraction pattern of the graphene itself to be obtained without background signal from the amorphous support.

7. Remove the objective aperture if inserted in Step 5a and record the characteristic hexagonal diffraction pattern from the sample, as shown in Figure 9d.

a. Long exposure times should be used if required to successfully record the diffraction pattern, due to the weak intensity of the diffraction peaks for very thin areas of the sheet.

b. To prevent damage of the CCD camera from the bright central beam it may be necessary to use the beam stopper or to acquire multiple exposures and average these with post processing.

8. Acquire electron diffraction patterns for 10 or more different areas of the graphene sheet. These should be separated by at least 1 µm and suspended over different holes in the carbon support.

    a. Note that the presence of the 0.34 nm (002) diffraction spots close to the central beam indicates that the sheet is folded and these areas should not be used for determination of the number of layers.

    b. For each diffraction pattern the corresponding BF TEM image and an image of the position of the selected area aperture used should also be recorded, as shown in Figure 9c, in order to aid interpretation of the diffraction data.

9. Remove the objective aperture and increase the magnification to perform lattice resolution imaging of edges or folded regions of the graphene sheet. Acquire both over- and underfocus images.

    a. Note that in some TEMs the resolution may not be great enough to allow the 0.34 nm (002) interlayer lattice spacing to be observed. Recall that the resolution limit is poorer at lower accelerating voltages and check manufacturer specification of point resolution for the appropriate conditions.

2.4.2 Data analysis

Tearing and folding of a CVD-grown graphene sheet is a common problem during the transfer of graphene to a TEM grid, as shown in Figure 9 which reveals typical TEM images of CVD-grown graphene sheets on a TEM grid. Tearing will appear as a gap in the continuous graphene sheet. A fold will appear as a region of material with two or more misoriented layers, often with a straight edge parallel to which the basal plane spacing may be observable. Comparison of the optical images of the CVD-grown graphene sheet before and after transfer to the TEM grid, as well as low magnification (5000× or less) BF TEM imaging can be used to observe the presence of folding where this has occurred.

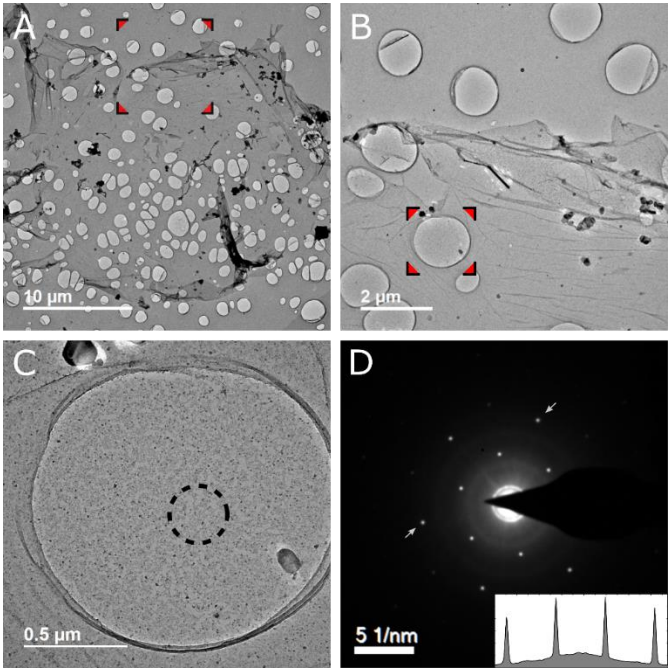

Figure 9: *BF TEM images of (a) a CVD-grown graphene sheet on a holey carbon film, with its typical creases and large lateral dimensions. The markers in (a) indicate an area of interest which appears at higher magnification in (b) showing some wrinkles in the sheet as well as folding near the edges. The marked area in (b), shown at higher magnification in (c), is an example of a suitable location to acquire diffraction information (at least 200 nm from any tears in the graphene, without visible wrinkles and where the sheet is suspended over vacuum). The black dashed circle indicates the position of the selected area aperture used to acquire the diffraction pattern in (d). The hexagonal spots in (d) are characteristic of graphene and the inset shows a characteristic intensity profile, which reveals that the sheet is single layer at the location.*

The characteristic hexagonal electron diffraction pattern obtained when graphene is viewed along the [001] direction is an unambiguous fingerprint that the material is graphitic. TEM electron diffraction should be used to distinguish single layer graphene from bilayer and few-layer graphene by comparing the intensities of the diffraction spots in the first and second diffraction rings [21, 22]. For single layer graphene the intensity of the spots in the outer hexagon is roughly equal to or less than that of the inner one. In contrast, for bilayer graphene the outer hexagon intensity is greater than the inner one. Figure 9d shows the diffraction pattern for 1LG.

If lattice resolution (0.34 nm) TEM/STEM imaging is achievable, a complementary approach to determining the number of graphene layers in the transferred sheets is to observe folded edges of the flakes. The TEM signature of folded regions can reveal the number of layers and appears similar to that observed for carbon nanotubes. The layer number is measured by

counting the dark lines at the edge as shown in Figure 10. Care should be taken to focus the specimen – acquire a focal series if unsure. One line in Figure 10a and two lines in Figure 10b can be observed, which represent single- and bilayer graphene, respectively.

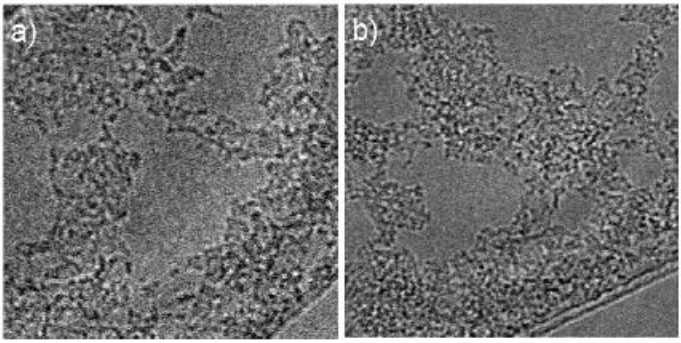

Figure 10: *BF images of (a) single layer and (b) bilayer graphene identified by the number of lines at the edges. From Ref. [23].*

Diffraction patterns can be utilised to identify and observe misoriented graphene layers known as turbostratic graphene, as shown in Figure 11. Disorder in the graphene sheet is identified by streaking of the diffraction spots. Turbostratic bilayer graphene regions are easily identified by the presence of 12 (or 18) spots in each ring rather than the 6 observed for single layer graphene or Bernal stacked graphite. The rotation angles between layers should be measured from the misorientation between neighbouring diffraction spots (Figure 11). However, this type of turbostratic graphene diffraction pattern may also result if a single layer folds back on itself during sheet transfer so this possibility should be investigated using the low magnification images.

Turbostratically stacked or folded graphene can also show Moiré patterns, as shown in Figure 12, the periodicity of which changes with rotation angle, and increasing angles (up to 59°) result in smaller Moiré periodicities. The overall percentage of single layer areas should be recorded, as well as the whether the areas of more than one layers are aligned turbostratically (with the rotation angles recorded) or Bernal-stacked.

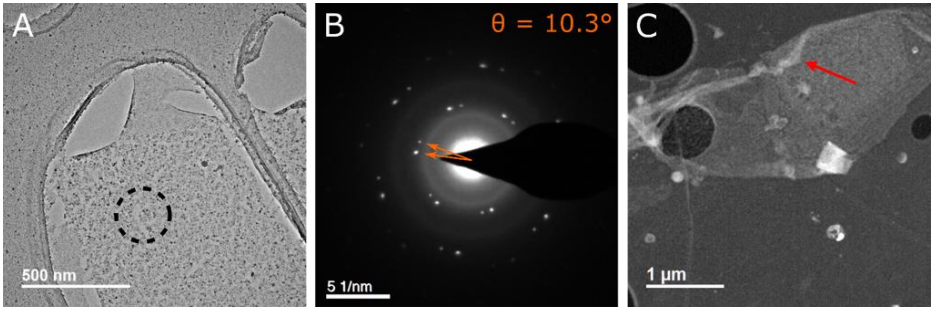

Figure 11: *(a) BF TEM image of suspended graphene. The black dashed circle indicates the position where the selected area aperture was inserted to acquire the diffraction pattern in (b). The presence of two independent hexagonal diffraction patterns is a signature of turbostratic graphene. Measuring the angle between the two hexagonal patterns gives the relative orientation of the two sheets, ϑ = 10.3°. (c) Dark field TEM imaging reveals a folded area of graphene, as shown by the red arrow.*

The typical number of layers observed with TEM should then be correlated with the Raman spectroscopy results. If the conclusions from the TEM measurements match the recorded measurements determined using Raman spectroscopy, the parameters reported using Raman spectroscopy can be used confidently. However, if these results do not match, issues such as turbostratic-stacking angles and substrate roughness should be considered and the conclusions adjusted so that both the observations using Raman spectroscopy and TEM are in agreement.

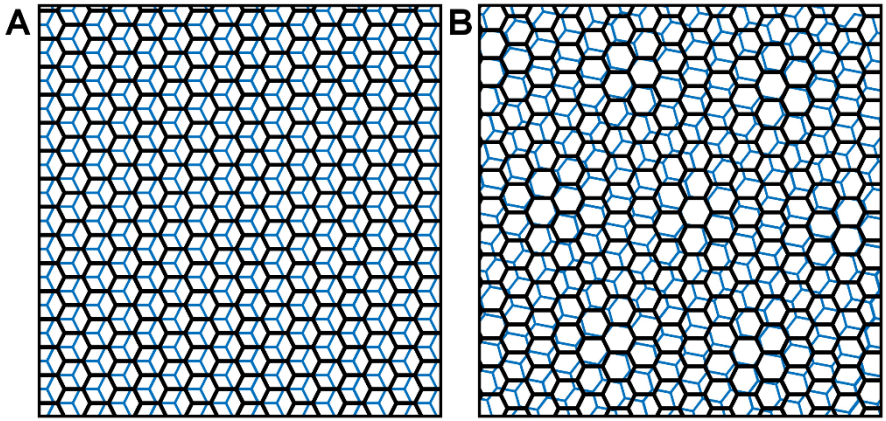

Figure 12: *Schematic representation of (a) AB stacked bilayer graphene and (b) turbostratic bilayer graphene revealing a Moiré pattern.*

The level of disorder observed in TEM should be qualitatively compared and confirmed with the Raman spectroscopy data obtained in Section 2.3.2.

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Chapter 3: Graphene flakes

- Quick analysis of flakes using Raman spectroscopy
- Preparation of flake samples
- Dimensional characterisation of flakes
- TEM dimensional characterisation of flakes
- Graphene lateral size and yield calculation

Graphene flakes may be present in either a powder or liquid dispersion form that contains flakes with a range of thicknesses, with a significant amount of flakes often having thicknesses that exceed the definition of 'few-layer graphene' [2], that is, flakes of graphite. Figure 13 outlines the overall measurement procedure for determining the structural properties of these flakes either in a powder or liquid dispersion form.

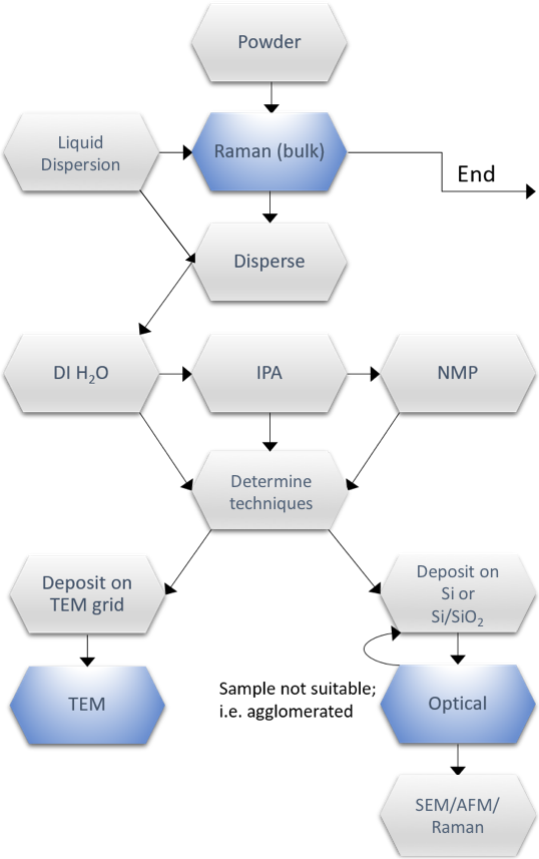

Figure 13: *Order and process of measurement techniques used to determine the structural properties of graphene flakes provided in either a powder or a liquid dispersion.*

A powder is required for the first rapid analysis step of Raman spectroscopy, as shown in Figure 13 and described in Section 3.1, which is used to determine if the material contains graphene and/or graphite flakes. Therefore if a dispersion has been supplied, the material will first need to be removed from the solvent, as detailed in Section 3.1.1.

As mentioned previously, this guide assumes that 1LG/2LG/FLG or graphite will be measured and as such, graphene oxide or functionalised graphene materials (which are outside the scope of this document) will not produce the same Raman spectroscopy results as those described in this guide. However, optical microscopy, scanning electron microscopy (SEM) and atomic force microscopy (AFM) characterisation of lateral flake size and flakes thicknesses can still be applied to materials such as graphene oxide and functionalised graphene.

The next step of characterisation uses either TEM or a combination of other techniques to determine the distribution of flake lateral sizes and the relationship with flake thickness. For this stage a liquid dispersion is required, therefore if the material was provided as a powder, it requires dispersing in a suitable solvent, as described in Section 3.2.1.

The dispersed flakes are then prepared on a TEM support grid or a Si/SiO_2 substrate accordingly. As described in Section 3.3, for deposition on Si/SiO_2, an optical microscopy step is used after the sample preparation to determine if the flakes have been appropriately deposited onto the substrate, or if the material has agglomerated so that it cannot be accurately measured. If optical microscopy determines the samples are not appropriate for analysis, the user must go back a step and the sample preparation steps must be performed differently. However, once optical microscopy results show an even deposition of the material across the substrate without agglomeration, further analysis with SEM, AFM and then Raman spectroscopy mapping can be performed. If instead a TEM investigation is undertaken, Section 3.4 should be consulted.

Once either 'SEM/AFM', 'SEM/AFM/Raman' or 'TEM' measurements have been performed on the sample, the median lateral flake size, the range of flake sizes, the graphene (1LG) yield and few-layer graphene (FLG) (or thinner) yield can be calculated, as discussed in Section 3.5.

3.1 Quick analysis of flakes using Raman spectroscopy

Firstly, the sample, in powder form, should be tested using confocal Raman spectroscopy to determine if the powder/dispersion supplied contains graphene and/or graphite flakes. This can also yield qualitative information on the structural properties of the material, including the level of disorder and the dimensions of the flakes. If the material is supplied as a liquid dispersion, then the dispersant must first be removed from the dispersion so it can be analysed in powder form.

If a powder has been provided, this should be analysed with the powder secured on adhesive tape (Section 3.1.2).

A small amount of the sample is required for this rapid Raman spectroscopy analysis step, which should not be confused with the processes used later for measurement of individual

flakes with AFM and Raman spectroscopy (detailed in Section 3.3.4) after further sample preparation.

3.1.1 Removing graphene from a liquid dispersion

1. In a vacuum filtration kit, use a membrane with pore size of ≤0.2 µm to ensure that the majority of the smaller flakes are retained on the membrane.
    a. The material of the membrane must be compatible with the solvent of the dispersion.
    b. Alumina or cellulose membranes should be used for common graphene solvents such as water, IPA or N-methylpyrrolidone (NMP).
2. A pressure of ~100 mbar should be applied for the vacuum filtration step.
3. Collect the dried material on top of the membrane at the end of the process as a supported or free-standing film.
    a. The thickness of the film produced should be at least 1 µm to provide a strong Raman signal during subsequent measurement and therefore a high enough concentration or large enough volume of dispersion will be required to provide a film that can be handled.

3.1.2 Sample preparation from a powder

1. Before handling a powder sample of nanomaterial, an appropriate risk assessment should be performed and the required personal protective equipment and safety processes employed.
2. Place double-sided adhesive tape on to a microscope slide.
3. Deposit a small amount of the powder on the adhesive tape, pressing down lightly with a spatula to ensure adhesion.
    a. To assess uniformity, material can be collected and prepared from more than one part of the batch (e.g. top, middle and bottom of the container). However as this step is for rapid analysis, a single sample is sufficient.
4. Once the material is secured on the adhesive tape, excess and unsecured material should be removed by tapping the microscope slide vertically.
    a. To prevent dust being raised, the material should be collected onto a wet paper towel.
    b. Figure 14 shows an example sample after preparation.

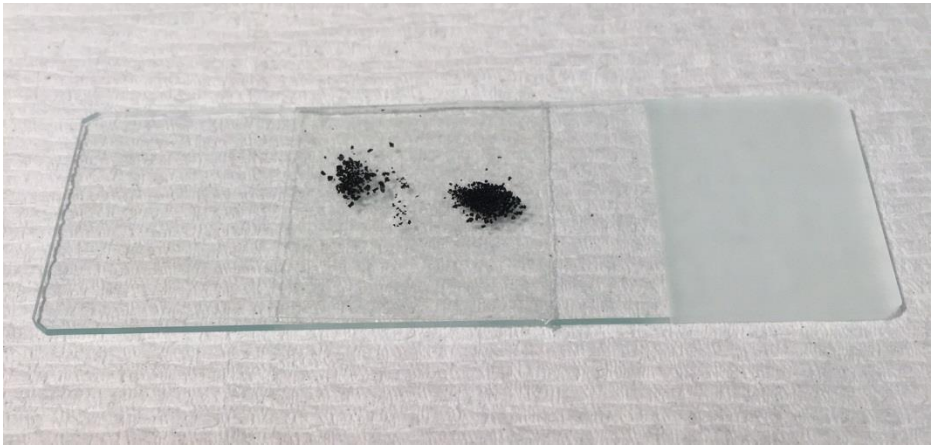

Figure 14: *Photograph of a powder, containing graphene and graphite flakes, deposited onto an adhesive tape.*

3.1.3 Raman spectroscopy of a film

As described in Section 2.3, Raman spectroscopy is a powerful technical for studying carbon nanostructures such as graphene.

As this step is to determine whether the sample contains graphene and/or graphite flakes and to provide a qualitative assessment of the level of disorder and/or size of the flakes, a less detailed method is required, compared to Raman spectroscopy steps in Sections 2.3 and 3.3.4. Raman spectroscopy should be performed in a backscattering geometry with preferably a 100× objective lens (NA = 0.9). The system should be calibrated prior to measurements. In this case of rapid measurement, a red or green laser line should be used, however, it should be noted that some of the peaks observed will be at different spectral positions, depending on the wavelength of the excitation laser.

The spectral range should be chosen such that the relevant Raman lines (D-line (~1350 cm^{-1}), G-line (~1580 cm^{-1}), 2D-line (~2700 cm^{-1})) and associated widths are included.

After locating to a measurement area with optical microscopy, set the Z-focus position such that the surface of the powder is in focus. Perform a single spectra Raman spectroscopy measurement, with a laser power of ~1 mW incident on the sample so as to make sure there is no damage to the sample, with an exposure of 5-10 seconds and 2 accumulations. This should provide a signal-to-noise ratio of at least 10; if not a longer measurement time can be used to increase the signal-to-noise ratio.

Measurements should be performed from a minimum of three different areas of the sample to understand the local variation across the sample. For samples prepared from a powder and on

an adhesive, the material is generally in the form of aggregates of many flakes. Therefore when recording the spectra, measurements should be made from at least three different aggregates.

To confirm the presence of graphene and/or graphite flakes, a sharp (<30 cm^{-1}) G-peak at approximately 1580 cm^{-1} and a 2D-peak at ~2700 cm^{-1} must be consistently observed in the Raman spectra, as shown in Figure 15. Note that the 2D-peak may not be a symmetric Lorentzian peak, as it would be for single layer graphene, instead comprising of several peaks. A prominent shoulder in the 2D-peak is indicative of layered material, with a thickness of over 10 graphene layers (i.e. graphite flakes). Restacked few-layer graphene flakes can also yield a single Lorentzian Raman 2D-peak. If these peaks are not present, further characterisation is not required, as the sample does not contain graphene or graphite flakes, however, a sufficient signal-to-noise ratio must be established before this conclusion can be made.

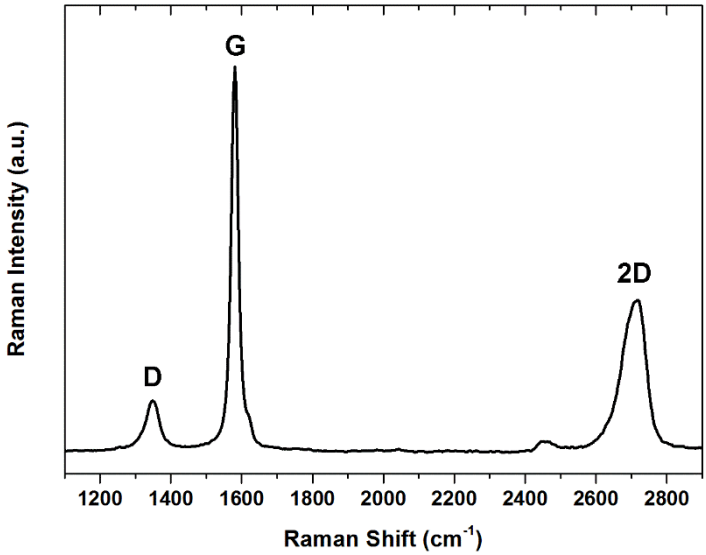

Figure 15: *Example Raman spectra of a powder containing graphene and graphite flakes. Adapted from Ref. [24].*

Whilst for CVD-grown graphene sheets, as described in Section 2.3, a very small or no D-peak will generally be observed with Raman spectroscopy, measurements of graphene flakes will typically reveal a D-peak, as shown in Figure 15. This is due to flake edges activating the D-band as well as basal plane defects. The intensity ratio I_D/I_G is therefore correlated to the lateral size of the flakes, with a larger ratio typically indicating flakes with smaller lateral dimensions. This I_D/I_G ratio should be recorded to compare to the results of the characterisation techniques subsequently used in the subsequent steps shown in Figure 13.

Note that if functionalised graphene or graphene oxide is present, Raman spectroscopy will show the D- and G-peaks, but not necessarily a 2D-peak, and the D- and G-peaks will have much larger FWHM values (>30 cm^{-1}) than expected. However, other carbon materials may also have these peaks, and so it is recommended that chemical characterisation is performed separately.

3.2 Preparation of flake samples

For further, more detailed, characterisation of the sample, the flakes must be prepared so that they are isolated on a substrate. This allows the next characterisation steps, as shown in Figure 13, to be performed, with the choice of a silicon substrate, Si/SiO_2 substrate or TEM grid dependent on the requirements of the characterisation techniques. For this stage a liquid dispersion is required, therefore if the material was provided as a powder, it requires dispersing in a suitable solvent.

Dispersal in several solvents in an order of preference should be attempted, starting with the most preferred (DI water), typically graphene or graphite flakes will stay dispersed in DI water only if a stabilising agent, such as a surfactant, is present as part of the manufacturing process. If this solvent does not disperse the material, isopropanol should be used, or lastly N-methylpyrrolidone (NMP) should be used as graphene disperses well in this solvent. However, due to the high boiling point of NMP (203 °C), this can affect the characterisation results in the form of solvent residue. Note that using a significant amount of ultrasonication to disperse the material can induce flake scission and therefore affect the structural characterisation results obtained for a sample. The amplitude (commonly expressed as power) and duration of ultrasonication should therefore be kept to the minimum required to disperse the flakes.

3.2.1 Dispersing graphene flakes

1. If the sample is provided as a powder, prepare it in an appropriate solvent, so that a concentration of approximately 0.1 mg/ml is achieved.
    a. The suitability of the solvent should be determined through the observation of any sedimentation of the material.
    b. Firstly, dispersion in DI water should be attempted, followed by dispersion in IPA and if both these solvents are not suitable, finally NMP should be used.
2. Place the dispersion in a glass vial or bottle and agitate.
3. Sonicate the dispersion for 10 mins in a table-top ultrasonic bath (30-40 kHz).
4. Leave the dispersion to stand.

Note, **if the sample is already provided as a dispersion**, this should be diluted to 0.1 mg/ml using the same solvent. If dispersed in water/surfactant, the dilution should be carried out using DI water, to reduce the level of surfactant deposited on the substrate. An example of a 0.1 mg/ml dispersion is shown in Figure 16, in cases where the concentration of the dispersion provided is not known and the dilution must be approximated.

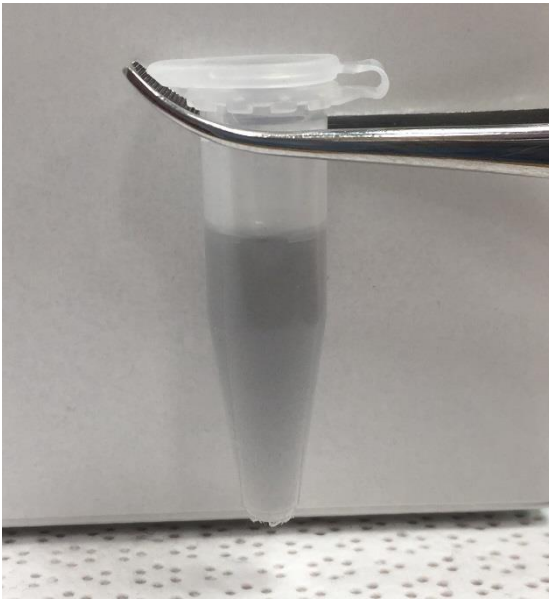

Figure 16: *Photograph of an example of a 1 mg/ml liquid dispersion of graphene and graphite flakes.*

3.2.2 Drop casting silicon or Si/SiO_2

To enable the measurement of flake dimensions for a range of flakes using optical microscopy, SEM, AFM or Raman spectroscopy, the prepared dispersion should be deposited onto a silicon (SEM) and a silicon dioxide (optical microscopy, AFM and Raman spectroscopy) substrate in such a way that a substantial fraction of the individual flakes are isolated from each other.

1. The silicon or Si/SiO_2 substrate should be cleaned, as described in Section 2.1.3.
2. Place the cleaned substrate on a hot plate and set the temperature to be slightly greater than the boiling point of the solvent used for the dispersion.
3. Drop cast 10 µl of the dispersion onto the substrate as shown in Figure 17.
    a. A well dispersed layer of flakes should then be left on the surface, as shown in Figure 18 using optical microscopy for the case of a Si/SiO_2.

4. The sample should then be left in a vacuum oven for 2 hours or more at 40 °C, to reduce solvent and/or surfactant residue.

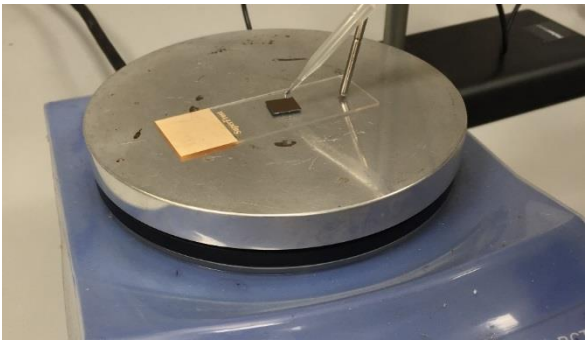

Figure 17: *Photograph of the drop casting process for a substrate on a hot plate.*

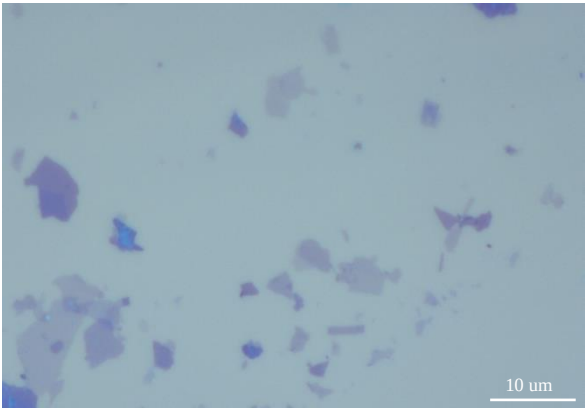

Figure 18: *Optical microscopy image of 1LG/2LG/FLG and graphite flakes deposited on a 90 nm thick layer of SiO_2 on a silicon wafer (Si/SiO_2).*

3.2.3 Deposition of flakes on a TEM grid

1. Clean the TEM support grid by washing in fresh isopropanol or similar solvent and baking in a vacuum at 200 °C for a few hours.

2. The flakes should be mounted onto the TEM support grid by simple drop casting with a pipette.

    a. To avoid agglomeration of the flakes, particularly if the solvent requires a long period of time to evaporate, wicking of the solution from the

underside of the grid after it has been deposited and/or heating the grid should be performed during drop casting.

b. An ideal coverage is achieved when there are many flakes within each grid square but not so many that individual flakes overlap, as shown in Figure 19. If there are too few flakes on the TEM grid, deposit more of the dispersion upon the substrate.

3. Before loading the grid into the TEM, dry the sample thoroughly to reduce the chance of contamination entering the microscope and interfering with imaging. This should be done by heating to ~150 °C in vacuum for 8 hours immediately prior to loading the sample.

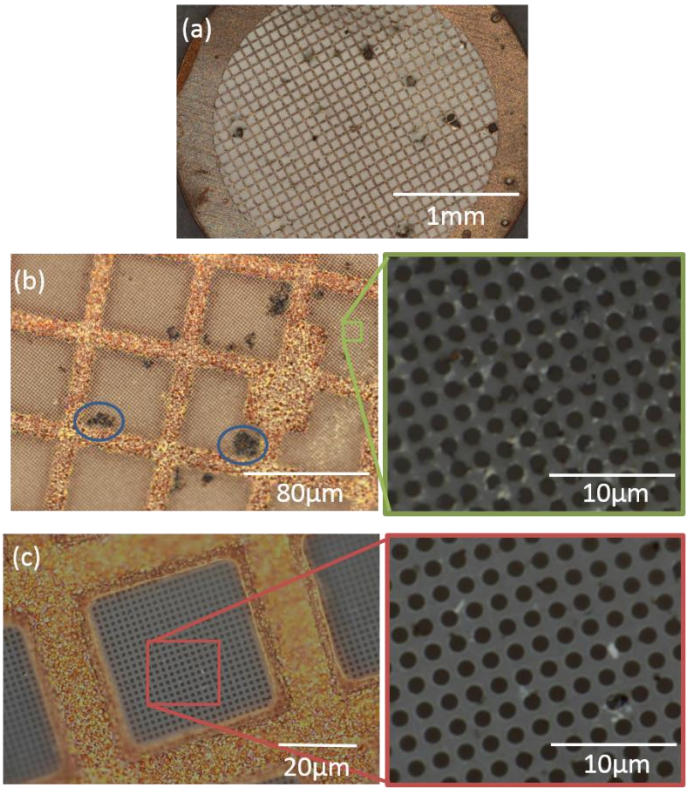

Figure 19: *Optical images of graphene flakes drop cast on a TEM support grid. (a) Low magnification image of the whole TEM grid. (b) Example of high density coverage of graphene flakes, which is not optimal for TEM imaging. Blue ovals highlight presence of regions with densely agglomerated flakes. (c) Suitable flake coverage for TEM imaging. Enlarged regions in (b) and (c) show the presence of micrometre-sized flakes which appear bright, superimposed on the regular holes of the TEM grid.*

3.3 Dimensional characterisation of flakes

As illustrated in Figure 13, once an initial measurement has confirmed the presence of graphene and/or graphite flakes, the range of lateral flake dimensions, associated flake thickness and level of disorder can be investigated using TEM or a combination of SEM, AFM and Raman spectroscopy, depending on the techniques available. For the latter option, the structural properties of the flakes should be determined using the combination of characterisation techniques shown in Figure 20.

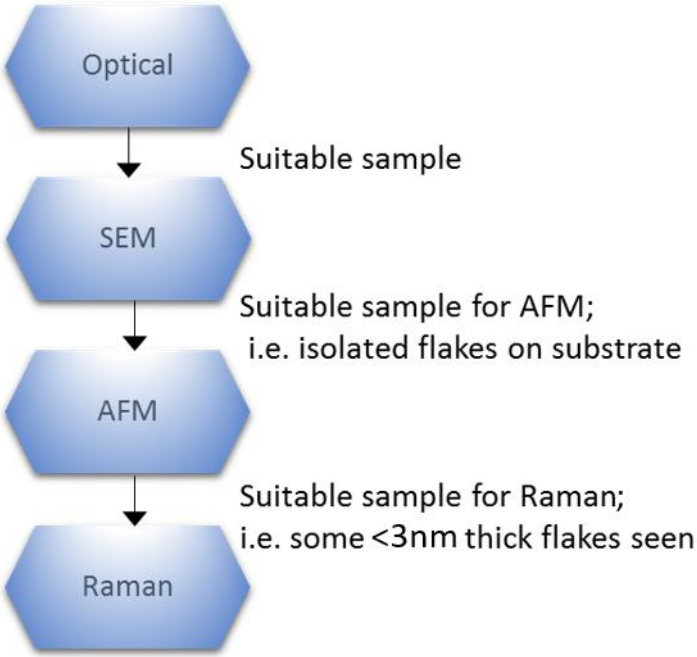

Figure 20: *Flow diagram and decision-making process for determining the range of lateral dimensions and thickness of graphene and/or graphite flakes.*

As previously described, a sample of the material should be prepared on both a silicon substrate and Si/SiO_2 substrate. From Figure 20, the Si/SiO_2 sample should then be investigated for suitability using optical microscopy as the first step, a qualitative understanding of the range of flake sizes can also be approximated. If the deposition of the material appears acceptable, the **silicon** sample should then be measured using SEM to determine the range of lateral flake sizes. If the sample is deemed suitable for AFM measurements after the SEM investigation, the Si/SiO_2 sample should be examined with AFM to determine the correlation between lateral flake size and flake thickness. If flakes of ~3 nm thickness are observed, these same flakes should subsequently be investigated using Raman

spectroscopy, to determine how the thickness relates to the number of layers more accurately, as well as attempt to determine the level of disorder.

3.3.1 Optical microscopy

Optical microscopy should be used for a rapid assessment of the suitability of the prepared sample for the determination of the lateral flake size and flake thickness.

To be able to measure the lateral size and thickness of the flakes, the flakes must be as isolated as possible, whilst also being as abundant as possible. This allows SEM and AFM measurements to be performed at a faster rate whilst also ensuring that the values obtained are for individual flakes and without contributions from other flakes.

Examples of flakes deposited onto Si/SiO_2 that can then be measured so as to determine the flake size and thickness are shown in Figure 18, these samples reveal either a spread of abundant isolated flakes or areas of the surface with such a spread of flakes that can be easily determined using optical microscopy. Care should be taken during optical microscopy analysis to ensure it is understood where the flakes are present compared to other material that effects the optical contrast, such as solvent residue causing changes in colouration of the substrate, as shown in Figure 21.

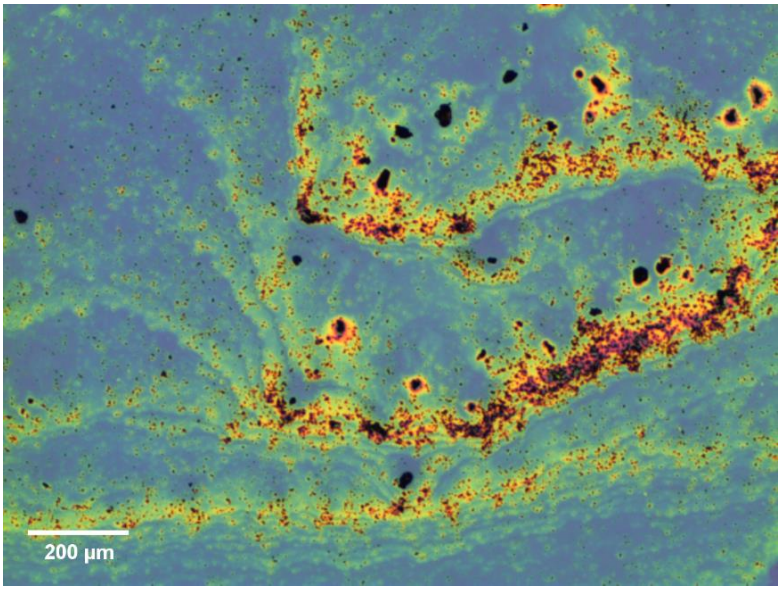

Figure 21: *Example optical microscopy image of residue left on a Si/SiO_2 substrate after deposition of a liquid dispersion of flakes, as shown by areas of varying colour.*

An optical image of a sample that cannot be used after preparation due to agglomeration of material is shown in Figure 22. Additionally, a substrate with little suitable material present will greatly reduce the rate of characterisation and should also be avoided. Different objectives lenses with different magnifications should be used to determine the general distribution of the material across the substrate. If the sample is not suitably prepared, the sample preparation process should be repeated with different concentrations of dispersion and/or different solvents, as described in Section 3.2.

If the material contains flakes with lateral dimensions that are large enough to resolve using optical microscopy, as shown in Figure 18, a better understanding of the sample is possible before SEM measurements are undertaken. In this way the optical microscopy imaging of the material can provide qualitative evidence of lateral flake size to corroborate the SEM imaging. Similarly, if individual flakes are not visible as they are too small, this also allows some assessment of the range of lateral flake sizes present.

Note that it is possible to estimate the thickness of a flake on a Si/SiO_2 substrate due to the fact that graphene is sufficiently transparent to add to an optical path, which changes its interference colour with respect to an empty wafer [3]. For a certain thickness of SiO_2, even a single layer has been found to give sufficient contrast to allow few micron-sized graphene crystallites to be identified among copious thicker flakes scattered over a millimetre-sized area, as described in Section 2.2.

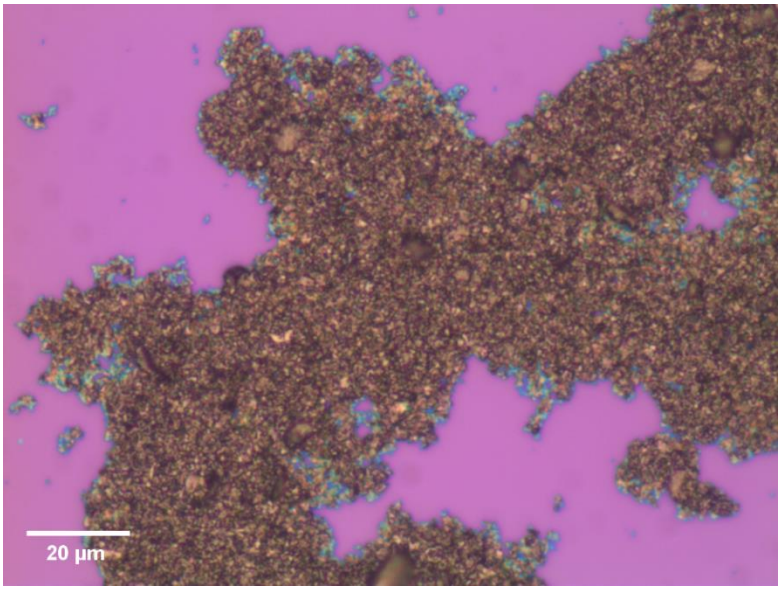

Figure 22: *Example optical microscopy image of a dispersion of flakes prepared on Si/SiO_2 that cannot be used for determining the dimensions of the flakes with other techniques due to agglomeration.*

3.3.2 SEM analysis

Scanning electron microscopy (SEM) can be used to determine the size of micro- and nanoscale objects. SEM uses the bombardment of a surface with electrons and the collection of the electrons after collision processes to image the surface structure. This technique has a much greater lateral resolution than optical microscopy due to the smaller wavelength of electrons compared to visible light. This allows this technique to achieve a lateral resolution of tens of nanometres. Thus, SEM should be used to measure the lateral sizes of the flakes.

Calibration of the lateral dimensions measured using an SEM should be performed regularly, using commercial calibration samples or nanoparticles of traceable dimensions [25].

Issues related to the characterisation of graphene using SEM include the charging effect created by the constant bombardment of a non-conducting surface such as silicon dioxide with electrons, as well as the deposition of carbonaceous material on the surfaces imaged by the electron beam.

These issues may not be totally overcome, but should be reduced through deposition of the graphene flakes onto a silicon substrate that only has a native oxide present rather than a thicker thermally-grown silicon dioxide layer. This reduces the charging effect observed as the sample substrate is more conductive. However, the silicon sample will have carbon deposited on the surface due to the SEM imaging and thus the AFM analysis should be performed on Si/SiO_2 samples to avoid any contribution of carbonaceous material to the measured thickness.

The SEM characterisation should also be used to further determine the suitability of the deposition of flakes, in addition to the previous optical microscopy step, for AFM analysis. An AFM instrument typically uses built-in optical microscopy to aid in the location of suitable areas to image with AFM, and therefore the use of a Si/SiO_2 substrate will aid the AFM measurement process.

Deposition of a conductive layer (typically gold) onto a sample surface prior to SEM imaging is common practice for non-conducting samples or surfaces that exhibit charging, However, due to the small dimensions of the graphene flakes being imaged, this kind of deposition can instead hamper the evaluation of the dimensions of the graphene flakes by depositing a comparable or thicker layer of conductive material. Thus deposition is not performed for SEM imaging of graphene flakes.

To determine the lateral distribution of flake sizes from graphene and/or graphite powders or dispersions, SEM images must be obtained for a statistically significant distribution of isolated flakes to determine the range and frequency of lateral flake size.

3.3.2.1 Measurement protocol

1. Mount the substrate into the SEM holder so that it is held rigidly and is well-supported, before securing the sample in the SEM and pumping down to vacuum for SEM measurements.
    a. The sample is not required for any further measurements after SEM imaging and so the sample can be mounted using an irreversible process if required.
2. Set the SEM configuration to image by collecting secondary electrons, typically with an accelerating voltage of 5 kV or less to minimize charging.
3. Identify the location of the sample surface through SEM imaging and optimise the system in terms of its aperture, working distance, stigmator and focus, to achieve the best resolution possible, as required for the specific SEM instrument.
    a. This optimisation process should be performed on a feature that is similar in size to the flakes, but that is not a flake of interest, as the long dwell time required for optimisation will affect the area.
4. Low magnification images (~10k magnification) should then be obtained for areas that contain flakes.
    a. When these areas of flakes are found, the sample is assessed for suitability for subsequent higher magnification SEM and AFM images. If the flakes are not abundant and isolated on the substrate, then the sample should be re-prepared.
5. Images of greater resolution and magnification should then be obtained for the flakes to allow subsequent image analysis.
    a. Higher magnification images reduces the uncertainty in lateral measurement, that is, the flakes should occupy a significant part of the image and the number of pixels should be sufficient that the lateral distance between pixels (specimen pixel size) is less than 10 nm.
    b. Images can be taken of multiple flakes at the same time as shown in Figure 23 or for specific flakes, with an aim to maximise the number of isolated flakes imaged.
    c. Importantly, a representative distribution of flakes of different sizes from across the substrate surface must be obtained. Selective selection of, for example, easier-to-image larger flakes will drastically skew the final results. Therefore a range of magnifications must be used and different areas of the sample must also be imaged.

6. Repeat Steps 4 and 5 until significantly more than 200 flakes have been imaged.

3.3.2.2 Data analysis

Once significantly more than 200 flakes have been imaged, the lateral dimensions of the flakes should be determined for 200 flakes or more. The lateral size of each flake is determined by measuring first the length and then the width (perpendicular to the length measurement) of the flakes and calculating the mean of the two values. Care should be taken that the first (length) measurement of the flake will also allow a representative width measurement (perpendicular to the length) to be obtained. An example of how a flake should and should not be measured is shown in Figure 23.

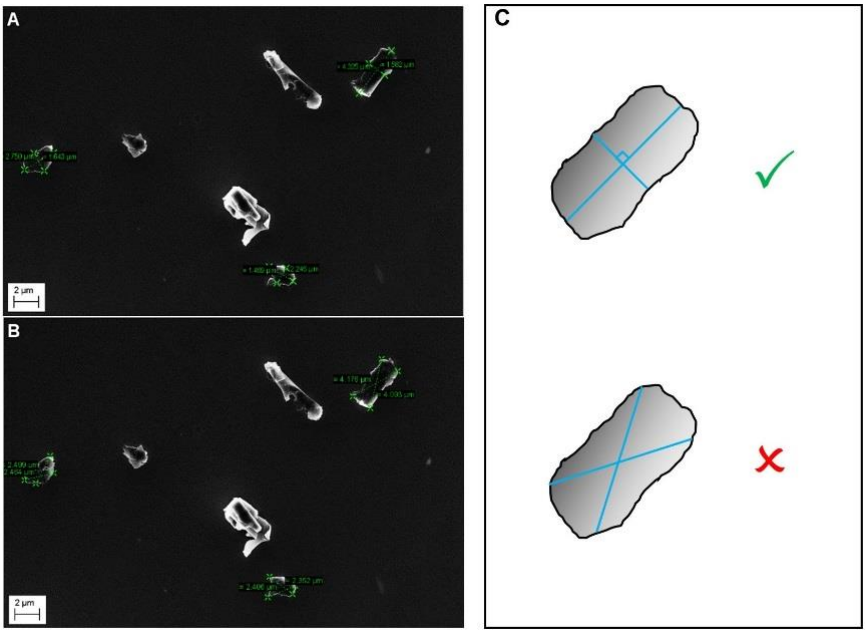

Figure 23: *Examples of lateral flake size measurements performed for an SEM image, (a) correctly, by first taking the length and then finding the width as a perpendicular bisector, and (b) incorrectly. (c) shows an enlarged schematic of the same process for clarity.*

Flakes should only be measured that are not ambiguous in their composition, that is, features that do not appear to be flakes should not be measured. Flakes that do not have the complete circumference of the flake visible, due to overlapping flakes, should also not be measured.

Once the lateral size of 200 flakes have been calculated, they should then be represented as a histogram with 20 bins. An example histogram is shown in Figure 24.

When comparing the spread of average lateral dimensions for more than one sample, the same sample may need to be represented in several histograms with different length-scales for a better comparison. For example, one sample may have lateral dimensions exclusively below 1 µm, whereas a second sample primarily has flakes below 1 µm in size, whilst having some in the range of 1-10 µm. The whole spread of the flake lateral dimensions should be shown for the second sample, but another histogram with a scale of 0-1 µm should also be shown to allow for a good comparison with the 1st sample.

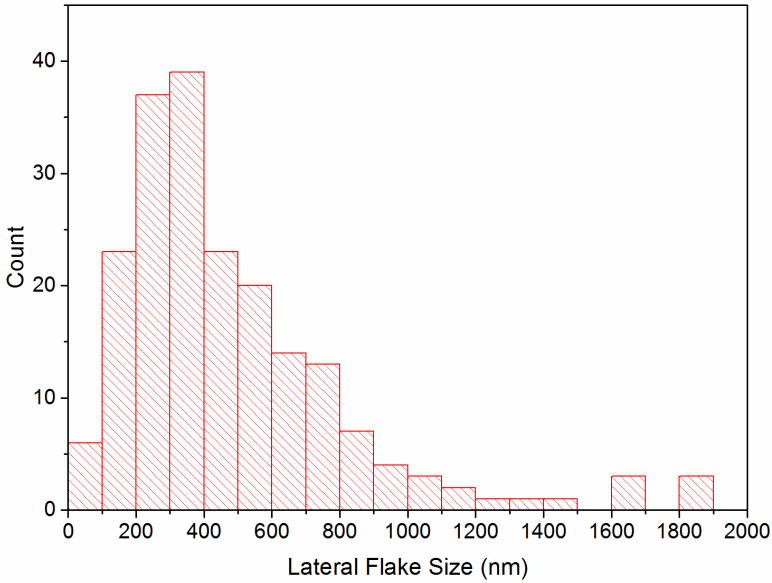

Figure 24: *Example histogram of the range of lateral flake sizes of a graphene and/or graphite flake powder sample, determined through SEM measurements.*

The largest source of uncertainty in the determination of the average lateral flake size is typically due to bias of the user, rather than the limits on the instrument itself. Thus, it is extremely important that the user does not preferentially measure certain shape or size flakes. The user should also record an associated uncertainty for each lateral measurement, taking issues such as the flake shape and image pixel separation into account. A minimum uncertainty value of 20 nm should be used for each flake measurement.

3.3.3 AFM measurements

Atomic force microscopy (AFM) is a scanning probe microscopy technique where the probe is a sharp apex mounted on a cantilever. The response of this cantilever to changes in topography allows the imaging of the topography of a surface with nanoscale lateral and height resolution.

When on a flat surface, such as Si/SiO_2, the dimensions of a graphene flake can thus be determined with this technique. AFM measurements should be performed in ambient conditions, with the instrument operating in a dynamic AFM mode allowing the thickness of flakes isolated on the substrate surface to be determined. These thickness values can be obtained alongside the lateral size measurements so any correlation between these properties can be understood.

The AFM system should be operated in a closed loop mode and the AFM scanners calibrated in X, Y, Z directions using traceable, calibrated lateral grids and step height standards that are available commercially.

The sample analysed by AFM should not have been previously investigated using SEM, and should consist of flakes deposited onto a Si/SiO_2 substrate, using a sample preparation method that has been shown by SEM to produce a large number of isolated flakes.

AFM measurements should be performed for material where the spread of lateral flake size has previously been determined using SEM, so that the AFM results can be used to correlate lateral size with flake thickness. To this end, isolated flakes of a range of lateral sizes corresponding to the range of lateral dimensions found using SEM, should be imaged.

3.3.3.1 Measurement protocol

1. Mount the substrate into the AFM so that it is held rigidly.
    a. The sample may be required for further Raman spectroscopy measurements after AFM imaging and so the substrate should be mounted in such a way that Raman spectroscopy can still be performed afterwards.
    b. If using an AFM-Raman system, Raman spectroscopy measurements can be performed as soon as a suitable flake is identified (see Section **Error! eference source not found.** for protocol).
2. Place an appropriate intermittent-contact mode AFM cantilever into the system and tune the oscillation frequency to be offset from the resonance frequency of the cantilever by 5 % of the resonance peak FWHM.
    a. Typical parameters for the cantilever are spring constant ~40 N/m, resonant frequency 240 kHz, and a probe apex size of 5-15 nm.
3. Locate an area of the surface where there is an abundance of isolated flakes using the built-in optical microscopy image of the AFM system.
4. Approach the surface with the AFM probe using the minimum oscillation amplitude possible to allow stable AFM feedback with a set-point of 80 % of the oscillation amplitude.

5. Set the fast scan direction to be perpendicular to the length of the cantilever and scan a large area to allow the identification of several flakes.
    a. A 20 × 20 µm scan size covers a large enough area to image multiple flakes but still achieve the resolution required so that it can be determined whether features are flakes or other material.
    b. A scan-line speed of less than 10 µm/sec with a resolution of 256 × 256 pixels should be used so as to image the area in a reasonable time but also allow stable imaging when the probe passes over high topography features.
    c. A set-point of ≤70 % of the free amplitude should be used and an appropriate feedback gain that provides as fast a response to topography changes as possible, without creating oscillation artefacts.
        i. The set-point can be reduced when imaging a flake and the step height monitored to determine if a lower set-point is required to measure a lower and more accurate flake thickness measurement.
        ii. However, a very low set-point value (i.e. a set-point of <40 % of the free amplitude) can lead to artefacts due to damage of the probe apex and should be avoided.
6. After a larger scan has been completed, AFM topographic images of the areas of interest within the larger scan should be obtained, using a smaller scan size, chosen so as to encompass an individual flake.
    a. Typical scan size is 2 µm and the number of pixels per image should be increased to 512 × 512 pixels to allow for increased precision during data analysis.
7. Repeat step 6 for the other areas of interest observed in the larger AFM image obtained in step 5.
8. Repeat steps 5, 6 and 7 so that a minimum of 20 flakes with lateral flake sizes that cover the range of lateral sizes shown by SEM have been reliably imaged.
9. The data should then be analysed to determine the thickness of the flakes versus the lateral size of the flakes.

3.3.3.2 Data analysis

The AFM topographic images should be used to calculate the mean of the width and length measurements as the lateral flake size, using the same procedure performed for SEM

measurements. For each flake, first the length measurement is obtained and then the width (perpendicular to the length measurement) of the flake is measured and the mean of the two values is then calculated. Care should be taken that the first (length) measurement of the flake will also allow a representative width measurement (perpendicular to the length) to be obtained. It is important to record the associated uncertainty in each lateral measurement in the same way as for the SEM lateral measurements, taking issues such as the flake shape and image pixel separation into account, with a minimum uncertainty value of 10 nm. An example of how the lateral flake size should and should not be measured is shown in Figure 25.

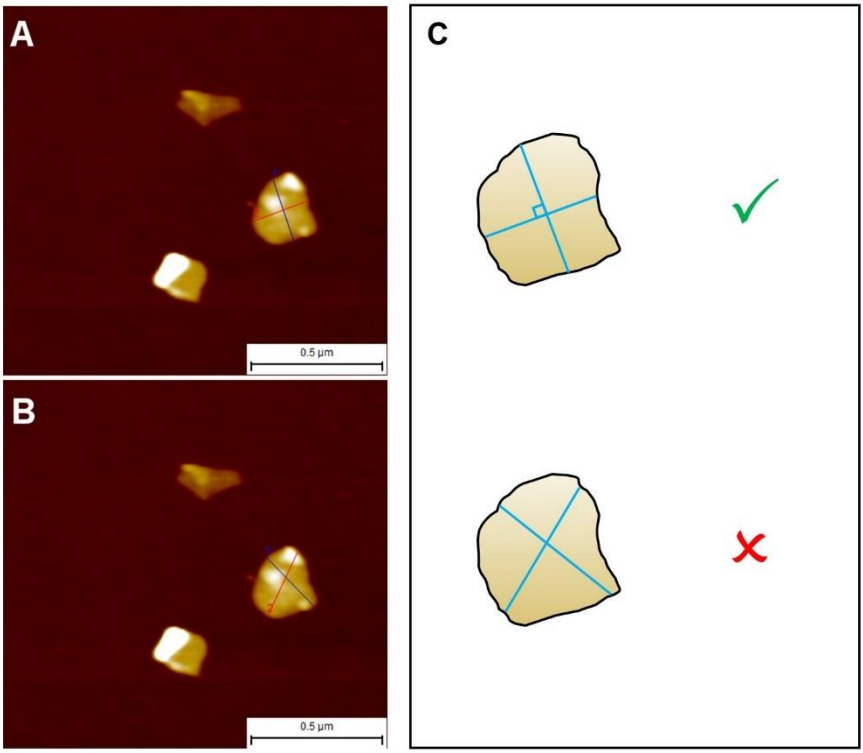

Figure 25: *Examples of lateral flake size measurements performed for an AFM image, (a) correctly, by first taking the length and then finding the width as a perpendicular bisector, and (b) incorrectly. (c) shows an enlarged schematic of the same process for clarity.*

Flakes should only be measured if they are not ambiguous in their composition, that is, features that do not appear to be flakes should not be measured. Flakes that do not have the complete circumference of the flake visible, due to overlaying flakes, should also not be analysed. Additionally, only flakes that are flat on the surface, i.e. their surface shows no sign of curvature and is parallel to the surface, should be measured. Otherwise the thickness

measurement may be incorrect due to a large flake laying upon a smaller flake that is not directly observed.

The thickness of each flake should be determined by analysing three horizontal profiles (that is, along the fast scanning axis) of the flake and measuring the position on the substrate next to the flake and the position at the apex of the step in topography due to the flake, as shown in Figure 26b. If two straight horizontal lines can be reliably superimposed onto the flake surface and substrate surface, then the difference between the two lines should be used for a more accurate measurement, as in Figure 26c. However, due to issues with contamination or nanoobjects upon the flake and/or layered terraces within the flake itself, this may not be possible and so the method in Figure 26b should be used instead.

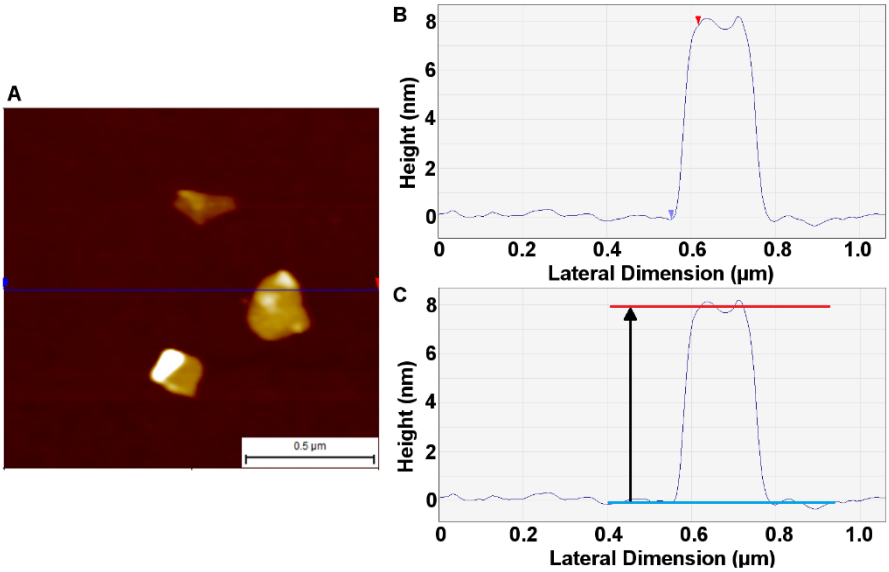

Figure 26: *(a) AFM topography image with the horizontal line profile (blue line) shown in (b) and (c), where (b) shows the two measurement points positions (blue and red triangles) and (c) shows the two line positions for determining the flake thickness.*

The height of the step from substrate to flake should be obtained from unprocessed data (that is, data directly measured from the Z-axis sensor) and used as the value of the thickness of flake. The standard deviation in the three values of thickness should be used as the associated uncertainty, unless this was less than the RMS roughness of the substrate, due to either the height resolution of the instrument or the actual roughness of the substrate or solvent residue, in which case the RMS roughness should be used. The uncertainty in the lateral size of each flake should be calculated from the lateral size of the slope of the edge of the flakes, as shown by the two measurement points in Figure 26b, and thus is due to either the lateral

measurement uncertainty of the instrument or the uncertainty in the location of the edge of the flake.

A graph should then be plotted for the sample, with at least 20 data points showing the thickness of the flakes (Y-axis) vs the average lateral dimension (X-axis), as shown in Figure 27.

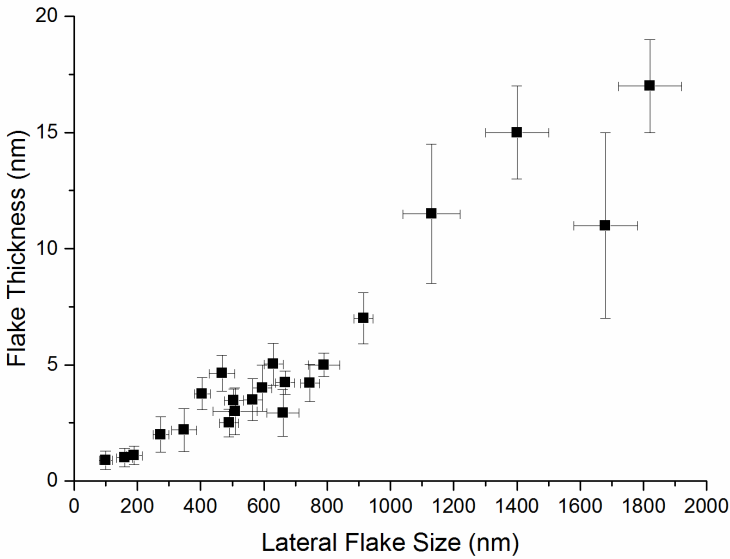

Figure 27: *Flake thickness vs lateral flake size from AFM measurement data for more than 20 flakes within a sample, spanning the range of lateral flake size found in initial SEM measurements.*

This value of thickness can be used to provide an estimate of the number of graphene layers in the flakes, with a thickness of ~0.34 nm corresponding to a single graphene layer [26]. However, there may be residue between the graphene and the substrate itself that has not been removed during the sample preparation stage, which should be taken into account when calculating the number of layers for flakes that may only be ~3 nm or less in thickness. The substrate positions of flakes of these dimensions should be recorded so that they can be investigated using Raman spectroscopy, to provide a more accurate determination of the number of layers, or otherwise Raman spectroscopy should be performed on the flakes using a combined AFM-Raman instrument.

If none of the measured flakes have a thickness of 3 nm or less, then Raman spectroscopy is not required and Section 3.5 should be referred to instead.

3.3.4 Raman spectroscopy of flakes

Raman spectroscopy can be used to provide a more accurate understanding of the number of layers of flakes than solely using AFM, which measures the flake thickness. As liquid dispersions typically lead to restacking of flakes when they are deposited on a substrate, it is imperative that only flakes previously assessed via AFM are probed, as the AFM topography maps can be used to confirm that only individual flakes are being investigated. If this accurate location and measurement of flakes cannot be achieved, the AFM thickness values should be used instead and Section 3.5 should be consulted.

For flakes identified through AFM measurements, Raman spectroscopy should be performed in a backscattering geometry to provide the best signal-to-noise ratio. The full details of calibration procedure, environment, laser excitation, laser power, laser spot diameter, spectral range and spectral resolution parameters that must be followed are detailed in Section 2.3.

To improve the accuracy of the thickness measurements of the flakes using AFM, Raman spectroscopy should be performed on all flakes with a thickness of ≤3 nm. Raman spectroscopy should also be performed on flakes with laterally larger than the Raman probe size so that a determination of the I_D/I_G intensity ratio can be determined without any contribution to the D-band from the edges of the flakes. If the flakes are smaller than the laser spot size, then I_D/I_G cannot be used to determine the defects concentration, because the D-peak due to the edges of the flakes will misrepresent the level of disorder in the flakes. At the same time if the flakes are too small there are practical implications of performing the measurement on the specific flake.

3.3.4.1 Measurement protocol

1. Secure the sample so that it is flat and will not be displaced with respect to the stage, when the stage is moved during sample scanning.
2. Measurement positions should be determined from the AFM measurements of selected flakes as previously described in Section 3.3.3.
3. After locating to the flake to be measured using optical microscopy and centering the probe position at the centre of the flake, set the Z-focus position by performing Raman spectroscopy measurements at different positions, with respect to the Z-axis, to determine the position of highest Raman signal.
    a. Approximate the focus position using optical microscopy, such that the substrate surface/flake is in focus.
    b. Set the instrument to measure 11 positions in the Z-axis, over a total travel area of 2 µm, which is 1 µm below the optical focus position to 1 µm above, using ≤1 mW laser power for a ≥500 nm diameter laser spot. A measurement time of 5 seconds should be used, but if this does not

provide a signal-to-noise ratio of at least 10 for the G- and 2D-peaks, a longer measurement time should be used to achieve this ratio.

4. Perform a Raman spectroscopy measurement at the position of highest Raman signal found in Step 3, with 10 second exposure, and 2 accumulations for an improved signal-to-noise ratio.

    a. The same laser power as in Step 3b should be used, to achieve a signal-to-noise ratio of 20. If the signal-to-noise ratio is less than 20 use a longer exposure time as required.

5. Repeat Step 4 for each flake at each measurement position as determined in Step 2, performing focusing Step 3 before each measurement.

6. After the spectra have been acquired, the data should then be analysed to determine the number of layers and level of disorder.

3.3.4.2 Data analysis

Firstly, the D- and G-peak area of interest (~1350 cm^{-1} and ~1580 cm^{-1} spectral positions respectively), and the 2D-peak (~2700 cm^{-1}) must be extracted for separate analysis. A baseline must be determined and substracted for each spectrum before peak fitting is performed, using Lorentzian peaks for the D-, G- and 2D-peaks, where the G-peak should only require 1 Lorentzian peak, as should the D-peak if it is present. Note that the D'-peak may also need to be taken into account, positioned at ~1620 cm^{-1} and present for defective graphene. At very high levels of disorder, the D'-peak merges with the G-peak, so they cannot be distinguished.

The key parameters that should be calculated for each spectra are:

- Peak intensity ratio for the G- and 2D-peaks, I_{2D}/I_G
- Peak intensity ratio for the D- and G-peaks, I_D/I_G
- The minimum number of Lorentzian peaks required to fit the 2D-peak

The value of I_D/I_G should be used as the measurand of the level of disorder of the graphene only for flakes that have lateral dimensions larger than the probe size of the Raman spectroscopy instrument (typically 500 nm - 1 µm). This is because the edges of flakes also produce a D-peak in the Raman spectra and so the measurements to determine I_D/I_G of the material must be taken at the centre of the flake where no contribution to the D-peak from the edges is recorded.

If a I_D/I_G ratio of >0.2 is observed, it is likely that chemical characterisation is required due to the possibility of functional groups being present, to allow a complete understanding of the material to be obtained. As described previously, chemical characterisation is beyond the scope of this guide. Additionally, this level of defects in a flake large enough that the flake edges are not being probed, yet thin enough that Raman spectroscopy has been decided upon

through Figure 20, may be indicative of electrochemical exfoliation or another process where functional groups are introduced. As such, the thickness of the flakes from the AFM measurements should be referred to instead of inferring the number of layers from Raman spectroscopy, as more studies are required before Raman spectroscopy can be used to reliably measure the number of layers of these flakes.

For individual flakes (as identified with AFM) that are Bernal-stacked, rather than restacked flakes when deposited from solution, the peak intensity ratio I_{2D}/I_G and the number of Lorentzian peaks required to fit the 2D-peak are used to determine the number of layers of the flake. For single layer flakes, the 2D peak can be described by a single Lorentzian peak, and if I_D/I_G is not significant, i.e. less than 0.2, $I_{2D}/I_G > 2$ is observed, as shown in Figure 28. However, $I_{2D}/I_G < 1$ and an asymmetric 2D-peak is observed for flakes with more than one layer, as shown for 2LG and FLG in Figure 28 and described in more detail in Ref. [27]. For flakes with a similarly-shaped 2D band as graphite and thus 3 or more layers, the thickness and associated uncertainty determined through AFM measurements should be used.

It should also be noted that issues in determining the number of graphene layers with Raman spectroscopy can be overcome through probing the C-modes [28] or other much weaker peaks [13] present for graphene that are related to the interaction of the graphene layers themselves in a flake. However, due to issues with requiring specialist filters or the signal-to-noise ratio of these peaks, these methods are not considered for flakes in this guide.

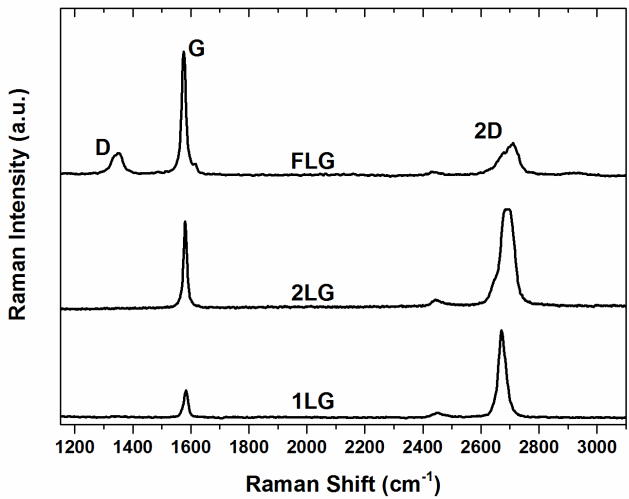

Figure 28: *Raman spectra for Bernal-stacked single layer graphene (1LG), bilayer graphene (2LG) and few-layer graphene (FLG) flakes, using a 532 nm excitation laser line. The I_{2D}/I_G Raman peak intensity ratio is greatest for 1LG and reduces as the number of layers increases. The 2D-peak is a single Lorentzian peak for 1LG, whereas the 2D-peak contains more than one Lorentzian peak for 2LG and FLG. The D-peak, typically due to the measurement of the edge of the flake, is also shown in the FLG spectra as an example.*

Using this method, the thickness of flakes in this sample that were determined using AFM can be correlated to the number of layers, as determined with Raman spectroscopy for flakes that had previously been measured using AFM.

3.4 TEM dimensional characterisation of flakes

In a transmission electron microscope (TEM) a high energy beam of electrons is passed through a thin electron transparent sample in a high vacuum environment, as described in more detail in Section 2.4.

TEM can be used to determine the lateral size and number of layers of flakes, as well as layer alignment, through diffraction contrast TEM imaging, lattice resolution imaging and selected area electron diffraction (SAED), which are achievable with most modern TEM instruments. It should be noted that for liquid-phase exfoliated graphene and/or graphite flakes, the presence of surfactants and common contaminants from the environment (H, C, O, Si, Na and Cl) can cause difficulties in imaging.

3.4.1 Measurement protocol

1. To reduce contamination the sample should be baked at ~150 °C for ~8 hours in a moderate vacuum immediately before loading the grid into the microscope.
2. Single or double tilt specimen holders can be used because it is not necessary to tilt the sample, it is possible to locate flakes lying flat on the support grid.
3. The TEM should be operated using an accelerating voltage of 80 kV or below in order to reduce the likelihood of knock-on damage degrading the sample during imaging.
    a. Note that although 80 kV is below the knock-on damage threshold for pristine graphene, defects and edges will have a lower threshold and have found to be susceptible to damage even at 80 kV.
    b. The quality of the vacuum in the microscope may also affect the damage susceptibility of graphene flakes (with UHV instruments seeing lower damage rates).
4. At moderate magnification in bright field (BF) TEM imaging mode, scan the grid until the first graphene flakes are identified. The positions that have been imaged should be tracked using the specimen tracking tools.
    a. If the flakes are very thin it is typically difficult to observe the flake.
    b. In BF TEM inserting a small objective aperture will improve the contrast relative to the carbon support film.

5. Record an image of the whole flake at a magnification that occupies most of the image with the flake itself, to allow the lateral flake size to be determined.

6. In TEM imaging mode, locate a flat region of the flake. Insert the smallest selected area aperture available and switch to diffraction mode. Remove the objective aperture if inserted and record the characteristic hexagonal selected area electron diffraction pattern from the sample.

    a. Long exposure times should be used if required to successfully record the diffraction pattern, due to weak intensity diffraction peaks for very thin flakes.

    b. To prevent damage of the CCD camera from the bright central beam use the beam stopper or acquire multiple exposures and average these with post processing.

7. 10 or more electron diffraction patterns should then be obtained for different areas of the flake.

    a. Note that the presence of the 0.34 nm (002) spots close to the central beam indicates that the flakes are folded and these areas should not be used for thickness determination.

    b. For each diffraction pattern the corresponding BF TEM image should also be recorded and an image of the position of the selected area aperture used in order to aid interpretation of the diffraction data.

    c. If the flake contains regions of different contrast it is necessary to take diffraction patterns from all these areas, using the SAED aperture to select different regions of interest.

    d. Note that the lateral dimensions of a SAED region are limited to approximately 100 nm (limited by spherical aberration which will introduce a small error in locating the region of the sample from which the selected area diffraction data originates). Consequently for very small flakes nanobeam diffraction mode should be used.

8. Remove the objective aperture and increase the magnification to perform lattice resolution characterisation of the graphene flakes.

    a. Note that in the some TEMs it is not possible to resolve the lattice fringes. The resolution limit is poorer at lower accelerating voltages and the manufacturer specification for the appropriate resolution limit should be consulted.

b. For bilayer or thicker samples, the 0.34 nm (002) fringe spacing is observed in folded regions of the flake, where these planes lie parallel to the incident electron beam. These should be imaged to confirm the flake thickness. As some flakes may include regions of differing thickness, how these lattice resolution images relate to the flake morphology observed in the low magnification images and to the electron diffraction patterns should be recorded. Additionally, if there is an image rotation associated with the increase in magnification this should also be recorded.

9. After measuring the flake, the next flake in this same grid square should be imaged.

    a. A total of 200 flakes should be measured per sample.

    b. Every flake within the grid square should be imaged before moving on to the next square.

    c. If every grid square does not need to be imaged to produce the desired number data points in Step 9a, squares with large separations between them should be selected (not only the squares closest to the centre) in case of local variations due to drop casting conditions.

    d. For each flake identified repeat the intermediate magnification imaging (Step 4 and 5), electron diffraction (Steps 6 and 7) and lattice resolution imaging (Step 8)

3.4.2 Data analysis

The characteristic hexagonal electron diffraction pattern obtained when graphene is viewed along the [001] direction, as shown in Figure 29, is an unambiguous fingerprint that the material is 1LG/2LG/FLG/graphite. To distinguish single layer graphene from bilayer and few-layer graphene with TEM electron diffraction, the intensities of the first and second ring of the diffraction spots should be compared [21, 22]. For single layer graphene the intensity of the outer hexagon spots is equal to or less than that of the inner one. In contrast, for bilayer graphene the outer hexagon intensity is higher than the inner one. Figure 29a and Figure 29b show diffraction patterns for single layer and bilayer graphene flakes respectively, with an intensity profile corresponding to between the arrows of the same colour. For thicker samples the outer spots increase further in intensity relative to the inner ones.

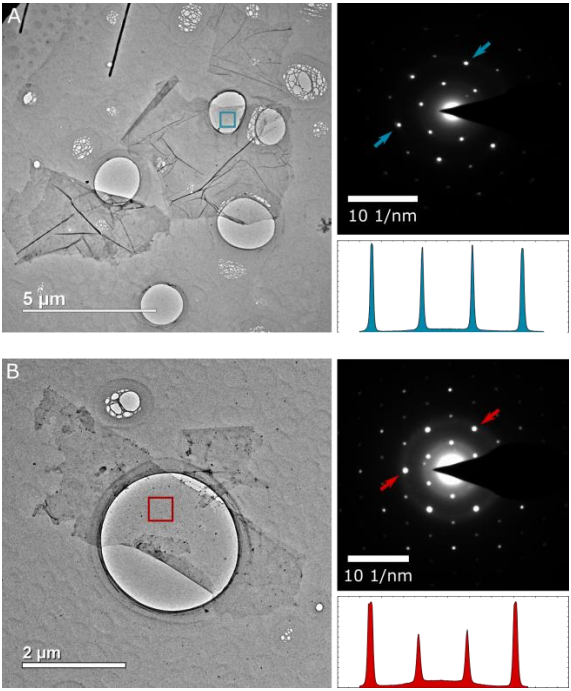

Figure 29: *BF TEM images, alongside diffraction patterns taken from the marked areas, of (a) single layer and (b) bilayer graphene. Intensity profile plots taken between the arrows are shown below the diffraction pattern.*

Electron diffraction can identify single layer, bilayer and few-layer graphene but cannot determine the precise number of layers. This can be performed if lattice resolution (0.34 nm) TEM/STEM imaging is achievable through observation of folded edges of the flakes at Scherzer defocus. The layer number should be measured by counting the bright lines at the edge, as shown in Figure 30 (and Figure 10). If difficulties are encountered during focussing, a focal series should be obtained to provide evidence of correct focussing. Liquid-phase exfoliated flakes are often thicker than CVD-grown graphene sheets so many more fringes are typically visible at the edge of the flakes, as can be observed in Figure 30.

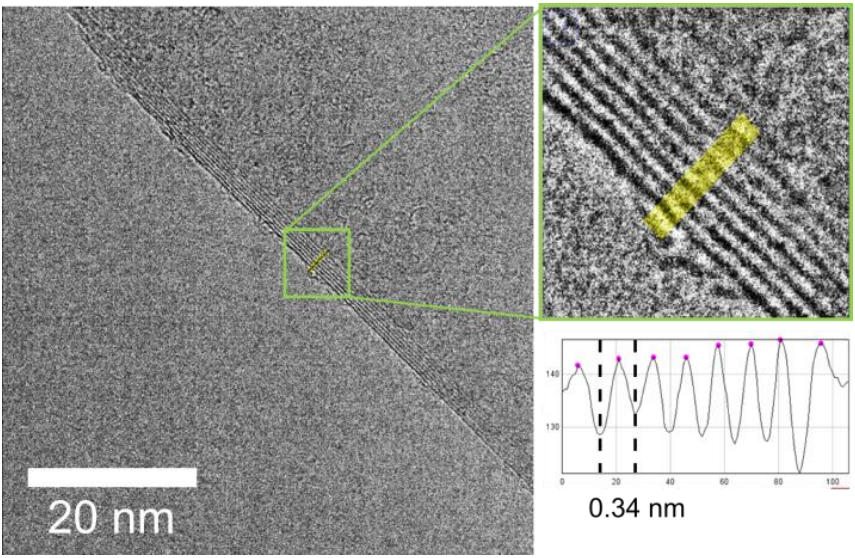

Figure 30: *BF TEM images of the edge of a flake. Higher magnification allows the number of layers to be determined directly from an intensity profile. In this case the dark fringes are counted, as Scherzer defocus is uncorrected, thus the flake is found to be 7 layers thick.*

Individual liquid-phase exfoliated flakes can often contain regions with variable thickness. In these cases it is necessary to take an average of the thickness measurements obtained from diffraction and high resolution imaging.

Despite efforts to reduce restacking during flake deposition, liquid-phase exfoliated samples often contain restacked flakes. These restacked flakes tend to be randomly oriented relative to each other so restacking can be identified by the presence of multiple diffraction spots. In this case 12 (or 18 or 24) spots are present in each diffraction ring rather than the 6 observed for single layer graphene or Bernal stacked graphite. Restacking of flakes, as well as folding of the individual flakes, produce Moiré patterns of large periodicity and diffraction data similar to turbostratic graphene, as shown in Figure 31.

The lateral flake size can be measured directly from the BF TEM images. For each observed flake measure the length and the width (perpendicular to the length) and then take the average of the two values for the average lateral size, as shown in Figure 32.

Once the lateral size of 200 flakes or more and the number of layers of at least 20 flakes, covering the range of lateral flake sizes observed, has been determined, a histogram of the lateral flake sizes should be produced and a scatter plot of flake thickness vs lateral flake size, as shown in Section 3.5. The measurement method should then be recorded as 'TEM'.

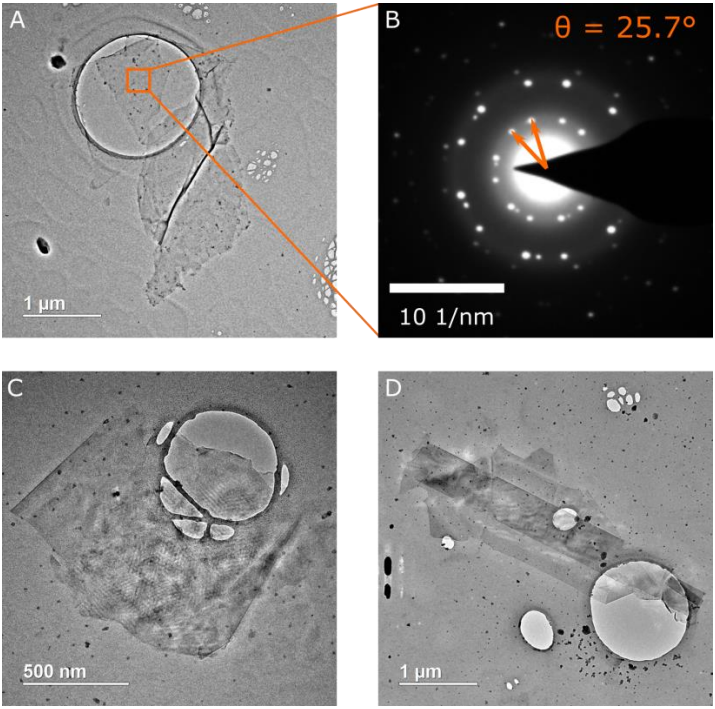

Figure 31: *TEM image of turbostratic graphene in (a), with a diffraction pattern showing 12 spots in (b). The angle measured is 25.7°. A folded graphene flake in (c) and a flake that has rolled up into a scroll-like structure in (d) both show Moiré patterns.*

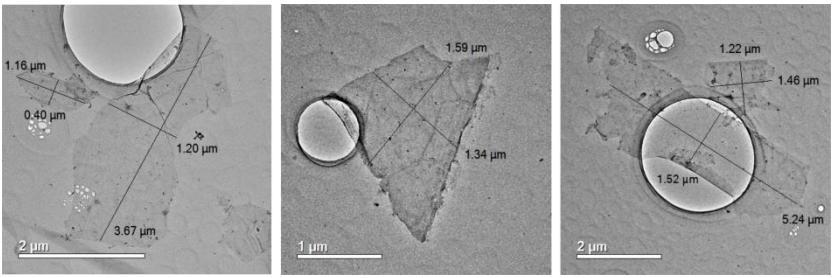

Figure 32: *BF TEM images with annotated measurements of the lateral sizes of typical liquid-phase exfoliated flakes.*

3.5 Graphene lateral size and yield calculation

The average lateral flake size and flake thickness data measured for a range of flakes should be used to calculate the percentage yield of either graphene flakes or few-layer graphene (and thinner) flakes, alongside the calculated median lateral flake size and lateral flake size range. This data should be provided from either the SEM and AFM measurements (Sections 3.3.2 and 3.3.3 respectively) or the TEM measurements (Section 3.4). Furthermore, if Raman spectroscopy data has been obtained (Section 3.3.4), this will improve the accuracy of the yield calculation for the SEM and AFM data. As there are three different combinations of techniques that can be used for calculating the graphene flake lateral size and thickness, the method(s) used to obtain the final results should be reported as either 'TEM', 'SEM/AFM' or 'SEM/AFM/Raman'. Note that the level of disorder should also be reported.

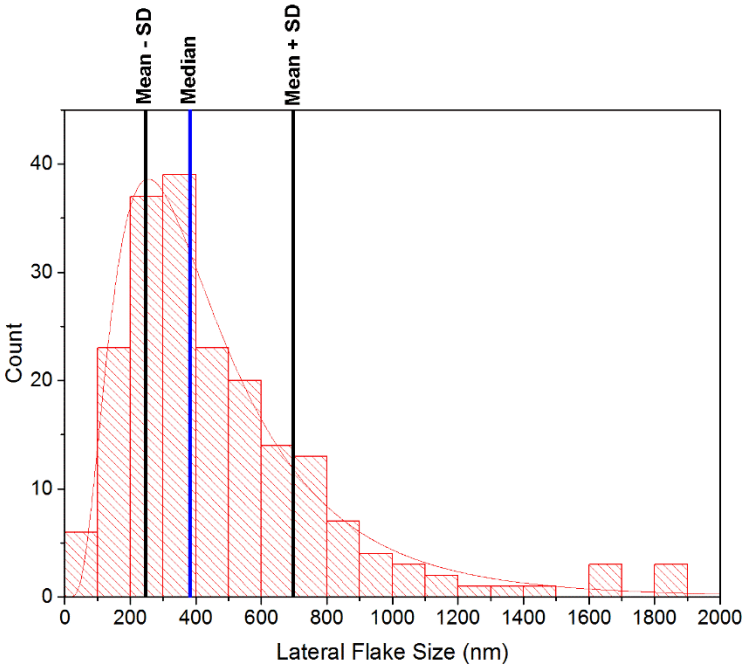

Figure 33: *Example histogram of average lateral flake size with a log-normal distribution plotted as a red line, median value as a vertical blue line and 68 % of values fall within the two vertical black lines (standard deviation from the log-normal mean).*

Firstly, the lateral flake size and lateral flake size range should be calculated from the average lateral flake size data, as shown in Figure 33 with a log-normal plot performed for the histogram of lateral flake size and vertical lines displaying the associated values to be

recorded. The log-normal plot should be used to calculate the median lateral flake size for the sample, this is then used as the recorded lateral flake size. The standard deviation from the mean of the log-normal distribution is then used as the range of lateral flake size.

The thickness vs lateral flake size data must then be plotted and a curve fit of the data should be performed. Typically, a linear positive correlation between flake thickness and lateral flake size is observed, as shown in Figure 34, due to the production method of top-down liquid-phase exfoliation that is commonly used. From the fitted data, a lateral flake size that corresponds to the thickness of a graphene layer (0.34 nm) should be calculated. The lateral flake size that corresponds to the thickness of ten layers of graphene (3.4 nm), i.e. the maximum thickness of few-layer graphene, should also be calculated. Note that it if no flakes of these thicknesses or below were measured the yield should be assessed as 0 %, as shown for 1LG in Figure 34. Furthermore, if no correlation is observed, then more thickness measurements should be performed to determine if there is a positive correlation, or if the material being investigated has no correlation between lateral flake size and thickness. For the latter conclusion, over 200 thickness versus lateral size measurements must have been performed, such that the thickness values can be used as a percentage yield for 1LG and FLG. If there is no correlation observed after thickness measurements for over 200 flakes, 'no correlation' should be reported alongside the method of measurement.

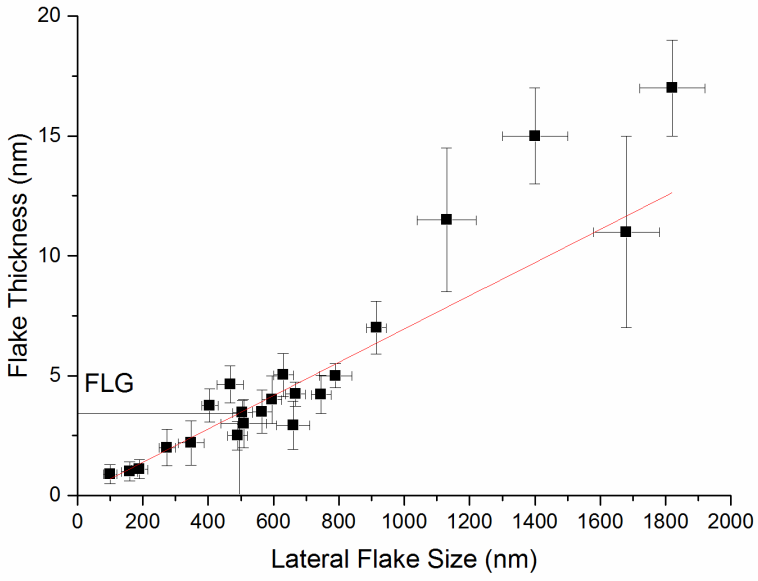

Figure 34: *Example of how to calculate the lateral flake size that corresponds to the 1LG and FLG flake thickness.*

Once the two lateral flake size values corresponding to 1LG and FLG have been obtained, they should be compared to the distribution of average lateral flake sizes. The 1LG or FLG lateral flake size value should be used to find the percentage of flakes that are equal to or lower than that value. The values should be superimposed on to the lateral flake size histogram as vertical lines, as shown in Figure 35. Figure 35 shows the lateral flake size data from Figure 33 and uses the example thickness vs lateral flakes size data shown in Figure 34.

Using the example of Figure 35, there is a histogram with 20 bins, summing to a total of 200 flakes measured, across the range of 0 to 2000 nm in lateral flake size. From the thickness data, the lateral flake size value for FLG and below is 495 nm. Therefore flakes that are FLG include 100 % of the first four bins and 95 % of the fifth bin, which is 15 of the 200 flakes accounted for and therefore 63.4 % of the total number of flakes are few-layer graphene (or thinner), which is value of the yield of FLG.

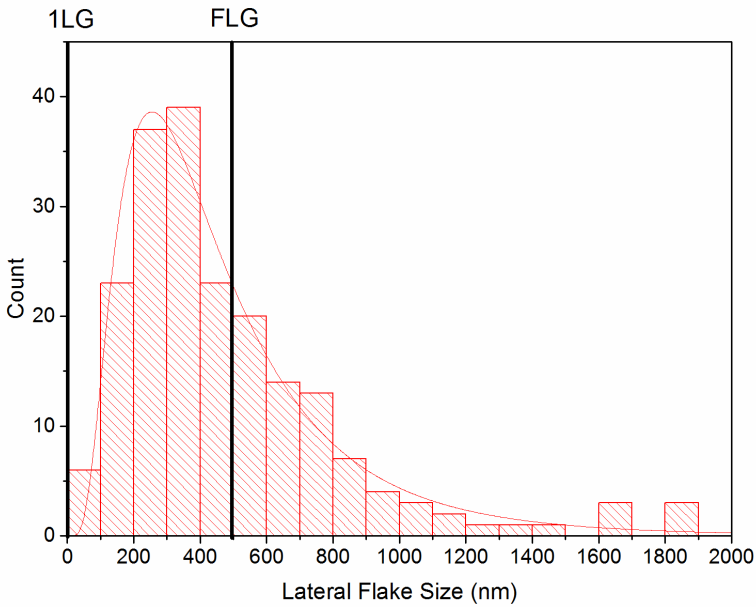

Figure 35: *Example of the calculation of the 1LG and FLG yield from a lateral flake size histogram.*

However, for the case where Raman spectroscopy has been performed on flakes of known AFM thickness and the analysis of Raman spectra has shown that flakes thicker than 0.34 nm correspond to single layer graphene, the lateral sizes for 1LG and FLG should be recalculated accordingly. For example, if flakes measured as 1.0 nm with AFM are shown to be single layer graphene with Raman spectroscopy, with a corresponding 0.34 nm step height for subsequent

layers (i.e. 2LG is ~1.34 nm and 3LG is ~1.68 nm thick), the number of layers, N, can be calculated using the equation below, where the 1LG thickness equals 1.0 nm:

$$N = \frac{[Flake\ Thickness\ (nm)] + 0.34\ nm - [1LG\ Thickness\ (nm)]}{0.34\ nm}$$

Therefore, the average value of the lateral flake size for 1LG now becomes 150 nm and the lateral flake size for FLG becomes 633 nm, as shown in Figure 36.

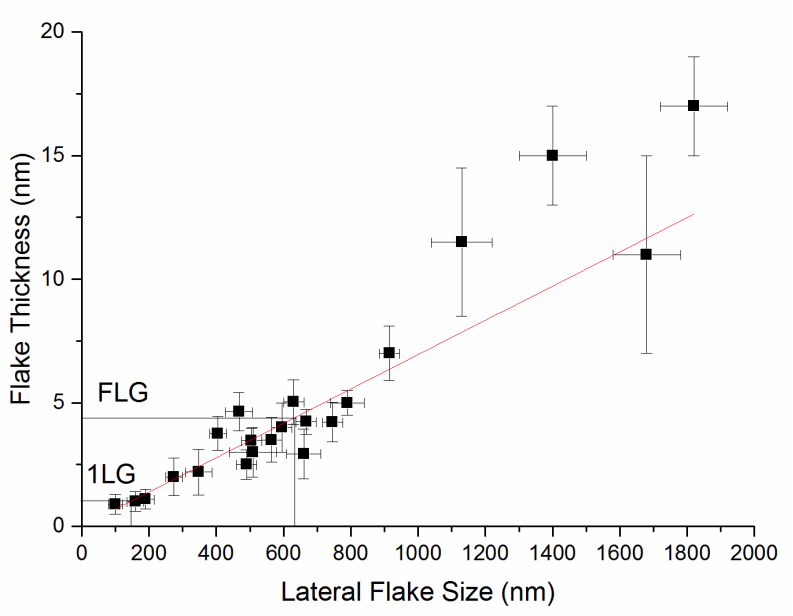

Figure 36: *Using the Raman spectroscopy measurements, Figure 34 is recalculated to produce new average values of the lateral flake size for 1LG and FLG.*

These results also require a recalculation in terms of yield, as shown in Figure 37. From this recalculation, the 1LG yield is equal to 8.4 %, the FLG yield is 76.3 %.

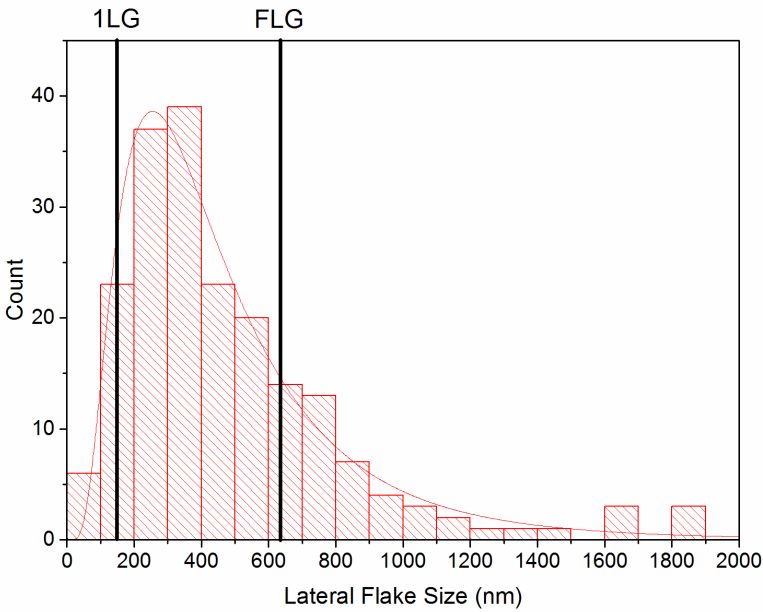

Figure 37: *Changes in assignment of 1LG and FLG lateral flake sizes in Figure 36 lead to changes in Figure 35 and revised values for the yields.*

To determine the level of uncertainty in the yields, the standard error in the slope calculated from the linear curve fit of the thickness vs lateral flake size (i.e. the red line in Figure 34 or Figure 36) must be used. In the case of Figure 36, the slope is 0.0070 ± 0.0003 i.e. the standard error is 4.3 %. In the case of Figure 37, for FLG this means that the yield must be calculated for 633 ± 27 nm lateral flake size, that is, 606 nm and 660 nm, to provide the associated uncertainty, in this case a yield of 74.4 % and 78.2 % respectively. This produces a final FLG yield of 76 ± 2 %. It should be noted that this uncertainty only considers random error and not any systematic error. As shown from the change in FLG yield from 63 % to 76 % (much larger than the calculated standard error) due to the recalculation of number of layers vs thickness from the Raman spectroscopy data, it is evident that understanding how the thickness measured by AFM relates to the number of layers is a key issue when considering the yield uncertainty. Thus, whether Raman spectroscopy has been used to improve the accuracy of the results or not is included in the results themselves (i.e. 'SEM/AFM' or 'SEM/AFM/Raman'). This is a perfect example of why users must perform a thorough and careful analysis of graphene flakes, as detailed in this guide, to produce reliable results.

Chapter 4: Conclusions and acknowledgements

4.1 Conclusions

Using this guide, users with previous characterisation expertise within industry can more reliably, quantitatively and comparably measure the structural properties of commercially-supplied graphene.

This guide can be used to measure the properties of both CVD-grown graphene and samples containing graphene flakes, determining the substrate coverage, percentage of single layer graphene, level of disorder and layer alignment for the former, as well as the lateral flake size range and corresponding thickness along with the level of disorder for the latter.

This guide will form the basis for future international standards in this area, specifically, standards currently under development within ISO/TC 229 'Nanotechnologies' for the characterisation of the structural properties of graphene in different forms. This guide will also be used for international interlaboratory comparison studies, particularly through VAMAS TWAS 41 'Graphene and Related 2D Materials'. This will lead to the continual improvement in the measurement of the structural properties of graphene, as well as reveal any reproducibility issues in performing these graphene measurements in different laboratories across the world.

4.2 Acknowledgements

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Characterisation of the Structure of Graphene

Good Practice Guide No. 145

Due to the many superlative properties of graphene, there are many application areas where these exciting nanomaterials may be disruptive, areas such as flexible electronics, nanocomposites, sensing, filtration membranes and energy storage. A major barrier is the inability to accurately measure or describe the material properties of the many different real-world 'graphene' products now available commercially. This guide describes the best practice for characterising the structural properties of graphene using a series of measurement techniques. Measurement protocols are detailed for both CVD-grown graphene on a substrate and graphene flakes present in a dispersion or powder form. The physical properties such as the coverage, number of layers/thickness, lateral flake size, the level of disorder, layer alignment, as well as the distribution and relationship of these properties, are determined through these protocols, which also include details on sample preparation and data analysis.

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