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Nanotechnologies — Structural characterization of graphene —

Part 1: Graphene from powders and dispersions

Nanotechnologies — Caractérisation structurelle du graphène — Partie 1: Graphène issu de poudres et de dispersions

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Foreword

ISO (the International Organization for Standardization) and IEC (the International Electrotechnical Commission) form the specialized system for worldwide standardization. National bodies that are members of ISO or IEC participate in the development of International Standards through technical committees established by the respective organization to deal with particular fields of technical activity. ISO and IEC technical committees collaborate in fields of mutual interest. Other international organizations, governmental and non-governmental, in liaison with ISO and IEC, also take part in the work.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives or www.iec.ch/members_experts/refdocs).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO and IEC shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents) or the IEC list of patent declarations received (see patents.iec.ch).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html. In the IEC, see www.iec.ch/understanding-standards.

This document was prepared jointly by Technical Committee ISO/TC 229, *Nanotechnologies*, and Technical Committee IEC/TC 113, *Nanotechnology for electrotechnical products and systems*.

A list of all parts in the ISO/IEC 21356 series can be found on the ISO and IEC websites.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html and www.iec.ch/national-committees.

Introduction

Due to the many superlative properties of graphene and related 2D materials, there are many application areas where these nanomaterials could be disruptive, areas such as flexible electronics, nanocomposites, sensing, filtration membranes and energy storage.

There are barriers to commercialisation that are impeding the progress of products containing graphene, which need to be overcome. One of these crucial barriers is answering the question "What is my material?". End-users of the raw materials containing graphene should be able to rely on the advertised properties of the commercial graphene on the global market, instilling trust and allowing worldwide trade. Reliable and repeatable measurement protocols are required to address this challenge.

This document provides a set of flow-charts for analysts to follow in order to determine the structural properties of graphene from powders and liquid dispersions (suspensions). Initially, a quick check should be undertaken to determine if graphene and/or graphitic material is present. If it is, then further detailed analysis is required to determine if the samples contain a mixture of single-layer graphene, bilayer graphene, few-layer graphene, graphene nanoplatelets and graphite particles. Depending on the methods used, the samples are typically analysed after deposition on a substrate. The document describes how to assess what measurements are required depending on the type of sample and includes decision trees and flow diagrams to aid the user. This document describes a selected set of measurands that are needed, namely:

a) the number of layers/thickness of the flakes;

b) the lateral dimensions of flakes;

c) layer alignment;

d) the level of disorder;

e) the estimated number fraction of graphene or few-layer graphene;

f) the specific surface area of the powder containing graphene.

The above physical properties of the material can change during its processing and lifetime, for example, the samples can become more agglomerated, obtain different surface functionalities. The above measurand list for the initial material defines their inherent characteristics that, along with the chosen manufacturing processes, will determine the performance of real-world products. Generally, different material properties can be important in different application areas, depending on the functional role of the material.

The document provides methods for structural characterization of individual flakes of graphene, bilayer graphene, graphene nanoplatelets and graphite particles isolated from powders and/or liquid dispersions. It does not provide methods for determination of whether the powders and/or dispersions are composed solely of these materials. No recommendation is provided as to when or how often to measure samples, although it is not expected this would be for every batch of the same material. It is up to the user to determine when, how often and which characterization routes described in this document to take. As with all microscopical investigations, care is needed in drawing statistical conclusions dependant on representative sampling.

A set of annexes provide example protocols on how to prepare and analyse the samples, sources of uncertainty and how to analyse the data. The methods used are Raman spectroscopy, scanning electron microscopy (SEM), atomic force microscopy (AFM), transmission electron microscopy (TEM) and the BET (Brunauer–Emmett–Teller) method.

Nanotechnologies — Structural characterization of graphene —

Part 1: Graphene from powders and dispersions

1 Scope

This document specifies the sequence of methods for characterizing the structural properties of graphene, bilayer graphene and graphene nanoplatelets from powders and liquid dispersions using a range of measurement techniques typically after the isolation of individual flakes on a substrate. The properties covered are the number of layers/thickness, the lateral flake size, the level of disorder, layer alignment and the specific surface area. Suggested measurement protocols, sample preparation routines and data analysis for the characterization of graphene from powders and dispersions are given.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO/TS 80004-1:2015, *Nanotechnologies — Vocabulary — Part 1: Core terms*

ISO/TS 80004-2:2015, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

ISO/TS 80004-6:2021, *Nanotechnologies — Vocabulary — Part 6: Nano-object characterization*

ISO/TS 80004-13:2017, *Nanotechnologies — Vocabulary — Part 13: Graphene and related two-dimensional (2D) materials*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-1:2015, ISO/TS 80004-2:2015, ISO/TS 80004-6:2021, ISO/TS 80004-13:2017 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
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) (3.3) and *few-layer graphene* (FLG) (3.4).

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

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.1]

3.2
graphite
allotropic form of the element carbon, consisting of *graphene layers* (3.1) stacked parallel to each other in a three-dimensional, crystalline, long-range order

Note 1 to entry: Adapted from the definition in the IUPAC Compendium of Chemical Terminology.

Note 2 to entry: There are two primary allotropic forms with different stacking arrangements: hexagonal and rhombohedral.

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.2]

3.3
bilayer graphene
2LG
two-dimensional material consisting of two well-defined stacked *graphene layers* (3.1)

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

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.6]

3.4
few-layer graphene
FLG
two-dimensional material consisting of three to ten well-defined stacked *graphene layers* (3.1)

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.10]

3.5
graphene nanoplate
graphene nanoplatelet
GNP
nanoplate consisting of *graphene layers* (3.1)

Note 1 to entry: GNPs typically have thickness of between 1 nm to 3 nm and lateral dimensions ranging from approximately 100 nm to 100 µm.

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.11]

3.6
lateral size
flake size
<2D material> lateral dimensions of a 2D material flake

Note 1 to entry: If the flake is approximately circular then this is typically measured using an equivalent circular diameter or if not via x, y measurements along and perpendicular to the longest side.

[SOURCE: ISO/TS 80004-13:2017, 3.4.1.15]

3.7
graphene oxide
GO
chemically modified *graphene* (3.1) prepared by oxidation and exfoliation of *graphite* (3.2), causing extensive oxidative modification of the basal plane

Note 1 to entry: Graphene oxide is a single-layer material with a high oxygen content, typically characterized by C/O atomic ratios of approximately 2,0 depending on the method of synthesis.

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.13]

3.8
reduced graphene oxide
rGO
reduced oxygen content form of *graphene oxide* (3.7)

Note 1 to entry: This can be produced by chemical, thermal, microwave, photo-chemical, photo-thermal or microbial/bacterial methods or by exfoliating reduced *graphite* oxide.

Note 2 to entry: If graphene oxide was fully reduced, then *graphene* (3.1) would be the product. However, in practice, some oxygen containing functional groups will remain and not all sp^3 bonds will return back to sp^2 configuration. Different reducing agents will lead to different carbon to oxygen ratios and different chemical compositions in reduced graphene oxide.

Note 3 to entry: It can take the form of several morphological variations such as platelets and worm-like structures.

[SOURCE: ISO/TS 80004-13:2017, 3.1.2.14]

4 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply.

NOTE The final "M", given as "microscopy", can be taken equally as "microscope" depending on the context.

1LG	single-layer graphene
2D	two dimensional
2LG	bilayer graphene
AFM	atomic force microscopy
BET method	Brunauer–Emmett–Teller method
CVD	chemical vapour deposition
FLG	few-layer graphene
FWHM	full-width at half maximum
GNP	graphene nanoplate or graphene nanoplatelet
GO	graphene oxide
NMP	1-methyl-2-pyrrolidinone also known as N-methylpyrrolidone
rGO	reduced graphene oxide
SAED	selected area electron diffraction
SEM	scanning electron microscopy
TEM	transmission electron microscopy

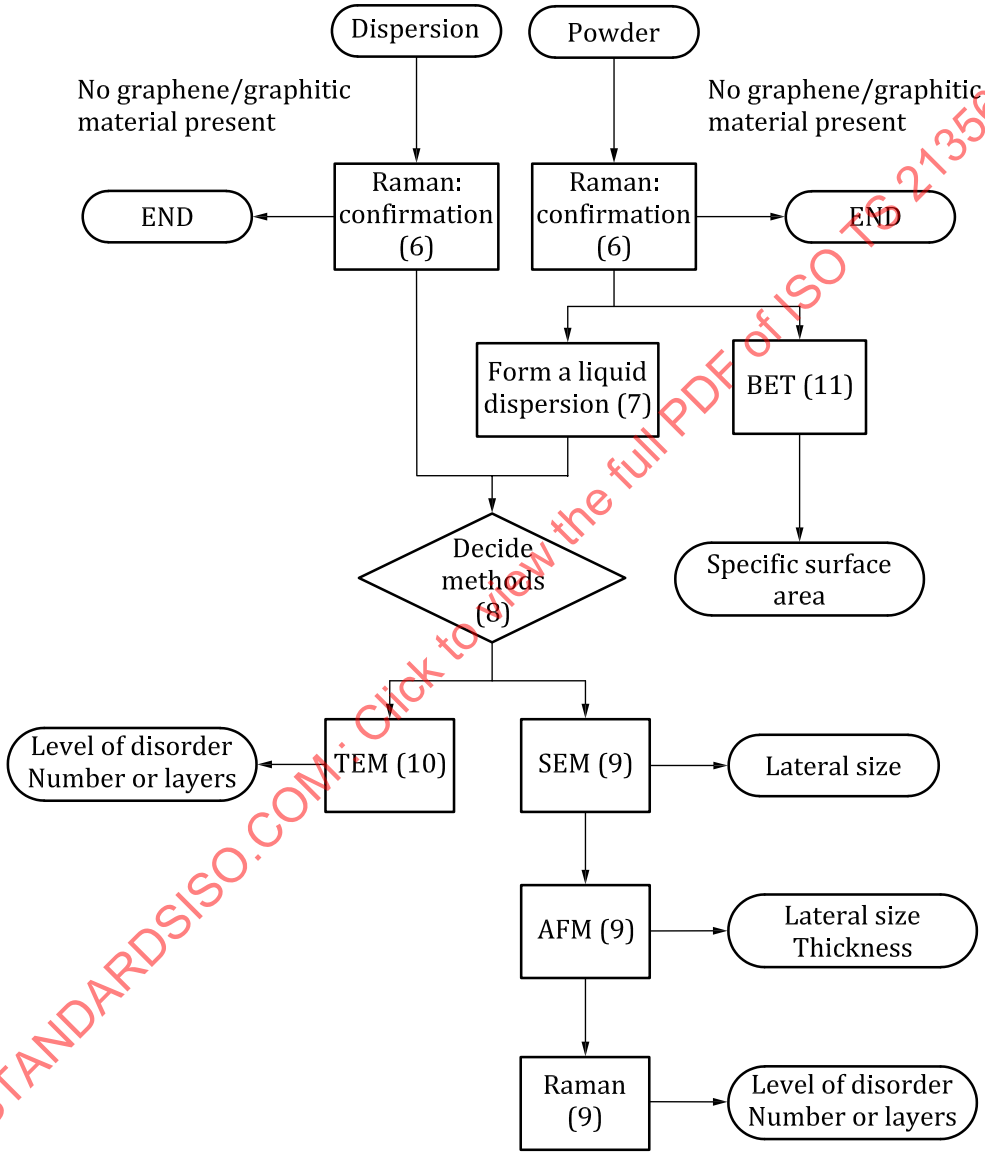
5 Sequence of measurement methods

This clause presents the sequence of measurement methods necessary to most efficiently characterize graphene, bilayer graphene, few-layer graphene and graphene nanoplatelets from powders and liquid dispersions (suspensions). In this document, graphene, bilayer graphene, few-layer graphene and

graphene nanoplatelets are in the form of flakes of limited lateral dimensions. However, samples also typically contain significant amounts of flakes having thicknesses that exceed ten layers, which are flakes of graphite by definition.

After an initial examination by Raman spectroscopy, and assuming the sample is graphene or graphitic in nature, a more detailed characterization should follow. Various characterization routes are then possible, as shown in Figure 1. The characterization method or methods to be used will depend on the time and equipment available and the measurands that the user requires.

NOTE 1 As the flakes are from a powder or liquid dispersion, they will typically require deposition onto a substrate before analysis.



NOTE The numbers in brackets refer to the clauses where the item is detailed.

Figure 1 — Overview of the sequence and process of the measurement methods used to determine the structural properties of graphene from a powder or liquid dispersion sample

Firstly, determine if the sample contains graphene and/or graphitic material, that is bilayer graphene, FLG, GNPs or graphite by undertaking a rapid analysis using Raman spectroscopy, as detailed in Clause 6 and Annex A. The sample needs to be in powder form deposited as a thin layer on a substrate, therefore if a liquid dispersion has been supplied, the material will first need to be removed from the solvent, as detailed in A.2.

Decide which methods to use as outlined in Clause 8. Either use TEM or a combination of SEM, AFM and Raman spectroscopy to determine the distribution of lateral flake sizes and the relationship with flake thickness. For this stage, clearly separated flakes on a substrate are required. To prepare these samples by deposition, a liquid dispersion is initially required, therefore, if the material was provided as a powder, it requires dispersing in a suitable solvent, as described in Clause 7, before subsequent deposition onto a suitable substrate with example procedures outlined in Annexes B and C.

If TEM is used (see Clause 10), prepare the sample on a TEM support grid as outlined in C.2, otherwise prepare the sample on a silicon dioxide on silicon substrate, B.2. Then use optical microscopy as a quick quality check to determine if the sample is too agglomerated and therefore cannot be accurately measured. Optimize the sample preparation until an even deposition of the material across the substrate occurs. Then undertake a combination of SEM, AFM and Raman spectroscopy measurements (see Clause 9 and Annex B) or TEM (see Clause 10, Annex C). SEM, AFM and Raman spectroscopy should be used in combination and not in individual isolation in order to determine the measurands listed in Figure 1.

If required, use BET to determine the specific surface area of the powder (see Clause 11 and Annex E).

Once all the necessary measurements have been undertaken, calculate the median lateral flake size, the range of flake sizes, the graphene 1LG number fraction and FLG number fraction, as discussed in Clause 12 and Annex D. Here, number fraction is the fraction by number of graphene or FLG over the total number of flakes, this can also be expressed as a percentage.

NOTE 2 It is assumed that the sample contains graphene/2LG/FLG/graphite. If the sample has different chemistries, for example contains graphene oxide or functionalised graphene, this will not produce the same Raman spectroscopy results as those described in this document. However, optical microscopy, SEM and AFM characterization of lateral dimensions and thicknesses (but not number of layers) can still be applied to these materials.

NOTE 3 There is currently no quantitative or standardised method for determining the specific surface area of the graphitic material when the sample is in or from a liquid dispersion form.

6 Rapid test for graphitic material using Raman spectroscopy

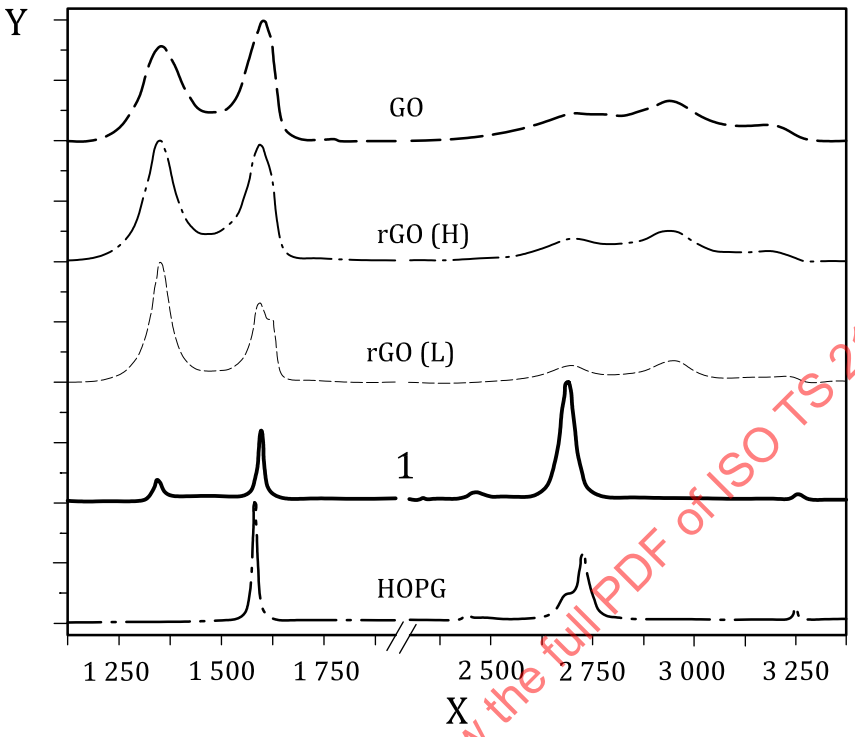
Firstly, test the sample, in powder form deposited on a substrate using Raman spectroscopy to determine whether the sample supplied contains graphene and/or graphitic material. This test can also provide qualitative information on the structural properties of the material, including the level of disorder and the dimensions of the flakes. If the sample is supplied in a liquid dispersion, then remove the liquid from the dispersion and analyse the sample in powder form.

A thin layer of powder is required for this rapid Raman spectroscopy analysis step. If a powder has been provided, this should be analysed with a significant amount of the sample secured on adhesive tape (see A.2) such that only the flakes rather than the substrate are analysed.

A measurement protocol and sample preparation method are detailed in Annex A.

To confirm the presence of graphitic material, a sharp (< 30 cm^{-1} full width at half maximum (FWHM)) G-peak at approximately 1 580 cm^{-1} and a 2D-peak (sometimes referred to as the G' peak) at approximately 2 700 cm^{-1} should be consistently observed in the Raman spectra as shown in the graphene spectrum in Figure 2. If an intense symmetric Lorentzian peak shape is found for the 2D-peak with close to or greater intensity than the G-peak, this suggests the sample could contain single-layer graphene. However, restacked few-layer graphene flakes can also show a single Raman 2D-peak. If the 2D-peak is not symmetric, this suggests flakes of multiple layers are present. A prominent shoulder in the 2D-peak is indicative of layered material, with a thickness of over ten graphene layers (i.e. graphite). If the G- and 2D-peaks are not present, further characterization is not required, as the sample does not contain graphene or graphite, however, a sufficient ratio of the Raman peak signal to background noise (S/N) ratio should be established before this conclusion can be made, see Annex A for example details. To improve the S/N ratio, longer acquisition times can be used, or averaging multiple scans with short acquisition times can be used.

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 for graphene. Here, additional chemical characterization should be undertaken to determine the oxygen content and any other components, which if found to be high means that the material is out of the scope of this document.



Key

X Raman shift, cm^{-1}

Y normalized intensity, arbitrary units

1 graphene

Figure 2 — Example Raman spectra of highly oriented pyrolytic graphite (HOPG), graphene, reduced graphene oxide with lower oxygen content [rGO(L)], reduced graphene oxide with higher oxygen content [rGO(H)] and graphene oxide (GO)

This step should not be confused with the processes used later for measurement of individual flakes with AFM and Raman spectroscopy (detailed in Clause 9 and Annex B) after further sample preparation.

NOTE 1 Adhesive tape is specified to stop the powder moving for both health and safety reasons and to stop possible electrostatic attraction and contamination of the lens.

NOTE 2 Chemical characterization of graphene including thermogravimetric analysis (TGA) and X-ray photoelectron spectroscopy (XPS) will be detailed in a further ISO document in development at the time of publication of this document.

NOTE 3 Other methods such as X-ray diffraction (XRD) can be used to determine the presence of graphitic material. Raman spectroscopy is used here as a rapid confirmation step, as Raman spectroscopy is also required for the detailed analysis of individual flakes (see Clause 9).

7 Preparing a liquid dispersion

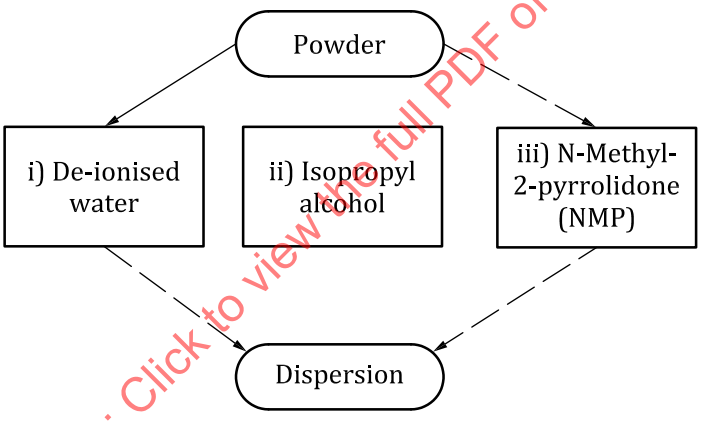
7.1 General

For further, more detailed characterization of the sample, the flakes should be prepared such that they are isolated on a substrate. This allows the next characterization steps, as shown in Figure 1, to be performed either using a combination of SEM, AFM and Raman spectroscopy with the sample on a silicon dioxide on silicon substrate, or TEM with the sample on a TEM grid. For the preparation of the flakes on a substrate, initially a liquid dispersion is required, therefore, if the material is provided as a powder, it requires dispersing in a suitable solvent.

7.2 Preparing a dispersion of the correct concentration

7.2.1 Powder samples

Disperse the powder in a solvent such that a concentration of approximately 0,1 mg/ml is achieved. The suitability of the solvent should be determined through the observation of how quick and how much, if any, sedimentation of the material occurs. There are a number of different solvents that can be used. Use the solvent that will disperse the powder and allow flakes to be characterized with the minimum of unwanted residue on the surface. The order of preference of three solvents is given in Figure 3.



NOTE An order of preference of the solvent to be used is outlined.

Figure 3 — Flowchart for the creation of a dispersion

Firstly, try to disperse the powder with deionised water. Place the liquid and powder in a glass vial or bottle and agitate. Sonicate the dispersion for up to a maximum of 10 min in a table-top ultrasonic bath at 30 kHz to 40 kHz. Longer sonication times can cause changes to the structural properties, including basal-plane scission (reducing the lateral size) and further exfoliation (reducing thickness/layer number). Observe the dispersion over a period of several minutes. If a significant amount of sedimentation occurs and occurs quickly then repeat the procedure using a different solvent.

If deionised water does not disperse the material, isopropanol should be used as the solvent using the same method. If this does not work, then N-methylpyrrolidone (NMP) should be used as the solvent as graphene disperses well in this. However, due to the high boiling point of NMP (203 °C), this can affect the characterization results in the form of solvent residue.

The deposition of the material onto a substrate is detailed in Clauses 9 and 10 and in particular in Annexes B and C.

Typically, graphene flakes will stay dispersed in deionised water only if a stabilizing agent, such as a surfactant, is present as part of the manufacturing process. However, it should be noted that significant use of surfactants can influence both the sample condition and the later measurement of the materials, see examples in B.2.

Using a significant amount of ultrasonication to disperse the material can induce flake scission and therefore affect the structural characterization 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. A comparison of flake size measurement as a function of the amplitude and duration of ultrasonication can be undertaken to check if flake scission occurs and to optimize sonication conditions, if required.

NOTE ISO/TS 22107[1)] provides general guidance on the definition of dispersibility and deals with processing and the achieved final dispersed state.

7.2.2 Samples already in a dispersion

If the sample is already provided as a dispersion, this should be diluted to approximately 0,1 mg/ml using the same solvent. However, if the solvent is a water/surfactant mix, the dilution should be carried out using deionised water, to reduce the level of surfactant.

NOTE In cases where the concentration of the dispersion provided is not known, the dilution needs to be approximated. This concentration is chosen such to produce dispersed flakes in solution and individual flakes on the substrate when cast.

8 Determination of methods

For detailed characterization, two characterization routes are possible, as shown in Figure 1. Determine whether to use a combination of SEM, AFM and Raman spectroscopy measurements (see Clause 9 and Annex B) or use TEM (see Clause 10 and Annex C). For powder samples, BET can be used to determine the specific surface area, as described in Clause 11 and Annex E. Which method or methods are used depends on the time and equipment available and the measurands that the user requires.

For either set of the microscopy methods, the samples shall be prepared firstly as a dispersion, as detailed in Clause 7, and then deposited on the correct substrate as discussed in B.2 or C.2.

9 Structural characterization using optical microscopy, SEM, AFM and Raman spectroscopy

This clause details the sequence of measurements to determine lateral flake dimensions, associated flake thickness, level of disorder and number of graphene layers using a combination of SEM, AFM and Raman spectroscopy. Use the methods as ordered in Figure 4.

1) Under preparation. Stage at the time of publication: ISO/DTS 22107:2021.

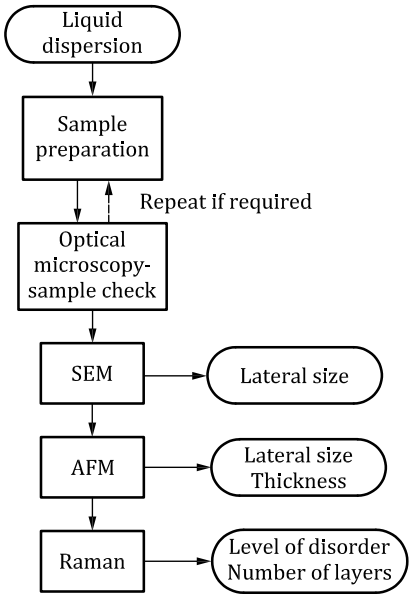


Figure 4 — Flow diagram and decision-making process for determining the range of lateral dimensions, thickness of flakes, number of layers and level of disorder

Firstly, the sample should be prepared from a liquid dispersion and placed on an appropriate substrate. Use optical microscopy to check the sample preparation. Once an appropriate sample has been produced, use SEM, AFM and Raman spectroscopy to characterize the sample and analyse the results to extract the measurands as detailed in Figure 4.

Sample preparation methods, measurement protocols and data analysis protocols are outlined in Annex B.

NOTE 1 The SEM measurements are performed on a different substrate and use different flakes to the AFM and Raman spectroscopy measurements.

NOTE 2 ISO 19749[2)] provides guidance for measuring size and shape distribution of nanoparticles including general principles, sample preparation, qualification of the SEM, image acquisition, particle and data analysis.

10 Structural characterization using TEM

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.

TEM can be used to determine the lateral size and number of layers in 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 flakes, the presence of surfactants and common contaminants from the environment (H, C, O, Si, Na and Cl) can cause difficulties in imaging.

Users should consult ISO 21363:2020 for useful information on instrument set up and particle analysis.

2) Under preparation. Stage at the time of publication: ISO/PRF 19749:2021.

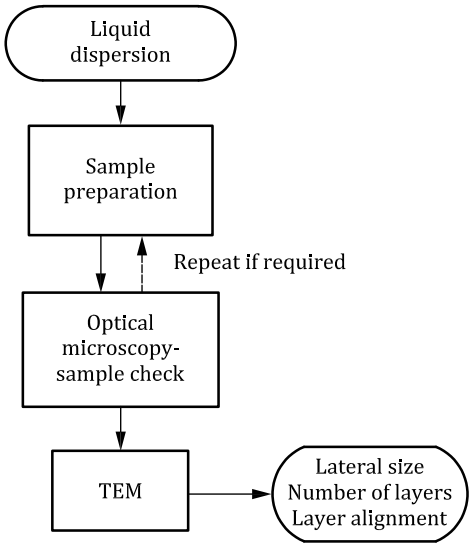


Figure 5 — Flowchart for TEM to determine lateral size, number of layers and layer alignment

Follow the order of operations as detailed on Figure 5 to determine the lateral size, number of layers and layer alignment of different flakes. The flakes should be deposited onto an appropriate TEM grid from a dispersion. Optical microscopy should be used to check the sample preparation and positions of flakes prior to analysis in the TEM. After TEM, the data should be analysed to determine the required measurands.

A sample preparation method, measurement protocol and data analysis method are detailed in Annex C.

11 Surface area determination using the BET method

The Brunauer–Emmett–Teller (BET) method determines the total specific surface area of disperse powders by measuring the amount of physically adsorbed gas. It utilizes the model developed by Brunauer, Emmett and Teller for interpreting gas adsorption isotherms. Use the BET method to determine the specific surface area of a powder sample.

A sample preparation method, measurement protocol and data analysis procedure are detailed in Annex E.

12 Graphene lateral size and number fraction calculation

Analyse the data produced from the dimensional characterization. Calculate the median lateral flake size, the range of flake sizes, the graphene 1LG and FLG number fraction and report which techniques are used to do this. A method to calculate this data is given in Annex D.

Annex A
(informative)

Rapid test for graphitic material using Raman spectroscopy

A.1 General

This annex details possible sample preparation steps and a measurement protocol for a rapid test to confirm the presence of graphene, bilayer graphene, graphene nanoplatelets (GNPs) and/or graphite using Raman spectroscopy.

A.2 Sample preparation

A.2.1 Sample preparation from a liquid dispersion

a) In a vacuum filtration kit, use a membrane with pore size of ≤0,2 µm to ensure that majority of the smaller flakes are retained on the membrane.

   1) The material of the membrane needs to be compatible with the solvent used to make the dispersion.

   2) Alumina or cellulose membranes should be used for common graphene solvents such as water, isopropanol or NMP.

b) A pressure of ~100 mbar should be applied for the vacuum filtration step.

c) Collect the dried material on top of the filter at the end of the process as a supported or free-standing graphene film.

   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 amount of dispersion will be required to provide a film that can be handled.

NOTE There is no need to accurately measure the film thickness; if the signal from the material is not high enough to perform the analysis in A.3, then the film is not thick enough.

A.2.2 Sample preparation from powder form

a) Before handling a powder sample of nano-objects, an appropriate risk assessment should be performed and the required engineering controls, personal protective equipment and safety processes employed.

b) Place double-sided adhesive tape on to a clean microscope slide.

c) Deposit a small amount of the powder on the adhesive tape, pressing down lightly with a spatula to ensure adhesion. Adhesive tape is specified to stop the powder from moving for both health and safety reasons and to stop possible electrostatic attraction of the powder to the microscope lens and hence contamination of the lens.

   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.

d) Once the material is secured on the adhesive tape, excess and unsecured material should be removed by tapping the microscope slide vertically. To prevent dust being raised, the material

should be collected onto a wet paper towel. An example is shown in Figure A.1. As described above, sufficient material, such as shown in Figure A.1 should be deposited to provide a strong Raman signal. If a signal is observed from the substrate, then more material should be deposited.

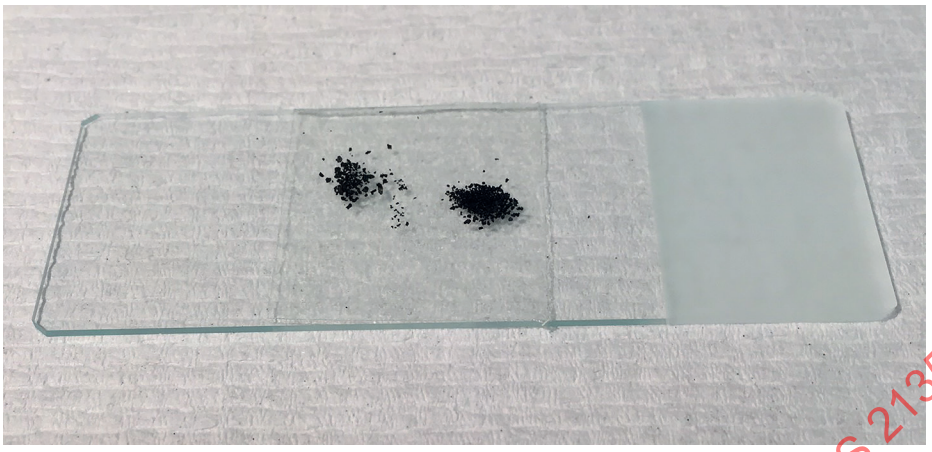


Figure A.1 — Photograph of a powder containing graphene deposited onto adhesive tape

NOTE An alternative sample preparation method would be to press the powder into a pellet.

A.3 Method

Raman spectroscopy should be undertaken in a backscattering geometry with preferably a 50 × or 100 × objective lens (NA ≥ 0,75). The system should be calibrated prior to measurements using the user's best practice. A red (typically 633 nm) or green (typically 532 nm or 514 nm) excitation laser should be used. The positions of 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-band (~1 350 cm^{-1}), G-band (~1 580 cm^{-1}), 2D-band (~2 700 cm^{-1})] and associated widths are included, so for example from 1 200 cm^{-1} to 3 000 cm^{-1}.

After locating a measurement area with the aid of optical microscopy, set the Z-focus position such that the surface of the powder is in focus. Perform a single Raman spectroscopy measurement with a laser power of less than 1 mW incident on the sample so as to minimize the damage to the sample, with an exposure of 5 s to 10 s and two accumulations. This should provide a Raman peak intensity to background noise (S/N) ratio of at least 10. If not, a longer measurement time can be used to increase the S/N ratio.

Measurements should be performed from a minimum of three different areas of the sample to understand the local variation across the sample as the material is generally in the form of aggregates.

To confirm the presence of graphene and/or graphite, a sharp (< 30 cm^{-1} FWHM) G-peak at ~1 580 cm^{-1} and a 2D-peak at ~2 700 cm^{-1} should be consistently observed in the Raman spectra. If an intense symmetric Lorentzian peak shape is found for the 2D-peak with close to or greater intensity than the G-peak, this suggests the sample contains single-layer graphene. However, restacked few-layer graphene flakes can also provide a single Raman 2D-peak. If the peak is not symmetric this suggests multiple layers are present. A prominent shoulder in the 2D-peak is indicative of layered material, with a thickness of over ten graphene layers (i.e. graphite). If the G- and 2D-peaks are not present, further characterization is not required, as the sample does not contain graphene or graphite, however, a sufficient S/N ratio should be established before this conclusion can be made.

Measurements of powders containing graphitic material also typically reveal a D-peak at approximately 1 350 cm^{-1}, as shown in Figure 2, due to flake edges activating the D-band as well as basal plane defects. The intensity ratio of the D-peak relative to the G-peak (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. Measure the I_D/I_G ratio and compare to the results of the later characterization methods subsequently used, following the flowchart shown in Figure 1.

NOTE If functionalised graphene or graphene oxide is present, Raman spectroscopy shows the D- and G-peaks, but not necessarily a 2D-peak, and the D- and G-peaks have much larger FWHM values (>30 cm^{-1}) than expected, for example, see References [5] and [6]. However, other carbon materials can also have these peaks, and so it is recommended that chemical characterization is performed separately (details will be provided in an ISO document on chemical characterization of graphene in development at the time of publication of this document).

Annex B
(informative)

Structural characterization protocol using SEM, AFM and Raman spectroscopy

B.1 General

This annex details a set of measurement protocols to determine lateral flake size using SEM, lateral flake size and thickness using AFM and the level of disorder and number of layers using Raman spectroscopy. Sample preparation should be undertaken as detailed in B.2 prior to analysis as detailed in B.3 through to B.5.

B.2 Sample preparation

B.2.1 Drop casting for SEM, AFM and Raman spectroscopy

To enable the measurement of flake dimensions for multiple flakes using optical microscopy, SEM, AFM or Raman spectroscopy, the prepared dispersion should be deposited onto two types of substrate, one substrate for SEM and one substrate for AFM and Raman spectroscopy.

For SEM, a silicon wafer with a thin native oxide should be used as a substrate. The oxide should be thin enough to ensure good conductivity and prevent charging while imaging using SEM. For AFM and Raman spectroscopy, a silicon wafer with a silicon dioxide layer of thickness of 300 ± 5 nm or 90 ± 5 nm should be used in order to maximize the optical contrast between the flakes and the substrate.

For all three methods, the flakes should be deposited in such a way that a substantial fraction of them are isolated from each other. For Raman spectroscopy, a flake should only be analysed if it is clearly separated from another by a distance of approximately 1 µm. This is to avoid the analysis of more than one flake at a time by the optical beam.

The procedure for deposition is as follows.

a) Prepare a stable dispersion as detailed in Clause 7.

b) The substrate should be cleaned as described in Annex F.

c) Place the cleaned substrate on a hot plate and set the temperature to be greater than the boiling point of the solvent used for the dispersion.

d) Thoroughly mix the dispersion by shaking and then quickly extract a representative sample into a pipette. Drop-cast a small volume of the dispersion onto the substrate (typically between 10 µl and 100 µl is sufficient). A well-dispersed layer of flakes should then be left on the surface. Examine under optical microscopy (see B.2.2) and determine whether the sample is suitable for analysis. If it is not, then the sample preparation process should be repeated with different concentrations and/or volumes of dispersion and/or different solvents. An example of a good sample is shown in Figure B.1.

e) The sample should then be left in a vacuum oven for 2 h or more at 40 °C in order to reduce solvent and/or surfactant residue.

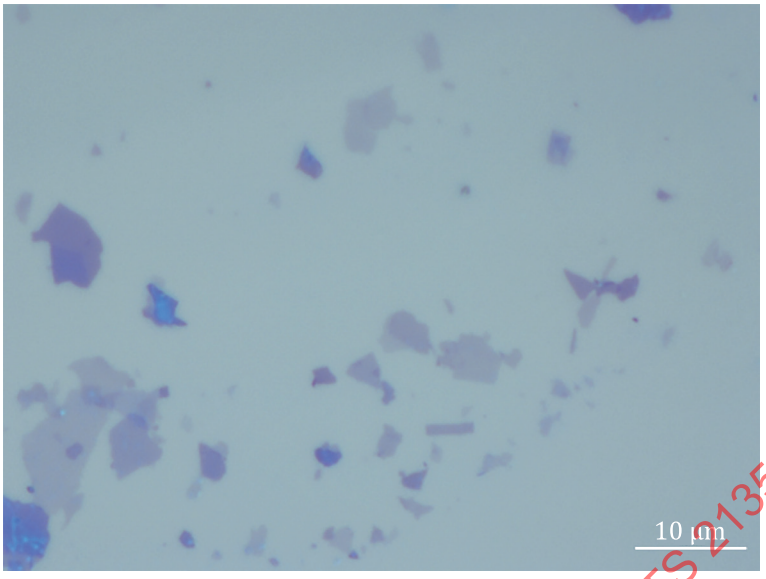


Figure B.1 — Optical microscopy image of 1LG/2LG/FLG and graphite flakes deposited on a 90 nm thick layer of silicon dioxide on silicon wafer to give an example of a well-prepared sample

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

B.2.2 Optical microscopy

Use optical microscopy at appropriate magnifications for a rapid assessment of the suitability of the prepared sample for further analysis. To be able to measure the lateral size and thickness of the flakes, the flakes should be as separated as possible, while also being as abundant as possible. This allows SEM and AFM measurements to be performed at a faster rate while also ensuring that the values obtained are for individual flakes and without contributions from other flakes.

Examples of flakes deposited onto a silicon dioxide on silicon substrate are shown in Figure B.1. 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 B.2.

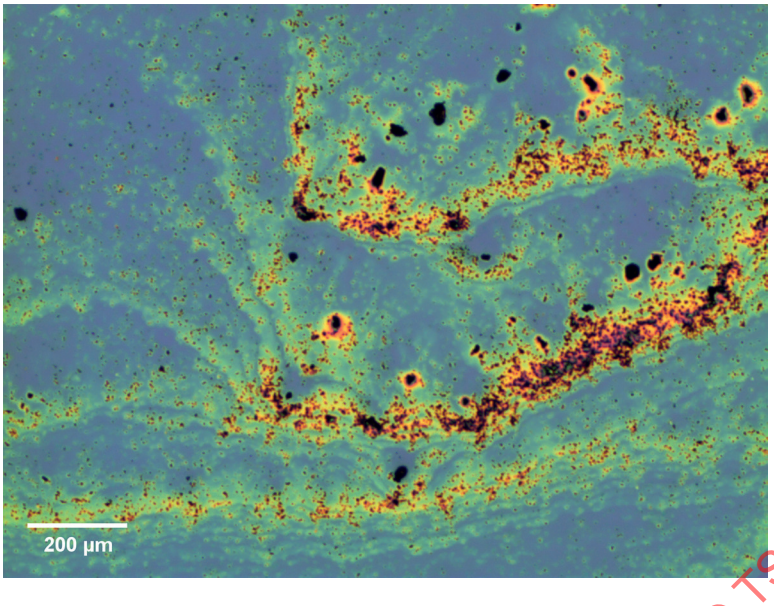


Figure B.2 — Example of an optical microscopy image of residue left on a silicon dioxide on silicon substrate after deposition of a liquid dispersion of flakes, as shown by areas of varying colour

An optical image of a sample that cannot be used after preparation due to agglomeration of material is shown in Figure B.3. Additionally, a substrate with little suitable material present will greatly reduce the rate of characterization and should also be avoided. Different objective 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, for example, due to too much solvent residue, then the sample preparation process should be repeated with different concentrations of dispersion and/or different solvents, as described in Clause 7 and B.2.1 and as shown in Figure 3.

If the material contains flakes with lateral dimensions that are large enough to resolve using optical microscopy, as shown in Figure B.1, 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 with the SEM data. Similarly, if individual flakes are not visible because they are too small, this allows for some assessment of the range of lateral flake sizes present.

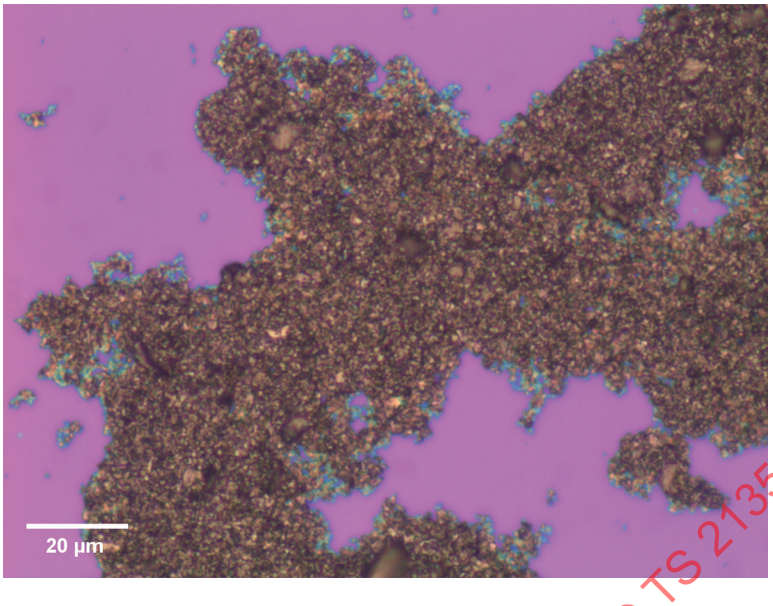


Figure B.3 — Example of an optical microscopy image of a dispersion of flakes prepared on a silicon dioxide on silicon substrate that cannot be used for determining the dimensions of the flakes using other techniques due to agglomeration

NOTE 1 It is possible to estimate the thickness of a flake on a silicon dioxide on silicon 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. For a thickness of silicon dioxide of 90 nm or 300 nm, even a single-layer has been found to give sufficient contrast to allow few micrometre-sized graphene crystallites to be identified among copious thicker flakes scattered over a millimetre-sized area. This is explained in Reference [7].

NOTE 2 If the flake sizes are all large, for example bigger than 1 µm, then an automated optical microscopy method can be used to image the graphene flakes and hence determine a flake size distribution.

B.3 SEM analysis

B.3.1 General

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. SEM has a lateral resolution of tens of nanometres which is a much higher lateral resolution than that of optical microscopy.

Measurement issues that could occur include charging effects and deposition of carbonaceous material on the surfaces imaged by the electron beam. These issues are unlikely to be fully overcome but can be reduced through good sample preparation. As previously mentioned, the flakes should be deposited onto a silicon substrate that only has a thin 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 undertaken on separate silicon dioxide on silicon substrates that have been prepared in the same way as described in B.2.1. This is done to avoid any contribution of added carbonaceous material to the measured flake thickness.

More than 200 flakes should be imaged. These should be well separated from each other to enable clear measurement of lateral flake size. The lateral dimensions of the flakes should be determined for at least 200 randomly chosen flakes where all analysable flakes in a given image should be included. The measurement protocol is outlined in B.3.2 and the data analysis in B.3.3.

B.3.2 Measurement protocol

a) Ensure that the SEM has been dimensionally calibrated using traceable calibrated dimensional calibration standards. The calibration should be valid for the working distance and accelerating voltage used in the subsequent measurements.

b) Mount the substrate into the SEM holder so that it is held rigidly and is well-supported, secure the sample in the SEM and pump down to vacuum.

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.

c) Set the SEM configuration to image by collecting secondary electrons, typically with an accelerating voltage of 5 kV or less to minimize charging.

d) Identify the location of the sample surface through SEM imaging and optimize 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.

This optimization 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 optimization will affect the area.

e) Low magnification images (~10 000 × magnification) should then be obtained for areas that contain flakes.

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.

f) Images of greater resolution and magnification should then be obtained for the flakes to allow subsequent image analysis. The magnification should be chosen such that the whole flake is in the field of view.

1) Higher magnification images reduce the uncertainty in lateral size measurement, that is, the flakes should occupy a significant part of the image and the number of pixels should be sufficient such that the lateral distance between pixels (specimen pixel size) is less than 10 nm.

2) Images can be taken of multiple flakes at the same time as shown in Figure B.4 or for specific flakes, with an aim to maximize the number of isolated flakes imaged.

3) Importantly, a representative distribution of flakes of different sizes from across the substrate surface shall be obtained. Selective selection of, for example, easier-to-image larger flakes will drastically skew the final results. Therefore, a range of magnifications should be used and different areas of the sample shall also be imaged.

g) Repeat steps e) and f) until more than 200 randomly chosen flakes have been imaged such that at least 200 isolated flakes can be analysed.

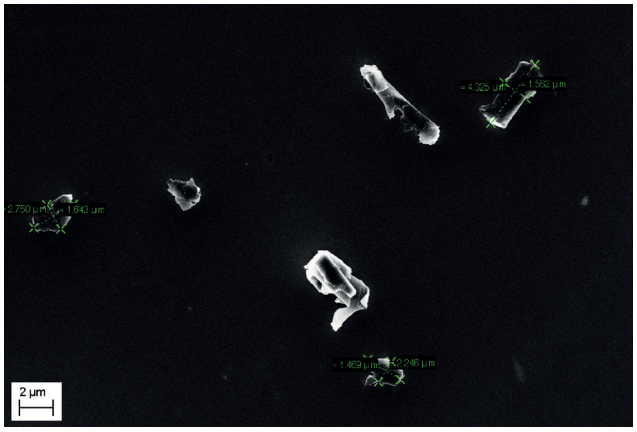
NOTE ISO 19749[3)] provides a general reference of guidance for measuring size and shape distribution of nanoparticles including general principles, sample preparation, qualification of the SEM, image acquisition, particle and data analysis.

B.3.3 Data analysis

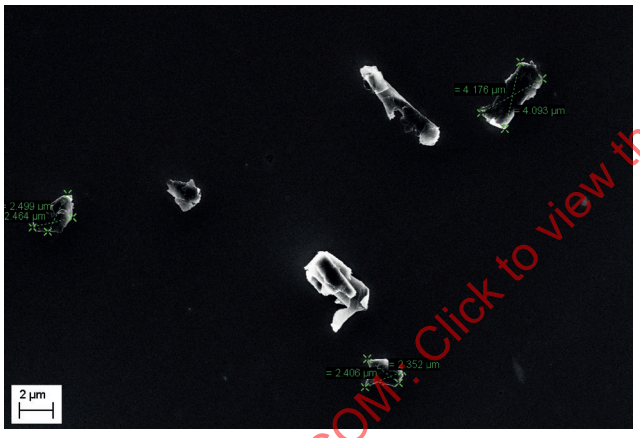
Once more than 200 flakes have been imaged, the lateral dimensions of the flakes should be determined for at least 200 randomly chosen flakes where all analysable flakes in a given image should be included. The lateral size of each flake is determined by measuring first the length and then the width (perpendicular to the length measurement) of the flakes and calculating the mean of the two values. Care should be taken so that the first (length) measurement of the flake also allows a representative width measurement to be obtained. The width should be taken as the perpendicular bisector of the

3) Under preparation. Stage at the time of publication: ISO/PRF 19749:2021.

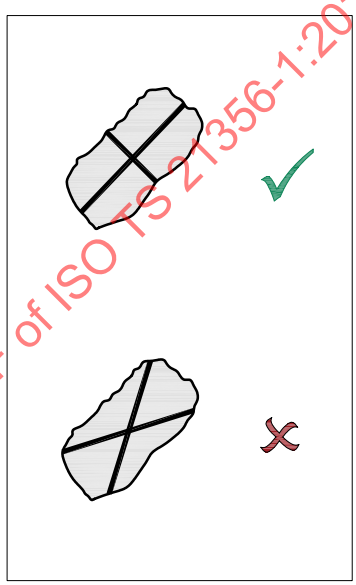
length, even where this is not the maximum perpendicular dimension. An example of how a flake should and should not be measured is shown in Figure B.4. Where the flake shape does not offer a clear choice of length by eye, the maximum Feret diameter can be taken as the length and the minimum Feret diameter can be taken as the width.



a) Correct analysis, by first taking the length and then finding the width as a perpendicular bisector



b) Incorrect measurement

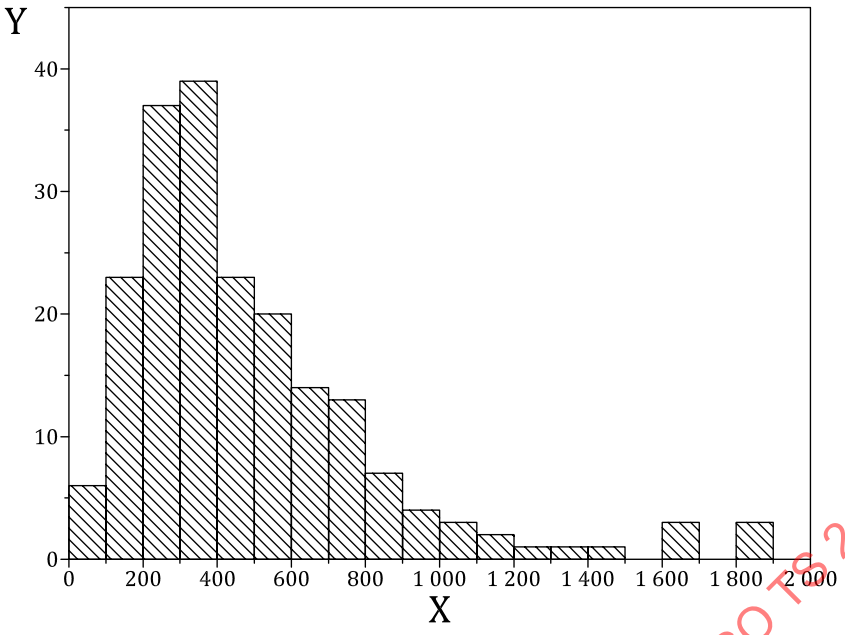


c) Schematic showing correct and incorrect measurements

Figure B.4 — Examples of lateral flake size measurements performed for a SEM image

Items 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. Flakes that are touching each other should also not be measured.

Once the lateral size of at least 200 flakes have been calculated, they should then be represented as a histogram with at least 20 bins. An example histogram is shown in Figure B.5.



Key

X lateral flake size, nm

Y number of flakes

Figure B.5 — Example histogram of the range of lateral flake sizes of a graphitic material, determined through SEM measurements

The largest source of bias in the determination of the average lateral flake size is typically due to the user and the flakes they choose to analyse, 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, but rather analyses all flakes that are possible in a given image.

The user should also record an uncertainty for each lateral size measurement, taking issues such as the flake shape, image pixel separation and instrumental calibration into account.

B.4 AFM analysis

B.4.1 General

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 height allows the imaging of the topography of a surface with nanoscale lateral and height resolution. When on a flat surface, such as silicon dioxide on silicon, the dimensions of a graphene flake including the thickness can thus be determined.

The thickness values can be obtained alongside the lateral size measurements, typically from SEM measurements, such that any correlation between these properties can be determined.

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 with AFM. For example, if SEM results reveal flakes with lateral sizes between 100 nm and 10 µm, then the AFM measurements should be undertaken on flakes with lateral sizes ranging between 100 nm and 10 µm.

B.4.2 Measurement protocol

a) The AFM system should be operated in a closed loop mode and the AFM scanners dimensionally calibrated in X, Y, Z directions using traceable, calibrated lateral grids and step height standards. Operate the AFM according to normal operating procedures using intermittent contact mode in ambient conditions.

b) The sample should not have been previously been used for SEM and should consist of flakes deposited onto a silicon dioxide on silicon substrate of oxide thickness of approximately 300 nm or 90 nm. Use the sample preparation method that has been shown by SEM to produce a large number of isolated flakes. Mount the sample into the AFM so that it is held rigidly.

   1) The sample could 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.

   2) If using a combined AFM-Raman system, Raman spectroscopy measurements (see B.5) can be undertaken straight after AFM on the same flake.

c) 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.

   Typical parameters for the cantilever are spring constant ~40 N/m, resonant frequency 240 kHz, and a probe apex size of 5 nm to 15 nm.

d) 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.

e) 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.

f) 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.

   1) A 20 µm × 20 µm scan size generally 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. If flakes are larger than the maximum scan size of the AFM, then SEM or optical techniques should be used to characterize the flakes.

   2) A scan-line speed of less than 10 µm/sec with a resolution of 256 pixels × 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.

   3) 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.

      1) 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.

      2) 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.

g) After a larger scan has been completed, AFM topographic images of the areas of interest (i.e. individual flakes) within the larger scan should be obtained, using a smaller scan size, chosen so as to encompass the individual flakes.

   Typical scan size is 2 µm and the number of pixels per image should be increased to 512 pixels × 512 pixels to allow for increased precision during data analysis.

h) Repeat step g) for the other areas of interest observed in the larger AFM image obtained in step f).

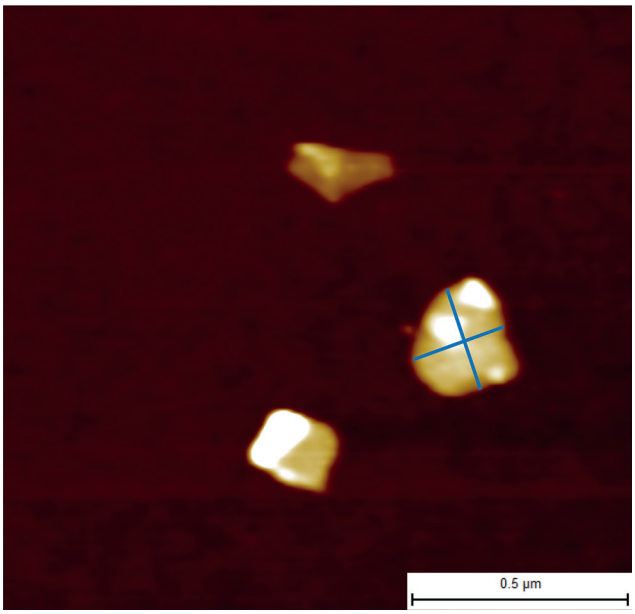
i) Repeat steps f), g) and h) so that a minimum of 20 flakes with lateral flake sizes that cover the range of lateral sizes shown by SEM have been reliably imaged.

j) The data should then be analysed to determine the thickness of the flakes versus the lateral size of the flakes.

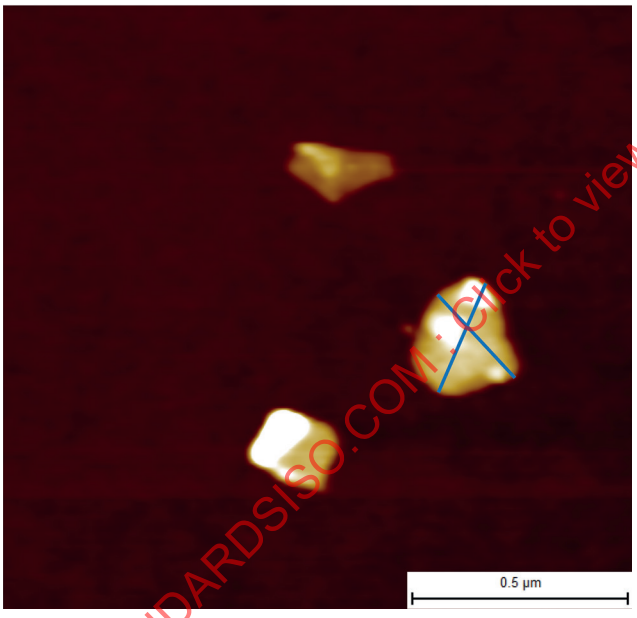
Ideally, the measurement of more flakes should be taken to give a statistically robust sample size, but this will be time consuming.

B.4.3 Data analysis

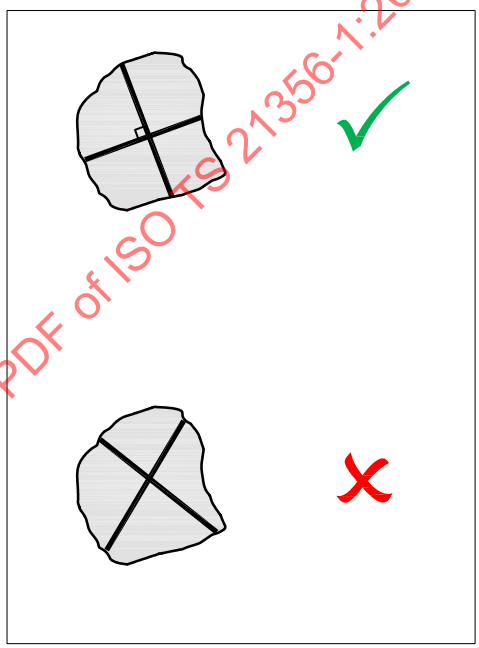
The AFM topographic images should be used to calculate the mean of the width and length measurements as the lateral flake size, using the same data analysis procedure performed for SEM measurements. For each flake, firstly obtain the length measurement and then measure the width (perpendicular to the length measurement) of the flake. Calculate 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 the lateral flake size should and should not be measured is shown in Figure B.6.



a) Correct analysis, by first taking the length and then finding the width as a perpendicular bisector



b) Incorrect measurement



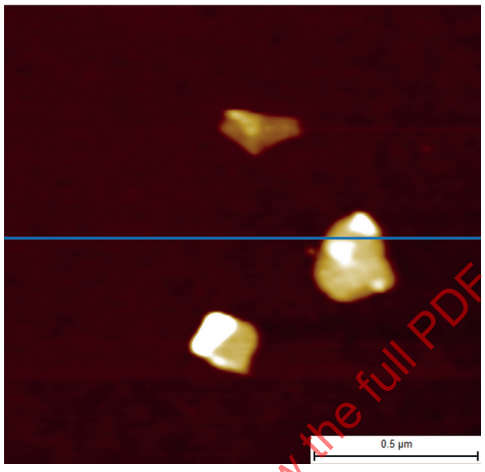
c) An enlarged schematic of the same process for clarity

Figure B.6 — Examples of lateral flake size measurements performed for an AFM image

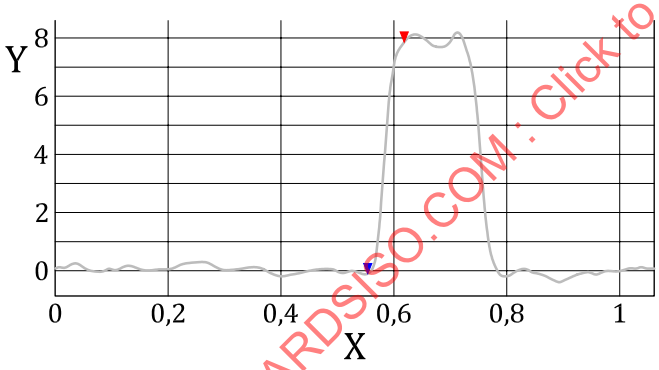
Flakes should only be measured if they are not ambiguous in their composition, that is, features that do not appear to be flakes should not be measured. Where the flake shape does not offer a clear choice of length by eye, the maximum Feret diameter can be taken as the length and the minimum Feret diameter can be taken as the width. Flakes that do not have the complete circumference of the flake visible, due to overlaying flakes, should 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.

It is important to determine the associated uncertainty in each lateral flake size measurement in a similar manner as for the SEM lateral size measurements. Here taking issues such as the irregularity of flake shape, image pixel separation and instrumental calibration factors into account.

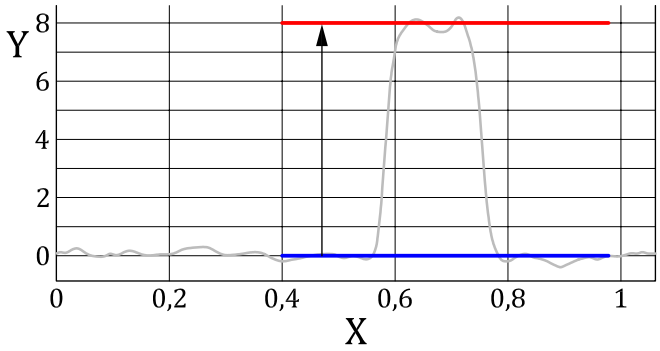
The thickness of each flake should be determined by taking a minimum of three height profiles through each flake to determine an average thickness for each flake. Data should be taken from unprocessed data, for example from the Z-axis sensor. The profiles should be taken along the fast scan axis. Measure the height difference between the substrate next to the flake and the position at the apex of the change in topography due to the flake, as shown in Figure B.7 b). If two obviously different height values can be measured at the edges of the flakes, then the lowest of the two should be taken. Alternatively, in the height profile, 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 B.7 c).



a) AFM topography image, with blue line section detailed in b) and c)



b) AFM height profile from a) highlighting the two measurement positions (blue and red triangles) for thickness measurement



c) AFM height profile showing the two-line positions (blue and red lines) for determining the flake, which in this case turns out to be a graphite flake

Key

X lateral dimension, µm

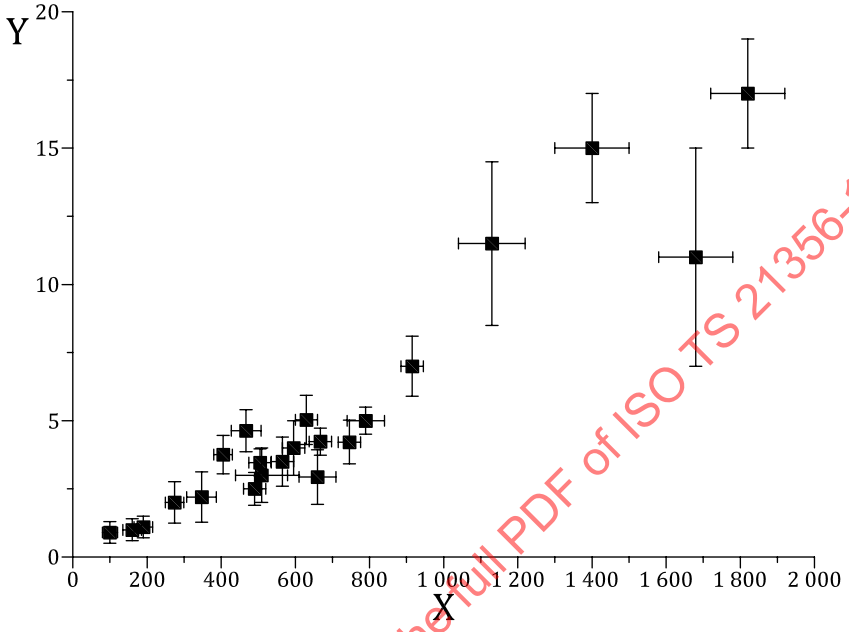
Y height, nm

Figure B.7 — Example of AFM measurement results

The standard deviation in the three values of thickness should be used as the estimated associated uncertainty, unless this is 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 B.7 b), 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) versus the average lateral dimension (X-axis), as shown in Figure B.8.



Key

X lateral flake size, nm

Y flake thickness, nm

Figure B.8 — Flake thickness versus lateral flake size from AFM measurement data for individual flakes 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. However, there can 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 are ~3 nm or less in thickness. The locations of these flakes on the substrate should be recorded so that the same flakes can be measured using Raman spectroscopy, in order to provide a more accurate determination of the number of layers. This can be done via optical microscopy via feature mapping using ideally the optics built into the AFM and Raman spectroscopy instruments. This can also then provide the typical offset in height due to the residue which can be applied to the population. Alternatively, 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.

B.5 Raman spectroscopy

B.5.1 General

Raman spectroscopy can be used to provide a more accurate understanding of the number of layers in a flake than solely using AFM, which measures the flake thickness rather than the number of layers directly. 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 analysed with Raman spectroscopy, as the AFM images can be used to confirm that only individual flakes are being investigated. If this accurate location and measurement of the same flakes cannot be achieved using Raman spectroscopy, the AFM thickness values should be used instead of the number of layers to find the 1LG and FLG number fractions, as described in Annex D.

Raman spectroscopy should be performed on all flakes with a thickness of ≤ 3 nm and on flakes with lateral sizes larger than the optical Raman probe size (typically 0,5 µm to 1 µm) such that a determination of the level of disorder from the intensity ratio of the D and G bands (I_D/I_G) can be determined without any contribution to the D-band from the edges of the flakes, otherwise there will be a misrepresentation of the level of disorder.

B.5.2 Measurement protocol

Raman spectroscopy should be performed in a 180° backscattering geometry to provide the best S/N ratio, with a 50 × or 100 × objective lens (NA ≥ 0,75), the details of which should be recorded. 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 first order Si peak for each laser excitation wavelength and grating. All raw data needs to be corrected in terms of spectral position accordingly. All measurements should be collected at room temperature, both the laboratory temperature and the humidity at the time of experiments needs to be recorded. Importantly, the temperature should remain constant (±2 °C) during the measurement procedure.

A green laser with a wavelength of 532 nm (2,33 eV) should be used for graphene on a silicon dioxide on silicon substrate. It should be noted that Raman spectroscopy measurements using different laser wavelengths cannot be directly compared for graphene. If the preferred 532 nm wavelength laser is not available, a 514 nm wavelength laser could be used. In all cases, the wavelength of the laser should be recorded along with the Raman spectra.

To avoid damaging the graphene material itself through localized heating effects, a total laser spot power (incident on the sample) of less than 1 mW should be used for a laser spot diameter of 500 nm or greater and acquisition times of less than 30 s for each measurement position. If required, the laser spot power should be measured using a power meter and the laser spot diameter should be measured using the edge of a graphene flake/sheet or ideally a carbon nanotube sample.

The spectral range should be chosen such that the relevant Raman lines [D-band (~1 350 cm^{-1}), G-band (~1 580 cm^{-1}), 2D-band (~2 700 cm^{-1})] and associated widths are included, either in the same spectrum or in two separate spectra. A suitable grating should be used that achieves a spectral resolution of ≤ 3 cm^{-1}. Typically, a Raman spectrum is obtained for the range 1 150 cm^{-1} to 3 100 cm^{-1}, in particular when measuring on a silicon substrate, as this avoids the second order silicon peak at ~1 000 cm^{-1}. However, in the most sensitive spectrometers, the third order of the silicon peak at ~1 450 cm^{-1} can be observed. This should not be confused with any carbon peak associated with graphene.

a) 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.

b) The measurements should be undertaken at the same positions as those used for the AFM measurements described in B.4. Locate the correct area and flake to be measured.

c) Use optical microscopy and position the probe spot 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.

    1) Approximate the focus position using optical microscopy, such that the substrate surface/flake is in focus.

    2) To optimize the signal strength by focusing the instrument in the Z-direction, 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 s should be used, but if this does not provide a S/N ratio of at least 10 for the G- and 2D-peaks, a longer measurement time should be used to achieve this ratio.

d) Perform a Raman spectroscopy measurement at the position of highest Raman signal found in step c), with 10 s exposure, and 2 accumulations for an improved S/N ratio.

The same laser power as in step b) should be used to achieve a S/N ratio of 20. If the S/N ratio is less than 20 use a longer exposure time as required.

e) Repeat step d) for each flake at each measurement position as determined in step b), performing focusing step c) before each measurement.

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

NOTE The 2D-peak is also sometimes referred to as the G'-peak.

B.5.3 Data analysis

Firstly, the D- and G-peak area of interest (~1 350 cm^{-1} and ~1 580 cm^{-1} spectral positions respectively), and the 2D-peak (~2 700 cm^{-1}) should be extracted for separate analysis. Determine and subtract a baseline for each area of interest, then fit Lorentzian peak shapes to the D-, G- and 2D-peaks, where the G-peak should only require one 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 ~1 620 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 spectrum are:

— the peak intensity ratio of the 2D- and G-peaks, I_{2D}/I_G;

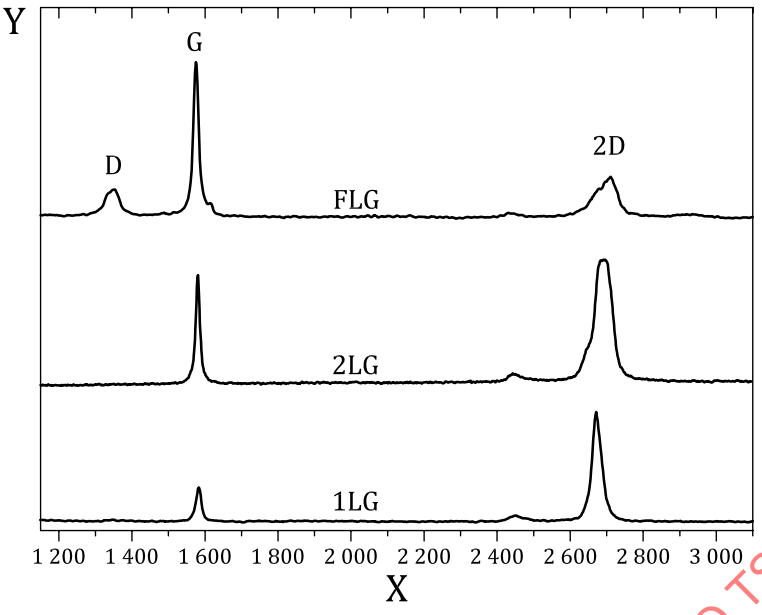
— the peak intensity ratio for the D- and G-peaks, I_D/I_G;

— 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 to 1 µm).

If an I_D/I_G ratio of > 0,2 is observed, it is likely that chemical characterization is required due to the possibility of functional groups being present, to allow a complete understanding of the material to be obtained. This 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 B.9. 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 B.8. For flakes with a similarly shaped 2D band as graphite and thus have three or more layers, the thickness and associated uncertainty determined through AFM measurements should be used.



Key

X Raman shift, cm^{-1}

Y normalized intensity, arbitrary units

NOTE 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.

Figure B.9 — Raman spectra for Bernal-stacked 1LG, 2LG and FLG flakes, using a 532 nm excitation laser line

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.

NOTE More information on asymmetric 2D-peak is described in Reference [8].

Annex C
(informative)

Structural characterization using TEM

C.1 General

This annex provides a measurement protocol using TEM to determine the lateral size, number of layers and layer alignment of graphene flakes.

See ISO 21363:2020 for the measurement protocol of size and size distribution of nanoparticles by TEM. It also includes useful information on instrument set up.

NOTE If the lateral sizes of flakes are large, for example over 3 µm then due to the limited field of view and resolution of TEMs at large field of views, measuring the lateral size of 200 representative flakes can prove time consuming or impossible. In this case, TEM is best suited to determining the number of layers and layer alignment only.

C.2 Sample preparation for TEM

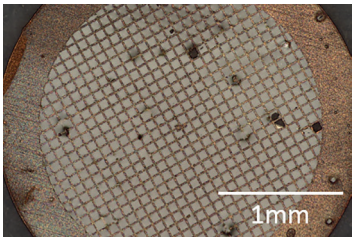
a) Use a TEM support grid with a diameter of 3 mm, consisting of an ordered array of ~1 µm diameter holes in an amorphous carbon film supported on a metal (e.g. copper) mesh. Clean the TEM grid by washing in fresh isopropanol or similar solvent and bake in a vacuum at 200 °C for a few hours.

b) The flakes should be mounted onto the TEM support grid by simple drop casting. Thoroughly mix the dispersion by shaking and then quickly extract 100 µl to 200 µl with a pipette to be mounted on the TEM grid.

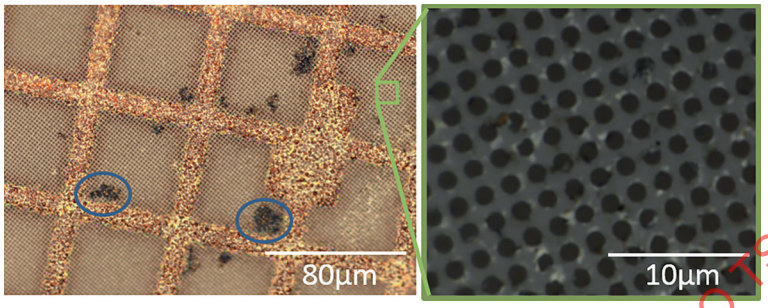
   1) To avoid agglomeration of the flakes, particularly if the solvent requires a long period of time to evaporate, wicking of the solution from the underside of the grid after it has been deposited and/or heating the grid should be performed during drop casting.

   2) An ideal coverage is achieved when there are many flakes within each grid square but not so many that individual flakes overlap. Use optical microscopy to estimate the suitability of sample preparation. If necessary, repeat the sample preparation by adjusting the concentration of the dispersion and/or the amount of dispersion dropped onto the grid until an ideal coverage is obtained, as highlighted in Figure C.1.

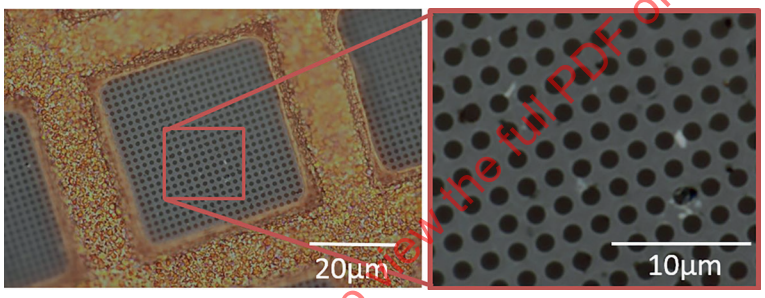
c) Before loading the grid into the TEM, dry the sample thoroughly to reduce the chance of contamination entering the microscope and interfering with imaging. This should be done by heating to ~150 °C in vacuum for 8 h immediately prior to loading the sample.



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



c) Suitable flake coverage for TEM imaging

NOTE 1 Blue ovals highlight the presence of regions with densely agglomerated flakes.

NOTE 2 The enlarged regions in Figure C.1 b) and Figure C.1 c) show the presence of micrometre-sized flakes which appear bright, superimposed on the regular holes of the TEM grid.

Figure C.1 — Optical images of graphene flakes drop cast on a TEM support grid

C.3 Measurement protocol

a) Operate the TEM according to typical procedures. The resolution of the TEM should be at least 0,2 nm.

b) 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.

c) Use an accelerating voltage of 80 kV or below in order to reduce the likelihood of knock-on damage degrading the sample during high-resolution imaging that requires higher doses of electron irradiation.

   1) 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.

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

d) 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 in the TEM control software. Relatively low magnifications will be needed for larger flakes.

1) If the flakes are very thin (one to three atomic layers) it is typically difficult to observe the flake.

2) In bright field TEM inserting a small objective aperture will improve the contrast relative to the carbon support film.

e) 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.

f) 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 (SAED) pattern from the sample.

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

2) 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.

g) Ten or more electron diffraction patterns should then be obtained for different areas of the flake.

1) Note that the presence of the 0,34 nm [002] spots in the diffraction pattern close to the central beam indicates that the flakes are folded, and these areas should not be used for thickness determination.

2) For each diffraction pattern the corresponding bright field 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.

3) 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.

4) 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 a small condenser aperture should be used resulting in a very small spot size.

h) Remove the objective aperture and increase the magnification to perform lattice resolution characterization of the graphene flakes.

1) Note that in 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.

2) For bilayer or thicker samples, the 0,34 nm (002) fringe spacing will be 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.

3) Additionally, if there is an image rotation associated with the increase in magnification this should also be recorded.

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

1) A total of at least 200 flakes should be measured per sample.

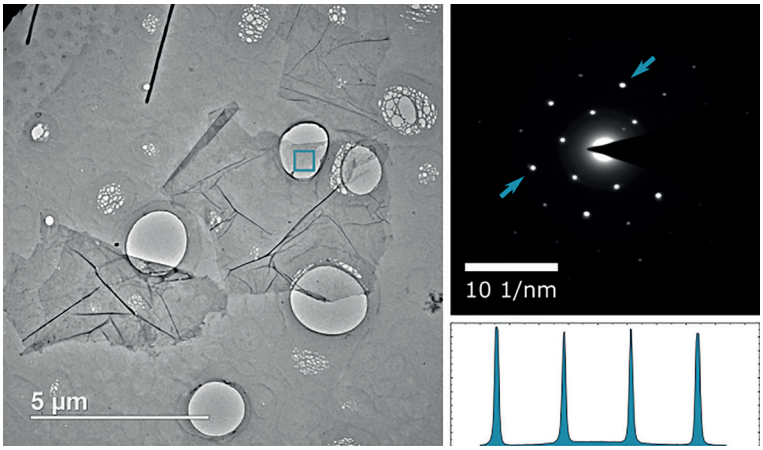
2) Every flake within the grid square should be imaged before moving on to the next square.

3) If every grid square does not need to be imaged to produce the desired number data points in step i), 1) above, 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 sample preparation.

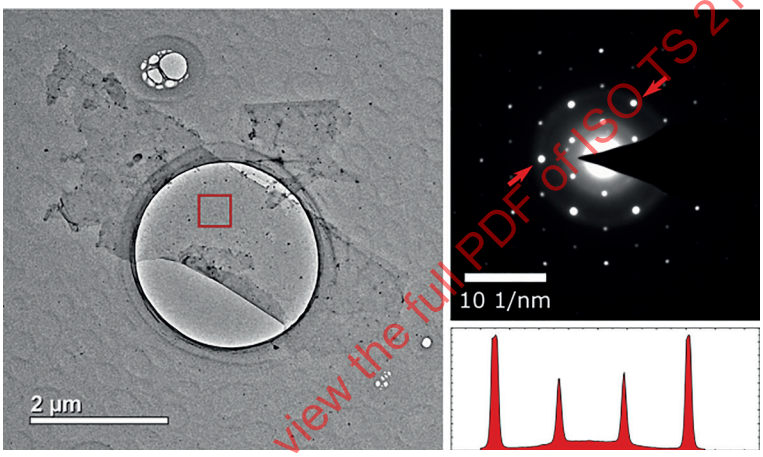
4) For each flake identified repeat the intermediate magnification imaging [steps d) and e)], electron diffraction [steps f) and g)] and lattice resolution imaging [step h)].

C.4 Data analysis

Look for the characteristic hexagonal electron diffraction pattern obtained when graphene is viewed along the [001] direction, as shown in Figure C.2. This is an unambiguous fingerprint that the material is 1LG/2LG/FLG/graphite. The in-plane (100) lattice spacing is 0,213 nm and the (110) lattice spacing is 0,123 nm. To distinguish single-layer graphene from bilayer and few-layer graphene with TEM electron diffraction, compare the intensities of the first and second ring of the diffraction spots. 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 C.2 a) and Figure C.2 b) 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 with more than two graphene layers, the outer spots increase further in intensity relative to the inner ones.



a) Single-layer graphene

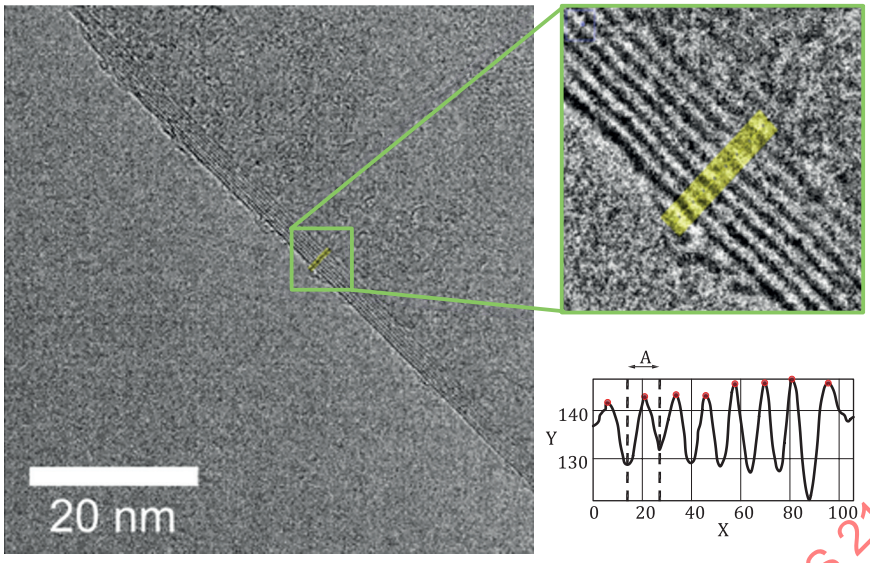


b) Bilayer graphene

NOTE Intensity profile plots taken between the arrows are shown below the diffraction pattern.

Figure C.2 — BF TEM images alongside diffraction patterns taken from the marked areas

Electron diffraction can identify single-layer, bilayer and few-layer graphene but cannot determine the precise number of layers for thicker samples. This can be performed if lattice resolution (0,34 nm) TEM/STEM imaging is achievable through observation of folded edges of the flakes. The layer number should be measured by counting the number of lines at the edge as shown in Figure C.3. In bright field TEM, contrast reversals can occur meaning that the atomic planes of graphene can either appear as bright fringes on a dark background or dark fringes on a brighter background depending on the imaging focus and local curvature. An error in the measurement due to this contrast inversion can lead to a thickness error of ±1 layer. Thin specimens imaged at Scherzer defocus in a conventional uncorrected TEM are expected to show atomic positions as dark and hence the graphene layers appear as dark fringes on a brighter background and the dark fringes are counted (see Figure C.3). If difficulties are encountered during focusing, a focal series should be obtained to provide evidence of correct focusing. However, absolute quantitative interpretation of phase contrast TEM data should be performed with the help of image simulations. Exfoliated graphitic 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 C.3. Measure the number of layers of at least 20 flakes.



Key

X lateral dimension, nm

Y intensity, arbitrary units

A 0,34 nm

NOTE 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 seven layers thick.

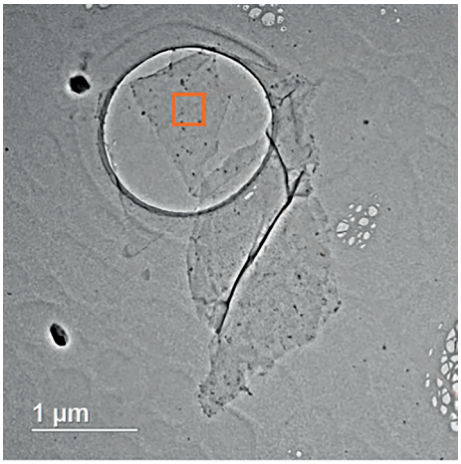
Figure C.3 — BF TEM images of the edge of a flake

Individual 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.

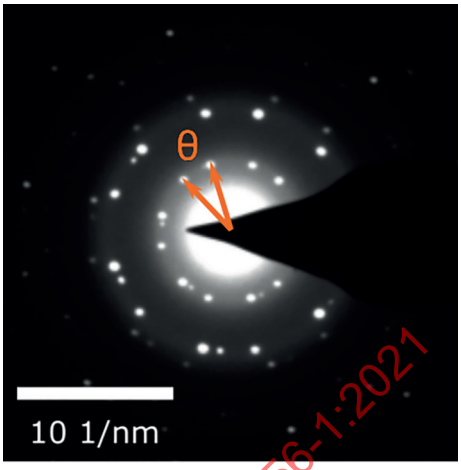
Despite efforts to reduce restacking during flake deposition, samples often contain restacked flakes. These restacked flakes, called twisted few-layer graphene, tend to be randomly oriented relative to each other so restacking can be identified by studying the morphology and by the presence of multiple 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 the ordered 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 graphite, as shown in Figure C.4.

Measure the lateral flake size directly from the bright field 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 C.5. Measure the flake size of at least 200 flakes in each sample.

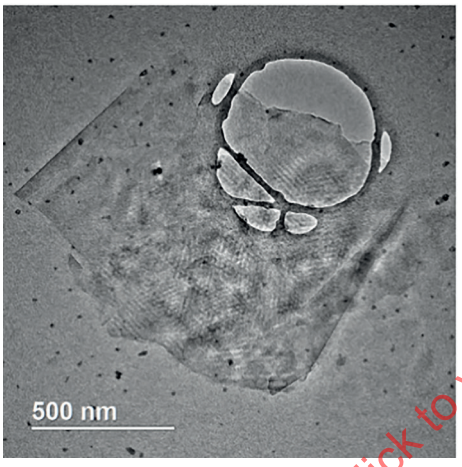
Once the lateral size of 200 flakes or more and the number of layers of at least 20 flakes, covering the range of lateral flake sizes observed, has been determined, produce a histogram of the lateral flake sizes and a scatter plot of flake thickness versus lateral flake size, as detailed in Annex D. The measurement method should be recorded as “TEM”.



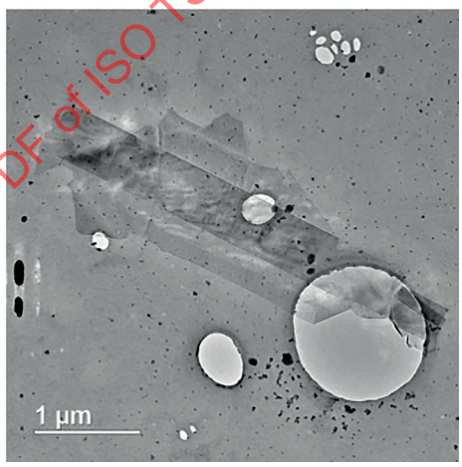
a) TEM image in which the orange box denotes area used for diffraction pattern in b)



b) Diffraction pattern showing 12 spots, taken from the orange box area in a) (θ = 25,7°)



c) TEM image showing a folded graphene flake, demonstrating the Moiré pattern



d) TEM image showing a flake that has rolled up into a scroll-like structure, demonstrating the Moiré pattern

Figure C.4 — TEM images of twisted few-layer graphene

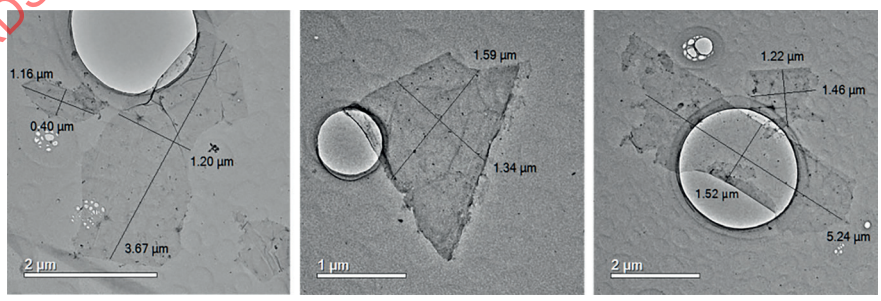


Figure C.5 — Bright field TEM images with annotated measurements of the lateral sizes of typical liquid-phase exfoliated flakes

Annex D
(informative)

Lateral size and number fraction calculation

D.1 General

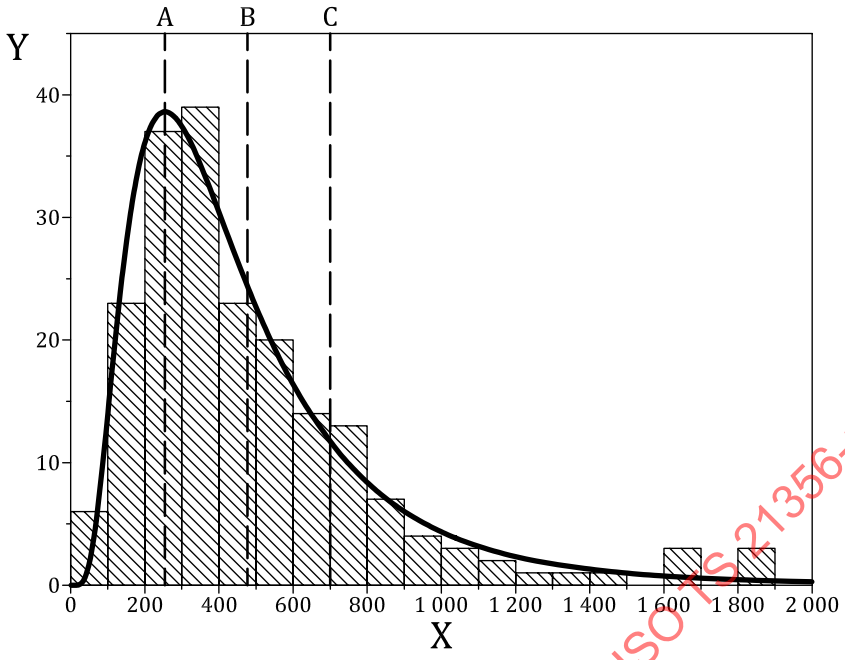
This annex describes the steps to be taken to calculate the average lateral flake size and an approach to calculate the number fraction of monolayer flakes and flakes with ten layers of graphene or fewer within the sample under test.

D.2 Average lateral flake size

This can be determined from the SEM, TEM or AFM data providing enough flakes have been measured by that method. The suggested minimum number of flakes that should be measured is 200. This is likely to be undertaken using SEM.

Plot the lateral flake size distribution as a histogram shown in Figure D.1. Fit the data to a log-normal distribution function. From the fit determine the median value and the standard deviation. The average lateral flake size to report is the median value and the range the standard deviation.

If a log-normal distribution function is not a good fit to the data, then a more appropriate fit function should be used.



Key

X lateral flake size, nm
Y number of flakes
A median – SD
B median
C median + SD

Figure D.1 — Example histogram of lateral flake size distribution with a log-normal distribution, where the median value (B) and standard deviation from median (A and C) are shown as vertical lines, where 68 % of the values fall between A and C

D.3 Number fraction

D.3.1 General

Three methods can be used to determine, within the sample under test, the number fraction of monolayer graphene and material with ten layers or fewer. Each method has advantages and disadvantages, use the method that provides the most accurate method, depending on the type of material under investigation, in the most appropriate timeframe. The number fraction can be determined by comparing data obtained from different methods. The three methods, not listed in order of preference are:

a) AFM only (D.3.2);

b) AFM thickness measurements correlated to SEM or TEM data (D.3.3);

c) Raman spectroscopy compared to AFM thickness measurements correlated to SEM or TEM data (D.3.4).

D.3.2 AFM only

This method requires AFM to have been used to measure at least 200 flakes and assumes the measured thickness on monolayer graphene is the same as the theoretical value (0,34 nm).

Plot lateral flake size versus average flake thickness. Determine the number of flakes with thicknesses less than or equal to 0,34 nm. The monolayer graphene number fraction is this number divided by the total number of flakes measured.

Similarly, the number fraction of flakes with ten layers or fewer can be calculated from the number of flakes with thicknesses less than or equal to 3,4 nm divided by the total number of flakes.

NOTE 1 The time taken to obtain 200 flakes by AFM is very time consuming which makes it along with NOTE 2 unlikely that this method will be used routinely.

NOTE 2 In reality, monolayer and 10-layer thickness values rarely match the theoretical values (see D.3.4) and so the number fraction reported here is at least a minimum value.

D.3.3 AFM combined with SEM or TEM

This method uses the data from AFM (see B.4) combined with SEM (see B.3) or TEM (see Annex C). This method assumes a linear correlation for the flakes between lateral flake size and thickness. The method also assumes that the measured thickness of monolayer or few-layer graphene is the same as the theoretical values.

Plot the lateral flake size versus thickness data obtained from AFM and fit a straight line to the data, as shown in Figure D.2. Typically, a linear positive correlation between flake thickness and lateral flake size is observed, as shown in Figure D.2, due to the production method of top-down liquid-phase exfoliation that is commonly used.

If no correlation is observed, then more measurements should be undertaken using AFM (see B.4) 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 flakes should be measured. If there is no correlation observed after thickness measurements for over 200 flakes, 'no correlation' should be reported. The number fraction can then be calculated for samples with over 200 flakes measured using AFM as described in D.3.2.

Assuming a straight-line correlation, from the fitted data, determine the AFM measured lateral flake size that corresponds to the theoretical thickness of a single graphene layer (0,34 nm). Also determine the AFM measured lateral flake size that corresponds to the thickness of ten layers of graphene (3,4 nm). In the example shown in Figure D.2, this is at approximately 495 nm.