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VAMAS TWA 41 - GRAPHENE AND RELATED 2D MATERIALS

PROJECT 12 - DISTRIBUTION OF LATERAL SIZE AND THICKNESS OF FEW-LAYER GRAPHENE FLAKES USING SEM AND AFM

SEM AND AFM MEASUREMENT PROTOCOL

K. DESPOTELIS, A. POLLARD, C. CLIFFORD, K. PATON

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Surface Technology Group
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ABSTRACT

This report details the measurement and data analysis protocol for Project 12 '***Distribution of lateral size and thickness of few-layer graphene flakes using SEM and AFM***' of Versailles Project on Advanced Materials and Standards (VAMAS) Technical Working Area (TWA) 41 "*Graphene and Related 2D Materials*", as part of the European Metrology Programme for Innovation and Research (EMPIR) 19NORM04 ISO-G-SCoPe project. This study is an international interlaboratory comparison of the measurement of the structural properties of graphene nanoplatelets (GNPs) deposited onto different Si/SiO_2 substrates for scanning electron microscopy (SEM) and atomic force microscopy (AFM) measurements. Participants will be asked to measure these samples, along with test samples to verify the calibration of their instruments and report the lateral flake size and thickness of the measured GNPs. The protocols for SEM and AFM measurements and related data analysis are described, with an aim of the study being to understand the sources of uncertainty in these types of measurements, as well as within- and in-between laboratory measurement variability, for a group of laboratories spread across the globe.

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Approved on behalf of NPLML by
Caterina Minelli, Science Area Leader.

CONTENTS

1. GENERAL

1.1 INTRODUCTION AND AIM

The aim of this international interlaboratory comparison (ILC) is to determine the lateral flake size distribution of graphene nanoplatelets (GNPs) using scanning electron microscopy (SEM), and to then correlate this distribution to measurements of lateral flake size and thickness using atomic force microscopy (AFM). The outcomes of this ILC will directly input in the future revision of ISO/TS 21356-1, providing an insight on method variability and objective data to build robust and widely applicable measurement procedures across different instrument models and laboratories. This work is undertaken as part of the European Metrology Programme for Innovation and Research (EMPIR) project 19NRM04 ISO-G-SCoPe.

Graphene is an exciting, advanced material, present in the form of flakes in powders or liquid dispersions, as well as in larger sheets grown through bottom-up processes. GNPs in flake form are already starting to find commercial application via small-to-medium enterprises (SMEs) to multi-national corporations, for a large range of application areas. There are currently over 100 commercial ‘graphene’ producers worldwide, including leading producers in Europe, with an 'on paper' offering of materials with vastly different properties and types. However, the activity of many suppliers (and buyers) is hindered due to materials being uncharacterised or poorly characterised, often consisting of graphite rather than GNPs or having large batch-to-batch variations. Products and applications suffer as a result. Thus, validated and disseminated measurement methods of GNPs are a key industry requirement.

The recently published ISO/TS 21356-1 ‘Structural characterisation of graphene from powders and liquid dispersions’ details protocols to characterise the lateral size and thickness of graphene and few-layer graphene flakes. However, these sections remain informative, until the typical variability in values obtained by different users/equipment can be evaluated through an ILC study and this VAMAS study provides such opportunity.

The standard provides guidance on how to measure the flake thickness and flake size distribution in one batch versus another. Precisely estimating these measurands at the nanoscale, especially thickness, requires a combination of robust measurement methods along with innovative metrology and correlative imaging.

1.2 CONFIDENTIALITY

The samples supplied in this ILC are not certified reference materials, but have been selected for this VAMAS comparison as the best available type of sample for this study. They are sent to the participants in confidence. If there are any problems with the received samples we invite the participants to immediately contact the lead participant (Dr Kostas Despotelis, kostas.despotelis@npl.co.uk) so that we can ascertain whether the problem is generic or restricted to a single sample. The results of this ILC will be anonymised and each laboratory will be assigned a code. Participants shall not publish any results from measurements performed on these samples without first consulting the lead participant.

2. SAMPLE DESCRIPTION

Do not unpack the samples until you are ready for the measurements. Before unpacking, read the rest of this protocol so that you are clear about the next steps. If any steps are unclear, please contact the lead participant as soon as possible for clarification.

Three samples are provided:

1) 1 cm × 1 cm sample: GNPs deposited onto an ISO-G-SCoPe grid on a SiO_2/Si wafer for AFM measurements and calibration, labelled as “AFM/X” (followed by a number).

2) SiO_2/Si wafer approximately 1 cm × 1 cm with GNPs deposited on it for SEM measurements, labelled as “SEM/X” (followed by a number).

3) 3 mm × 3 mm calibration grid for SEM calibration placed in a unique plastic bag and labelled as “Pelcotec G-1T um Grid Calibration Standard Unmounted”.

Please inspect the packaging to check if the samples were damaged during transit.

Specific sample information and sample codes are given also in the covering email.

These samples are not deemed hazardous as they are treated as an ‘article’, but every care should be taken to avoid contact with the skin and eyes. Good laboratory practice should be observed, and participants can refer to the supplied SDS for further details on the type of particles that are deposited onto the substrate. Note that this SDS is for a similar material and so the dimensions are not expected to be directly comparable.

For the samples for SEM measurements, a silicon wafer with a thin native oxide surface layer has been used as a substrate. The oxide is thin enough to ensure good conductivity and minimise charging during SEM imaging. For the samples for AFM measurements, a silicon wafer with a silicon dioxide layer of thickness of (300 ± 5) nm has been used in order to maximize the optical contrast between the flakes and the substrate.

The GNP samples for SEM and AFM are provided in sample containers as shown in Figure 1, **where the surface with the GNP flakes is facing downwards**. Additionaly, the non GNP side (backside facing upwards) of every AFM sample has been marked with a black dot to assist participants in identyfing it due to similar colour contrast of both surfaces.

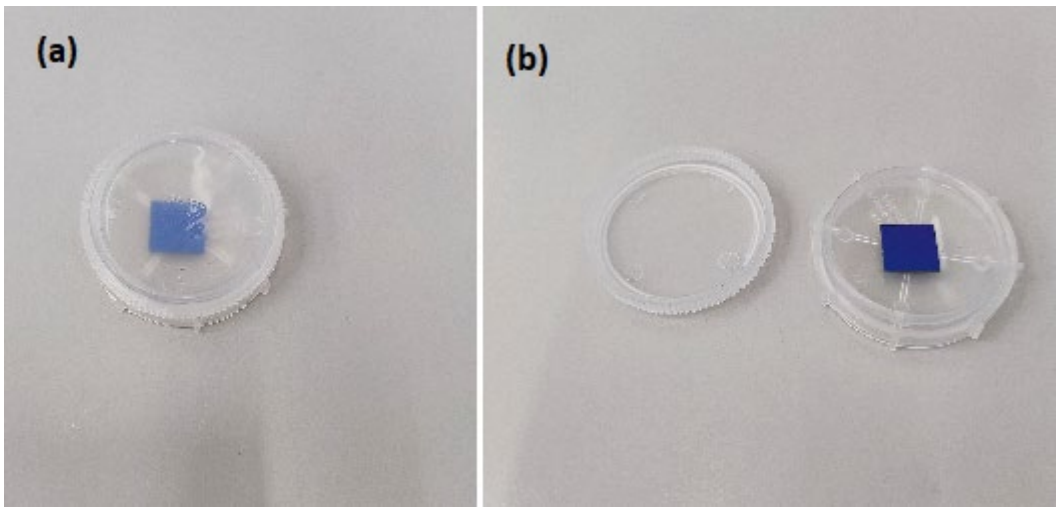


Figure 1 Photographs of a sample when (a) sealed and (b) open with ‘spider’ removed.

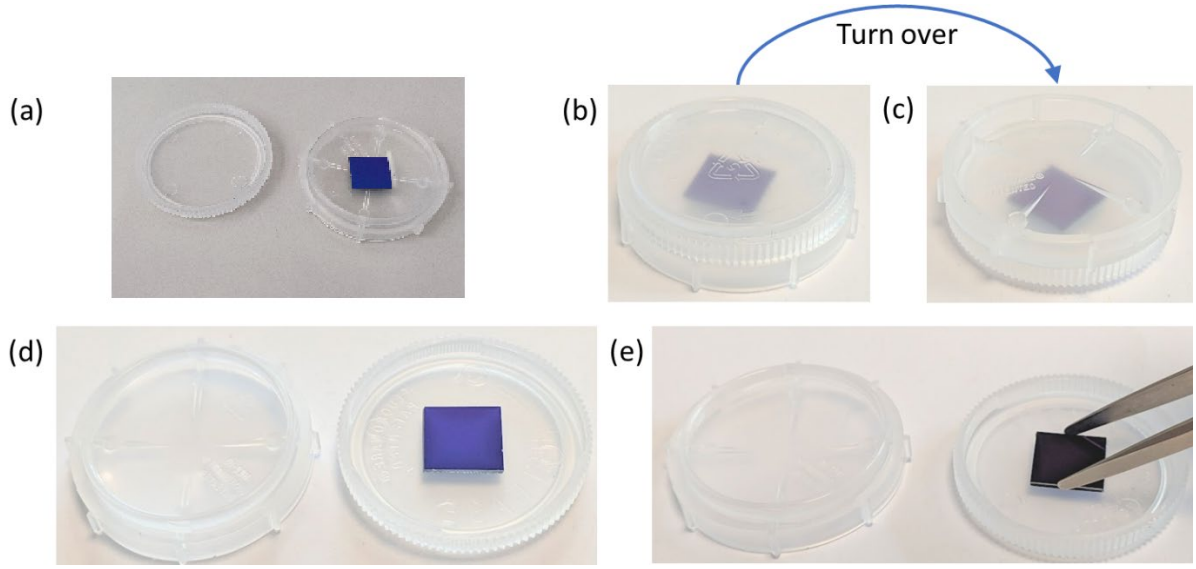


Figure 2 (a) The substrate in the base of sample container, with the back side facing up and 'spider' removed. To remove the substrate from the sample container, (b) close the lid then (c) turn the sample container upside down with the substrate still inside it. (d) Open the sample container upside down such that the substrate is in the lid of the sample container with the GNP side facing up. (e) Remove the substrate by holding the edges of the substrate with the edge of the metal tweezers.

To handle the sample, first open the sample container and remove the plastic 'spider' that secures the substrate within the sample container. After removing the spider, the substrate will be orientated with the backside facing up and the surface of interest (GNP side) facing down (Figure 2a). As the GNP side of the wafer is facing down, the wafer will need to be flipped 180° along either the vertical or horizontal axis, to reveal the GNP side. The most reliable way to flip the wafer is to put the lid back on the sample container (without the spider in place), flip the container with the wafer inside it, and then open the container upside down such that the GNP side is facing upwards (b-d). After this, hold the edges of the wafer using metal tweezers and place it onto the sample holder of your system.

The SEM calibration sample is provided in a Gel-Pak® box with the grid face up. Carefully remove the sample using tweezers holding the edge of the substrate.

3. SAMPLE ASSESSMENT USING OPTICAL MICROSCOPY

Use optical microscopy at appropriate magnifications (e.g. 50 ×) for a rapid assessment of the sample to ensure it is suitable to measure and locate areas appropriate for further analysis. Place the SEM and AFM samples in turn under the optical microscope and adjust focus and magnification and inspect the sample. If sample damage or unexpected features are observed, please contact the lead participant as soon as possible.

Find and record areas on the sample where the flakes are generally well separated, while also being relatively abundant. This allows SEM and AFM measurements to be performed at a faster rate while also ensuring that the measured values are obtained for individual rather than agglomerated flakes.

An example of a suitable area of the sample to investigate, where flakes have been deposited onto the substrate used for AFM, is shown in Figure 3. Care should be taken during optical microscopy analysis to ensure it is understood where the flakes are present as opposed to other material that may affect the optical contrast and should be avoided, such as solvent residues causing changes in colouration of the substrate as shown in Figure 4.

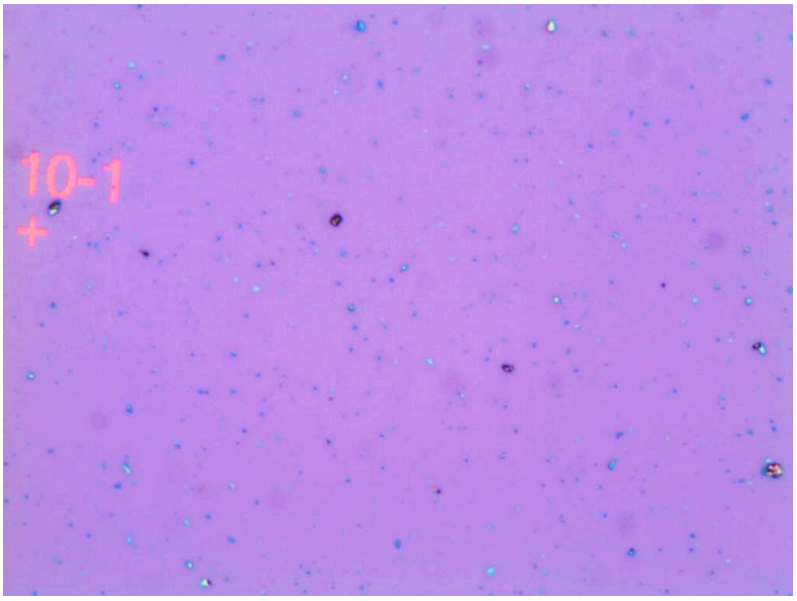


Figure 3 Optical microscopy image of GNP flakes deposited onto a silicon wafer with a 300 nm thick layer of silicon dioxide (markings for numbered area of wafer also shown in pink), showing an example of a good area to be analysed.

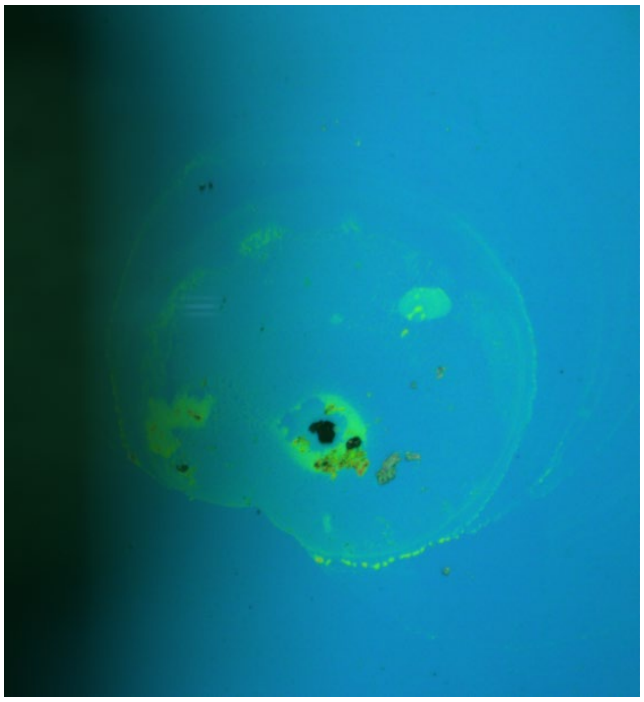


Figure 4 Example of an optical microscopy image acquired with a 20 × objective lens showing residues left on a silicon dioxide on silicon substrate after deposition of a liquid dispersion of GNPs, as shown by areas of varying colour. Such areas should be avoided during measurement.

4. SEM ANALYSIS

4.1 GENERAL

In scanning electron microscopy (SEM) an electron beam is focused onto the specimen and as the beam moves across the sample interacts with its surface and the emitted backscattered or secondary electrons are collected and their intensity is measured. This process enables sample imaging and measurements of the size of micro- and nanoscale objects. 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 practice in sample preparation. As previously mentioned, the GNP flakes have been deposited onto a silicon substrate with a thin native oxide layer at the surface rather than a thicker thermally-grown silicon dioxide layer. This minimizes the charging effect because the sample substrate is more conductive. However, SEM imaging will cause carbon deposition on the silicon sample and thus the AFM analysis is undertaken on separate substrates. This is to avoid any contribution of added carbonaceous material to the measured flake thickness.

A minimum of 200 flakes shall be imaged with SEM. Each of these flakes shall be well separated from the others to enable accurate identification of the flake boundaries. The lateral dimensions of the flakes shall be determined for at least 200 randomly chosen flakes where all flakes suitable for analysis (this is explained in detail later on) in a given SEM image should be included.

Follow the measurement protocol and data analysis as presented in detail below.

4.2 MEASUREMENT PROTOCOL

4.2.1 Key parameters to consider before performing measurements

a) The SEM instrument shall be dimensionally calibrated using traceable dimensional calibration standards at an appropriate magnification for the measurements. The calibration shall be valid for the working distance and accelerating voltage used in the subsequent measurements.

b) The SEM sample shall be mounted into the SEM holder so that it is held rigidly, is well-supported and secured. If possible, the SEM calibration sample should be mounted at the same time.

Note that while the sample should be mounted securely, it may also need to be further analysed at another laboratory. For this reason, a method that allows the sample to be safely removed and does not contaminate the sample is preferred. For example, the sample’s surface should not be scratched using the tweezers during mounting nor the wafer broken while trying to remove it.

c) The SEM imaging configuration shall be set to collecting secondary electrons or another detector available in your system, typically with an accelerating voltage of 5 kV or less to minimize charging.

d) The sample surface shall be located through SEM imaging and the system optimised in terms of its aperture, working distance, stigmator and focus to achieve the best resolution possible, as given for the specific SEM instrument.

This optimisation process shall be performed on a feature that is similar in size to the flakes, but that is not a flake of interest, as the long dwell time required for optimisation will affect the area.

4.2.2 SEM measurement method

Step 1) Locate an area of the SEM sample where there are abundant and isolated flakes on the substrate.

Step 2) Acquire a low magnification image (~10 000 × magnification) of this area.

Step 3) Acquire images of higher resolution and magnification for the flakes (or single flake) to allow subsequent image analysis. The magnification should be set such that the whole flake is in the field of view and the pixel size is less than 10 nm.

Images with magnification higher than 20 000 × reduce the uncertainty in lateral size measurement, that is, the flakes are likely to occupy a significant part of the image allowing ease of manual measurement.

Images of multiple flakes together can be taken or, if time permits, of individual flakes separately as shown in Figure 5, aiming at maximizing the number of isolated flakes imaged.

Ensure that if a same flake is imaged multiple times, e.g. at different magnifications, it is processed only once during the data analysis.

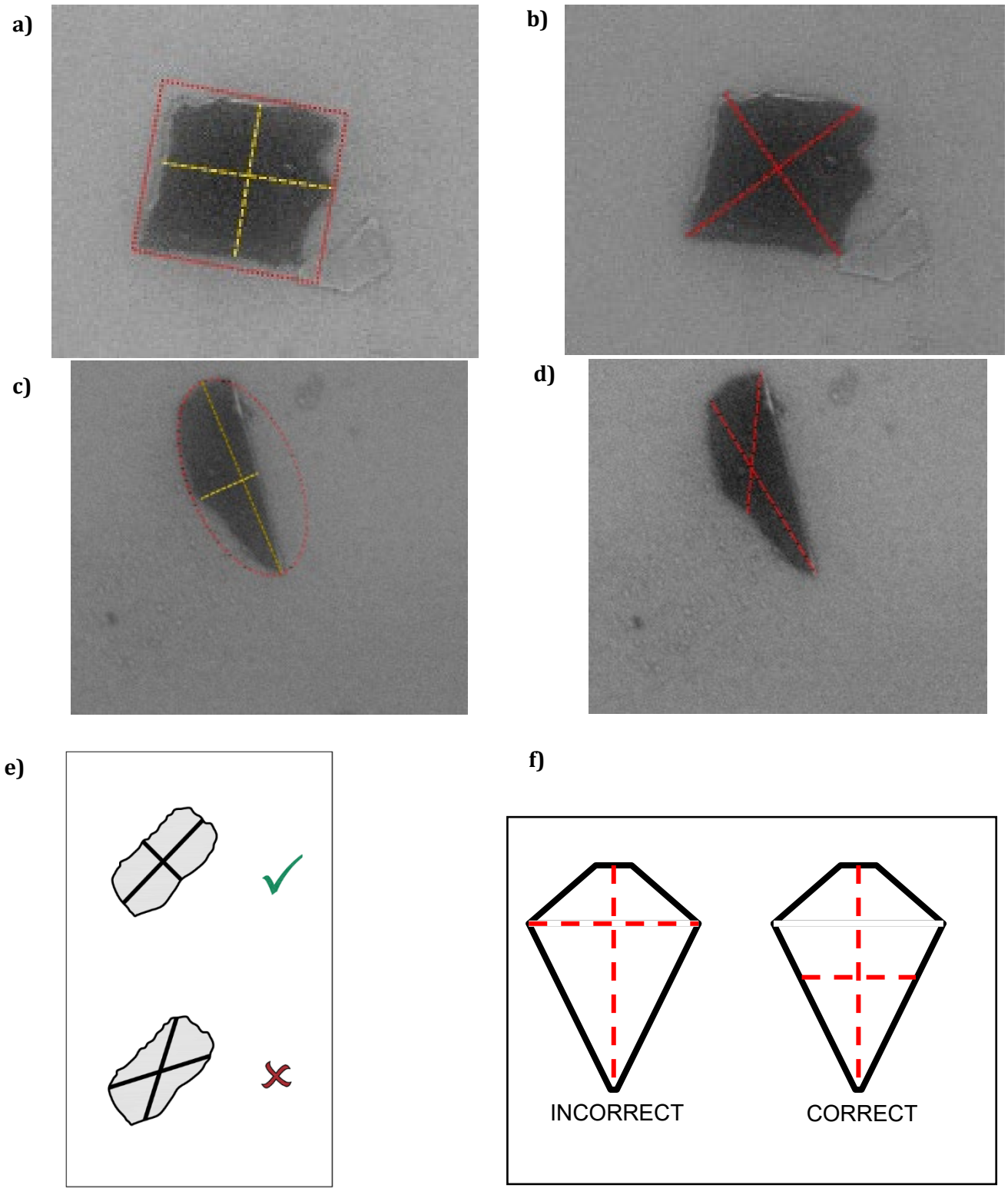


Figure 5 Examples of the correct and incorrect ways to measure the lateral flake size from an SEM image. a,c) Correct analysis, by first taking the length and then finding the width as a perpendicular bisector, the rectangle and ellipse are added as a guide to the eye, b,d) Incorrect measurement, e-f) Schematics showing correct and incorrect measurements.

Step 4) Repeat steps 1-3 such that at least 200 isolated flakes are analysed. A representative distribution of flakes of different sizes from across the substrate surface shall be obtained.

Preferential selection of, for example, easier-to-image larger flakes will drastically skew the final results. Therefore, a range of magnifications shall be used and different areas of the sample shall also be imaged.

Step 5) Once the imaging of the GNP flakes has been completed, the SEM calibration grid provided with the GNP SEM sample shall be imaged using the same imaging parameters, including magnification, as used for the GNP SEM sample. Image three different areas of the sample at the same higher magnification, making sure that each image covers at least 5 square features horizontally and 5 square features vertically (~ 20 000 × - 40 000 × magnification).

Examples of lower and higher magnification SEM images of the calibration grid are shown in Figures 6a) and 6b) respectively. A higher magnification image as the one presented in 6b) shall be used for the calibration. If the grid appears to be defective, such as the one presented in Figure 6c) where squares of the grid are connected to each other without clear gaps between them, then these areas should be avoided.

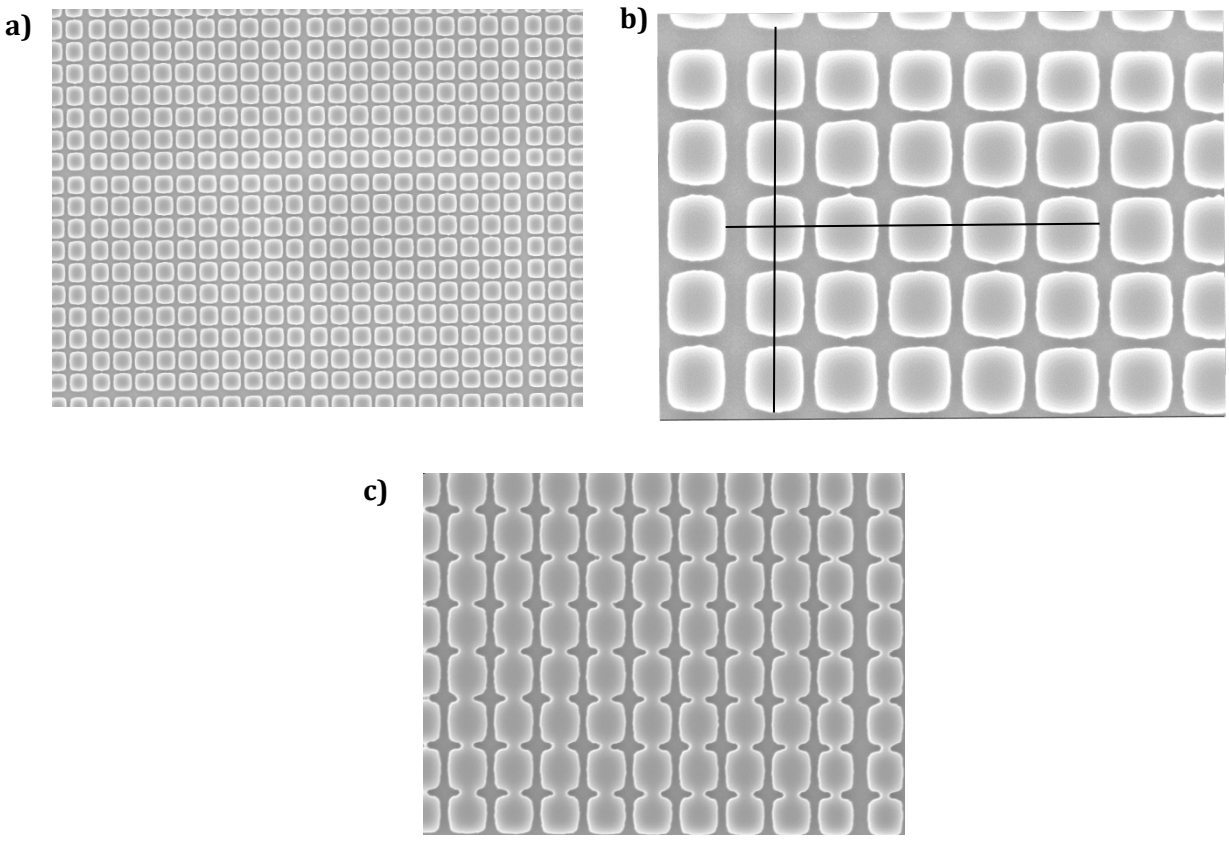


Figure 6 SEM images of the SEM calibration grid provided with the samples. a) Example of low magnification SEM image of the grid b) Example of higher magnification SEM image of the grid that shall be used to measure the average grid spacing from profile lines. c) Defective areas of the grid (e.g., connected squares in this instance) shall not be used for the calibration.

4.3 IMAGE AND DATA ANALYSIS

4.3.1 Calibration grid analysis

Measure the lateral and vertical pitch of the three different areas of the grid that were imaged at higher magnification as in Figure 6b.

The grid pitch should be measured by drawing a line-profile parallel to the square feature's sides across 5 features and then dividing the distance by 5 in order to calculate the distance between equivalent points of each feature. One line profile should be obtained on the horizontal axis, and another on the vertical axis. Figure 6b shows an example of such lines. This same procedure should be repeated for the 3 areas of the calibration that were imaged. This procedure shall be performed with the same software that has been used for the analysis of the flakes.

The pitch (average of the 3 measurements) of each axis shall be reported using the Measurement report Excel spreadsheet. The standard deviation of the 3 measurements of the pitch of each axis shall also be included.

Note that a Fast Fourier Transform analysis can be used if preferred, to calculate the pitch for each axis (see reference [3] for more information).

4.3.2 Particle lateral size analysis

The lateral dimensions of the flakes shall be determined for at least 200 randomly chosen flakes, where all flakes suitable for analysis in a given image shall be measured. As a reminder, a flake is classified as suitable for analysis when the entirety of the flake can be observed, with no ambiguous features in its composition. Features that do not appear to be flakes shall not be measured. Flakes that do not have the complete circumference visible due to overlapping flakes, shall also not be measured. Flakes that are in contact with each other shall also not be measured.

The largest source of bias in the measurement of the average lateral flake size is typically due to the users 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 flakes of specific shapes or sizes, but rather analyses all flakes that are possible in a given image.

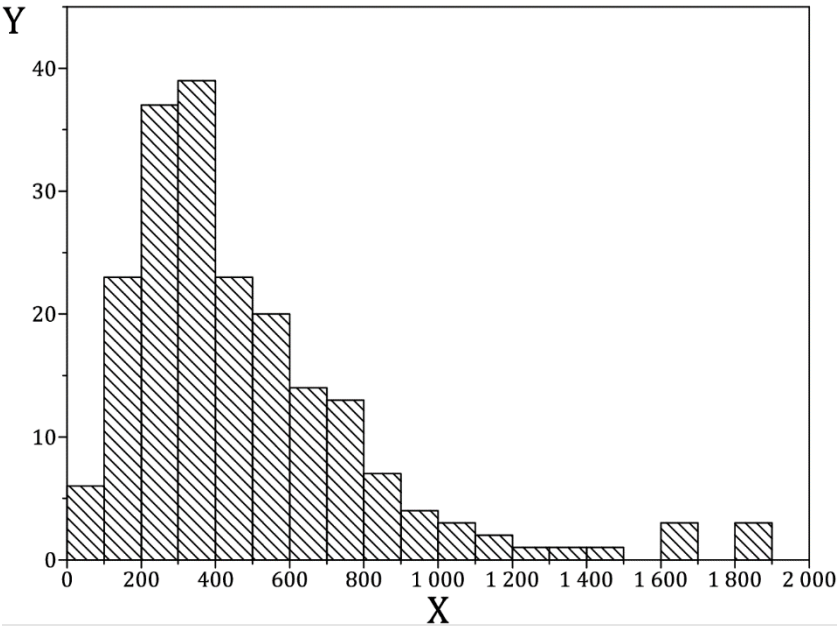
The dimensions of each flake are determined by measuring the length and then the width of the flake. Examples of how a flake shall and shall not be measured are shown in Figure 5. The length and width of the flake shall be defined so that they would approximately be the length and width of the smallest rectangle or ellipse that best represent the particle (see examples in Figures 6a and 6c). Both the length and width segments must fall within the boundaries of the particle.

The flake width is defined as the segment within the flake that is perpendicular to the length and divides it in two equal parts (perpendicular bisector segment). Care should be taken in the choice of the flake length so to obtain a representative width. Note that this may not be the maximum perpendicular dimension, as for example shown in Figure 5f. The bottom image in Figure 5e shows incorrect measurements of the flake dimensions because firstly, the length is not that of the smallest ellipse or rectangle containing the flake and secondly, the width is not the perpendicular bisector segment. Where the flake shape does not offer a clear choice of length, the minimum Feret diameter can be used as the width and the length taken as the perpendicular bisector of the width.

The user shall also record an uncertainty for each length and width measurement, taking parameters such as the flake shape, image pixel dimension and instrumental calibration into

account. The lateral size of a flake is then defined as the average mean of the length and width values and the standard deviation in length and width values shall be recorded as the associated uncertainty.

Once the lateral size of at least 200 flakes have been measured, the values shall then be represented as a histogram with at least 20 bins. An example histogram of a sample (different to the one in this study) is shown in Figure 7.



Key

X lateral flake size, nm

Y number of flakes

Figure 7 Example histogram of the range of lateral flake sizes of GNPs of at least 200 flakes, measured by SEM of a graphene sample from a different study.

The histogram shall then be fitted to a lognormal distribution. The median lateral size, mean lateral size and the standard deviation of the distribution shall be reported.

4.4 REPORTING THE SEM RESULTS

Results should be reported in the supplied SEM Measurement report Excel spreadsheet where full details of the experimental conditions must be given, including:

- Instrument used.
- Environmental conditions (temperature and humidity).
- Accelerating voltage used.
- Working distance used during imaging.
- Aperture size used.
- Detector used for imaging.
- Details of SEM dimensional calibration.

Full details of all data analysis must also be given, as well as the software used, including:

- Number of flakes analysed (≥ 200).

- The measurement uncertainty included for each particle recorded.
- The minimum and maximum particle lateral size measured in the sample.
- Lognormal median lateral size and standard deviation of the sample.
- Measured pitch of calibration grid in X- and Y-directions with an associated uncertainty.

5. AFM ANALYSIS

5.1 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 relatively flat surface, such as silicon dioxide, the dimensions of a GNP flake including the thickness can be measured.

In this study, AFM is used to measure the thicknesses of the GNPs. These values are then correlated to the flake lateral sizes. For this reason, AFM measurements should be performed for material where the spread of lateral flake size has previously been measured by SEM. To this end, at least 20 isolated GNPs of a range of lateral sizes should be imaged by AFM. The range of lateral flake size should reflect the range of lateral dimensions measured by SEM. 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.

Follow the measurement protocol and data analysis as presented in detail below.

5.2 MEASUREMENT PROTOCOL

5.2.1 Key parameters to consider before measurements

a) The AFM system shall be operated in a closed loop mode and AFM operation shall be performed according to normal operating procedures, using intermittent contact mode in ambient conditions. Ensure that any necessary instrument calibrations are up to date. For AFM this includes the Z piezoelectric scanner calibration. For the calibration of the Z axis, an in-house procedure shall be used.

The conditions used shall be included in the Measurement Report Excel spreadsheet supplied, including any deviations from the requirements outlined in this protocol.

b) The sample consists of flakes deposited onto an ISO-G-SCoPe grid consisting of silicon dioxide, on a silicon substrate with an oxide thickness of approximately 300 nm. Each substrate has a central calibration grid, a unique serial number and numbered areas in order to identify the exact locations where measurements are performed, as shown in Figure 8. Note that this type of substrate is for AFM measurements only. The sample must be mounted into the AFM in such a way so that it is held rigidly, while making sure that it can be removed later without damage or modification.

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 full width at half maximum (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) Use the built-in optical microscopy imaging capability of your AFM system to locate the specified area of the substrate within the numbered squares, that has been recommended as ideal for measurements (for further details see advisory file supplied with the sample). Once the appropriate area has been identified, position the AFM probe appropriately, as depicted in Figure 8. Figure 9 is also shown for reference, as an image of the calibration grid located at the centre of the sample.

e) Approach the surface with the AFM probe using the minimum free oscillation amplitude possible to allow stable AFM feedback, for example with a set-point of 80 % of the oscillation (target) amplitude.

f) Set the fast scan direction to be perpendicular (90 degrees scanning angle) to the length of the cantilever and scan a large area to allow the identification of several flakes.

1) A scan-line speed of less than 10 µm/sec with a resolution of 256 pixels × 256 pixels shall be used to image the area in a reasonable time but also allow stable imaging when the probe passes over high topography features.

2) A set-point of ≤ 70 % of the free amplitude shall be used and an appropriate feedback gain that provides as fast a response to topography changes as possible, without creating oscillation artefacts.

i) The set-point can be reduced when imaging a flake and the step height monitored to determine if a lower set-point is required to measure a smaller and more accurate flake thickness measurement.

ii) However, a very low set-point value (for example a set-point of < 40 % of the free amplitude) can lead to artefacts due to damage of the probe apex and should be avoided.

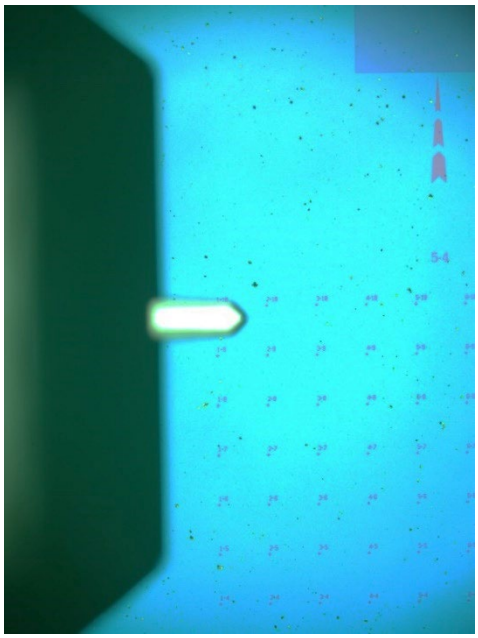


Figure 8 AFM tip placement over a location that has been identified as appropriate for measurements within the numbered areas of the substrate. (AFM optical microscope magnification is 20 ×).

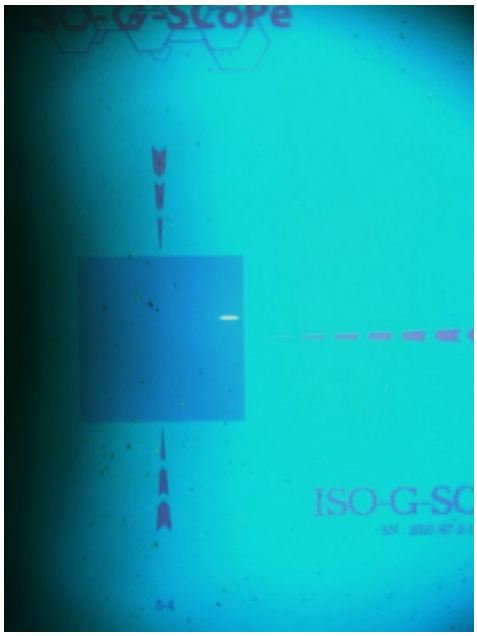


Figure 9 The calibration grid located at the centre of the substrate. The unique serial number of the substrate is also visible in the right lower part of the image (AFM optical microscope magnification is 20 ×).

5.2.2 AFM measurement method

Step 1) Start the measurements by obtaining a larger scan of the area of interest, for example a 20 µm × 20 µm scan as shown in Figure 10.

Step 2) In the AFM topographic image (Figure 10 here) identify features that are potential candidates for more detailed imaging, that is, individual flakes that appear to be primary particles and are not ambiguous in their composition. Avoid flakes or particles that appear to be a stack of different particles or appear to have significant height differences within one feature.

Step 3) Image the selected individual particles at high magnification and resolution (Figure 11). An ideal scan size for individual flakes would be between $(0.5\ \mu m)^2$ and $(2\ \mu m)^2$ and the number of pixels per image should be increased to 512 pixels or more to allow for increased precision during data analysis.

Step 4) Repeat steps 1-3. If during this step a particle is deemed as not appropriate for measurements, disregard this image, and continue with the next one until **topographic images of at least 20 individual primary particles have been obtained.**

- The raw images should be saved without any kind of image processing.

Step 5) Finally, acquire three 10 µm × 10 µm AFM images of the calibration grid located at the centre of the substrate, with each image from a different area of the calibration grid. This should be obtained using the same scanning parameters (scan speed, scanning angle etc.) as per the previous measurements of GNPs. An example AFM image of the grid is shown in Figure 12a.

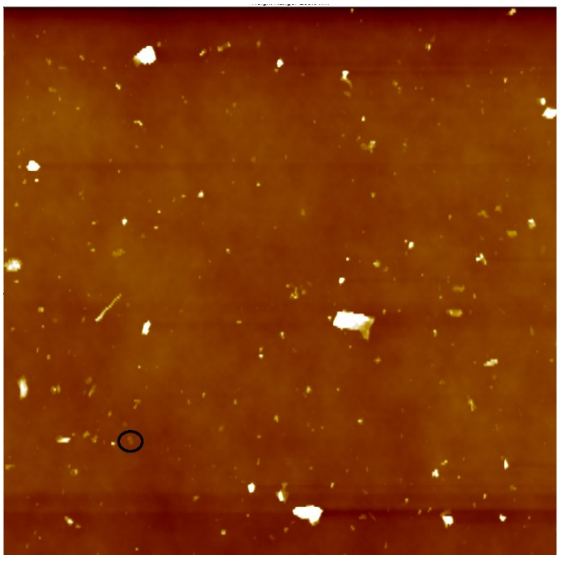


Figure 10 An example large scale topographic image of the area of interest. Individual particles can then be identified as candidates for high resolution imaging, as shown by the black circle. Image size 20 µm × 20 µm.

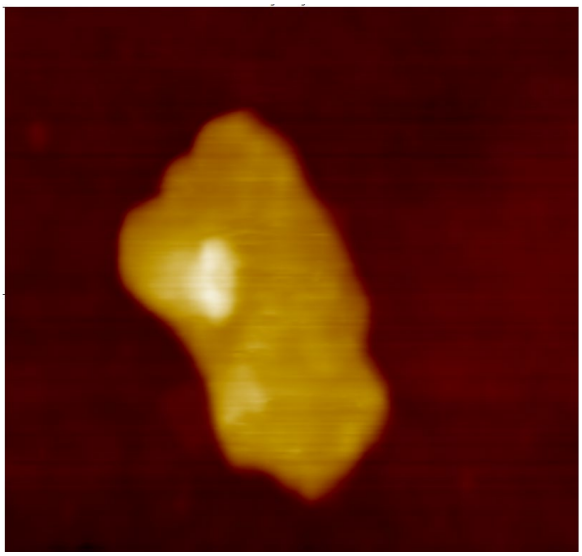


Figure 11 An example high resolution topographic image of an individual particle selected from the larger scan image (black circle in Figure 10). No scale bar is included to this image or others, so as to not bias the reader.

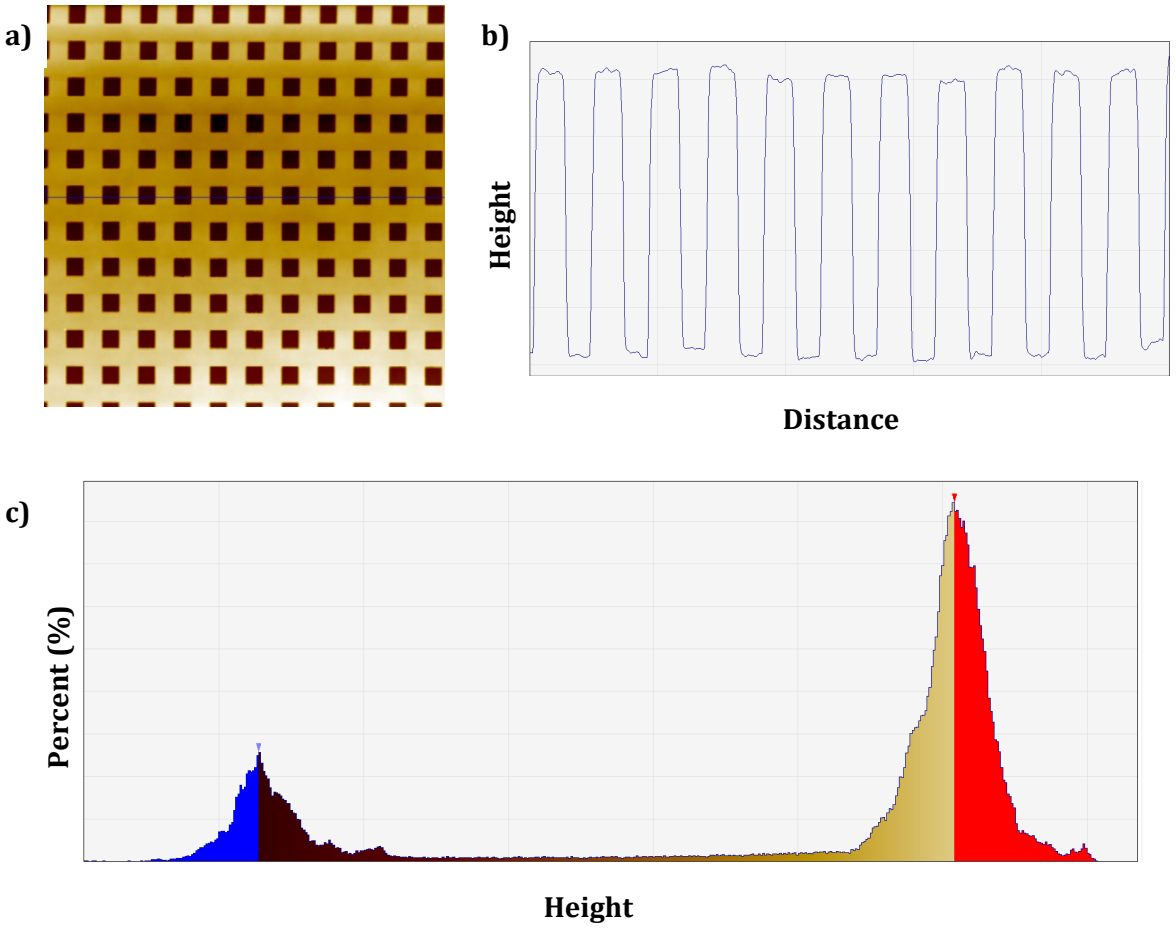


Figure 12 Examples of imaging and analysis of the calibration grid. a) 10 µm × 10 µm AFM height image of the calibration grid located at the centre of the sample, b) A line profile along the fast scan direction (blue line in (a)) has been obtained in the height image from which the pitch of the calibration grid can be calculated, c) Histogram of heights obtained from the height image. The difference between the two peaks can provide the average height difference for the grid in the image area.

5.3 IMAGE AND DATA ANALYSIS

5.3.1 Calibration grid analysis

Once an image of the calibration grid is obtained, a globally applied plane correction (first order or maximum 2nd order) shall be performed on the raw image to correct any possible image tilt. The X- and Y-axis of the grid are defined as having directions parallel to the sides of the grid squares. As shown in Figure 12a) a line profile in the AFM height image shall be obtained. The pitch of the grid is estimated using the AFM analysis software of your system, as presented in Figure 12b).

From the images of this grid, average values of the pitch in X- and Y- directions shall be obtained by measuring the distance between 10 features and then dividing the distance by 10 in order to calculate the distance between equivalent points of each feature. Analysis shall be done with a profile along the X-axis and subsequently a profile along the Y-axis. This same procedure shall be performed for the 3 AFM images, each image from a different area of the grid in the sample and shall be undertaken for the X-axis and Y-axis separately.

Note that a Fast Fourier Transform analysis can be used if preferred, to calculate the pitch for each axis (see reference [3] for details).

The pitch (average of 3 measurements) of each axis should be reported using the AFM Measurement report Excel spreadsheet, using the same software for analysis as was used for the analysis of the flakes. The standard deviation of the 3 measurements shall also be included for the pitch of each axis.

The height difference in the grid shall then be measured by creating a histogram of heights from the AFM height image. This should exhibit two peaks and the height difference can be measured from the distance between the modes of the two peaks, as shown in Figure 12c). This procedure may require any image bowing to be accounted for via image processing, depending on the system used (as described above). The height value of the calibration grid should be recorded in the AFM Measurement report Excel spreadsheet provided as the average value for the three images, with the standard deviation of the three values recorded as the uncertainty.

5.3.2 Particle analysis

AFM images that appear to have any type of artefacts SHALL NOT be analysed as this will result in extremely high uncertainty of measurements. Two examples of AFM images with artefacts are shown in Figure 13. In Figure 13a) a ‘double-tip’ effect results in a double edge at the left side of the particle and in Figure 13b) a drift of the sample or ‘flying tip’ results in poor tracking of the surface and line artefacts. AFM artefacts are not limited to the ones presented here, so care should be taken to ensure that the image quality is adequate for the purpose of this study.

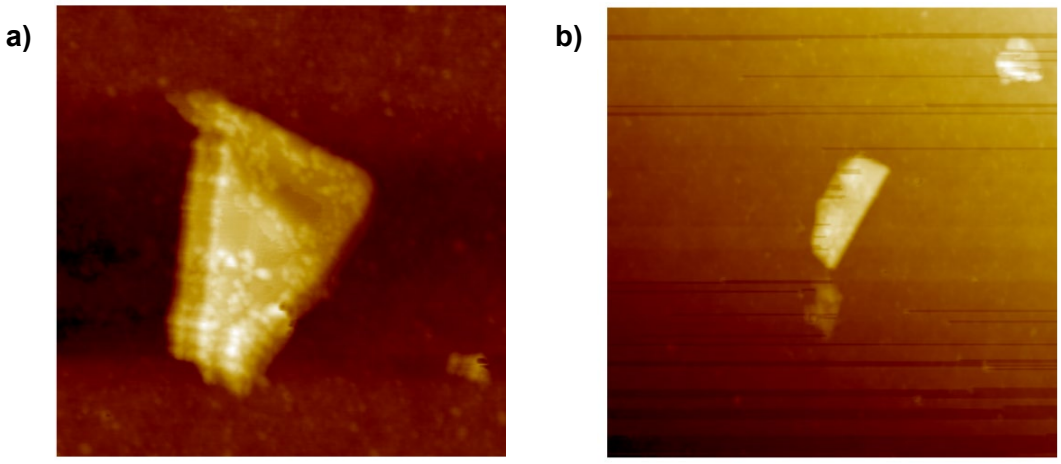


Figure 13 Examples of AFM images with artefacts present. a) A ‘double-tip’ effect resulting in a double edge on the left side of the particle, b) Poor tracking of the surface resulting in line artefacts. No scale bar is included to this image or others, so as to not bias the reader.

The AFM topographic images shall be used to measure the mean of the width and length measurements as the lateral flake size. It is best practice to use the raw AFM data files for analysis. In particular, the first option would be using the Z-axis sensor image and if this is not available then the height AFM images. Any kind of image processing should be avoided

when saving the images during the AFM experiment, simply save the unprocessed raw images. During analysis of the data, a globally applied plane correction (1st order) on the raw data to correct any possible tilting effects is acceptable and recommended. However, if significant bowing is observed, further flattening (2nd order plane correct) may be required, ideally using masking (excluding) graphene particles so that the software does not include features else than the substrate as part of the fitting routine.

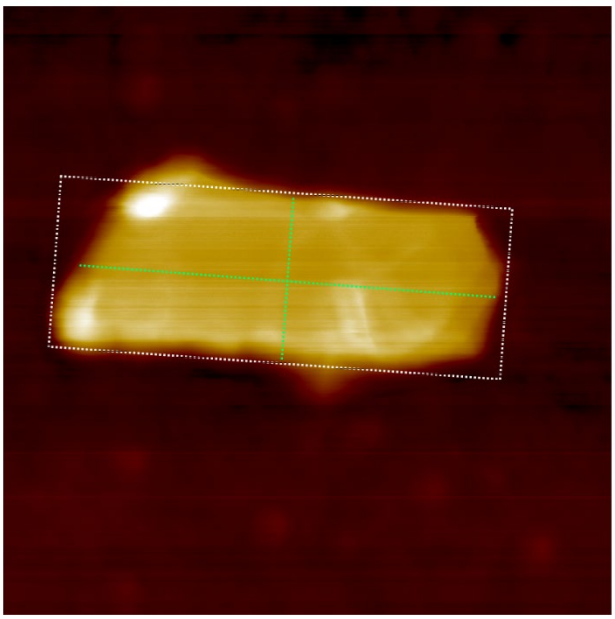
Using a similar data analysis procedure as the one performed for SEM measurements, the dimensions of each flake are determined by measuring the length and then the width of the flake, where these two values shall be defined so to approximately be the length and width of the smallest equivalent rectangle or ellipse that would best represent the particle (see examples in Figures 5a and 5c). Both the length and width must fall within the boundaries of the particle.

The width is defined as the segment within the flake that is perpendicular to the length and divides it in two equal parts (perpendicular bisector segment). Care should be taken in the choice of the flake length so to obtain a representative width. Note that this may not be the maximum perpendicular dimension, as for example shown in Figure 5f. Where the flake shape does not offer a clear choice of length, the minimum Feret diameter can be used as the width and the length taken as the perpendicular bisector of the width.

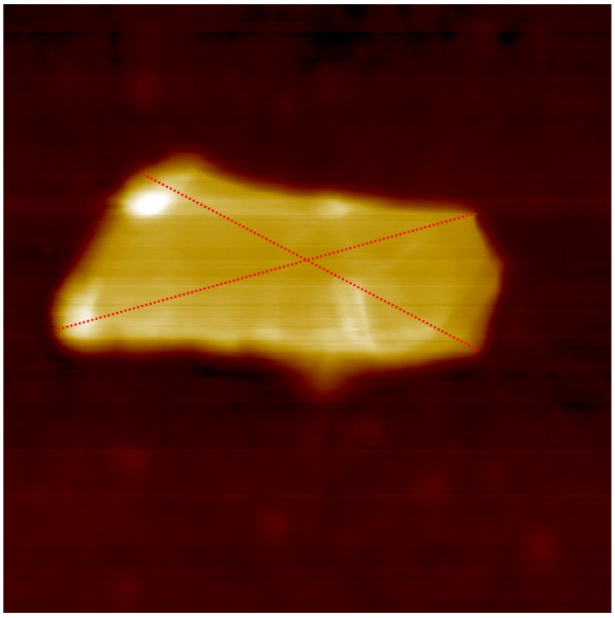
In addition, when acquiring length and width measurements from AFM images, a maximum and a minimum value for the length and width measurements shall be obtained as shown in the profile schematic in Figure 14c. The maximum value shall be obtained from the position at the apex of the change of the topography due to the flake (base of the flake) and the minimum value shall be obtained at the position where the flat part of the flake begins (top of the flake). **The length is then the average of the maximum and minimum length**. Similarly, the width is the average of the maximum and minimum width in the same way.

Finally, the lateral size of a flake is defined as the average mean of the length and width values and the standard deviation in average length and average width values shall be recorded as the associated uncertainty. An example of how the lateral flake size shall and shall not be measured in AFM images is shown in Figure 14.

a)



b)



c)

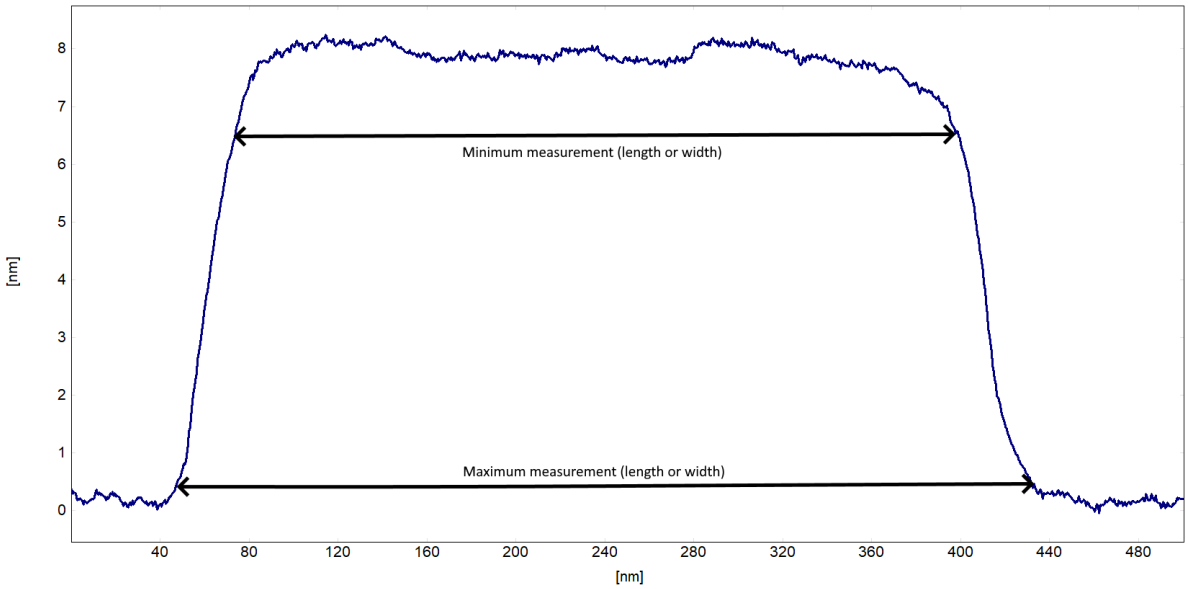


Figure 14 Examples of correct and incorrect lateral flake size measurements performed for an AFM image. a) Correct analysis, a rectangular representing approximately the flake is used for guidance to first acquire the length of the flake and then the width as the perpendicular bisector of the length. b) Incorrect measurement of the same flake. c) Schematic profile of this flake showing how minimum and maximum length and width measurements shall be acquired.

a)

b)

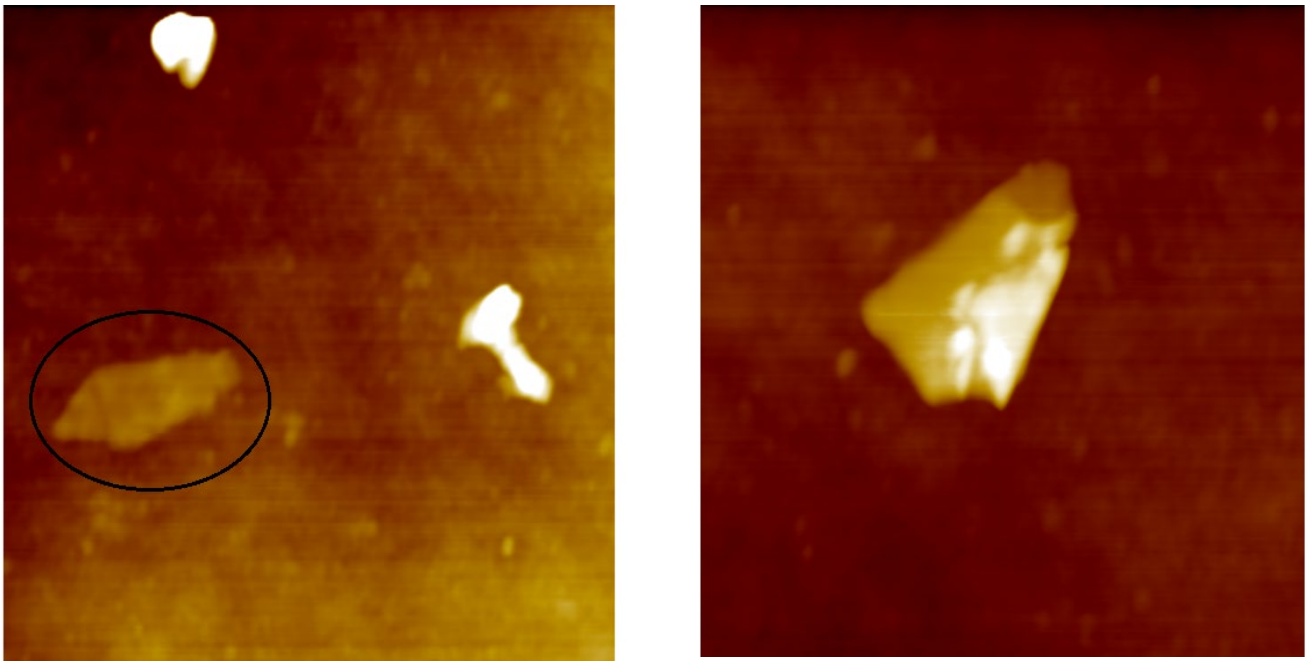


Figure 15 Examples of flakes observed in high resolution AFM images. a) Features that are identified as flakes lying flat on the surface (such as the flake highlighted by a black circle), are appropriate for measurements, whereas the brighter features are not. b) Flakes such as this one, that appear to have residue, significant height differences, folds or other structural inconsistencies shall be avoided.

Flakes shall only be measured if they are not ambiguous in their composition, that is, features that do not appear to be individual primary flakes shall not be measured. Flakes that do not have the complete circumference visible due to overlaying flakes or appear as agglomerated/aggregated particles shall 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 of the substrate, shall be measured. Otherwise, for example, the thickness measurement may be incorrect due to a large flake laying upon a smaller flake that is not directly observed. Examples of flakes that shall and shall not be measured are presented in Figure 15 above.

The thickness of each flake shall be measured by taking three height profiles through each flake and averaging the resulting thickness. The profiles shall be taken along the fast scan direction as shown in Figure 16a and shall be evenly spaced across the flake. Where possible, individual peaks on the flake surface should be avoided.

In each profile, measure the height difference between the substrate and the flake heights. The height of the substrate shall be measured as the average height of the substrate adjacent to the flake by superimposing a straight line. This can either be a fitted average if software permits or, if not, by approximation.

Similarly, the flake height shall be measured as the average height of the flat region of the flake (plateau), by superimposing a straight line either fitted if permitted or by approximation. If two obviously different height values can be measured at the edges of the flakes, as seen in Figure 16b-d, then the lowest of the two shall be reported.

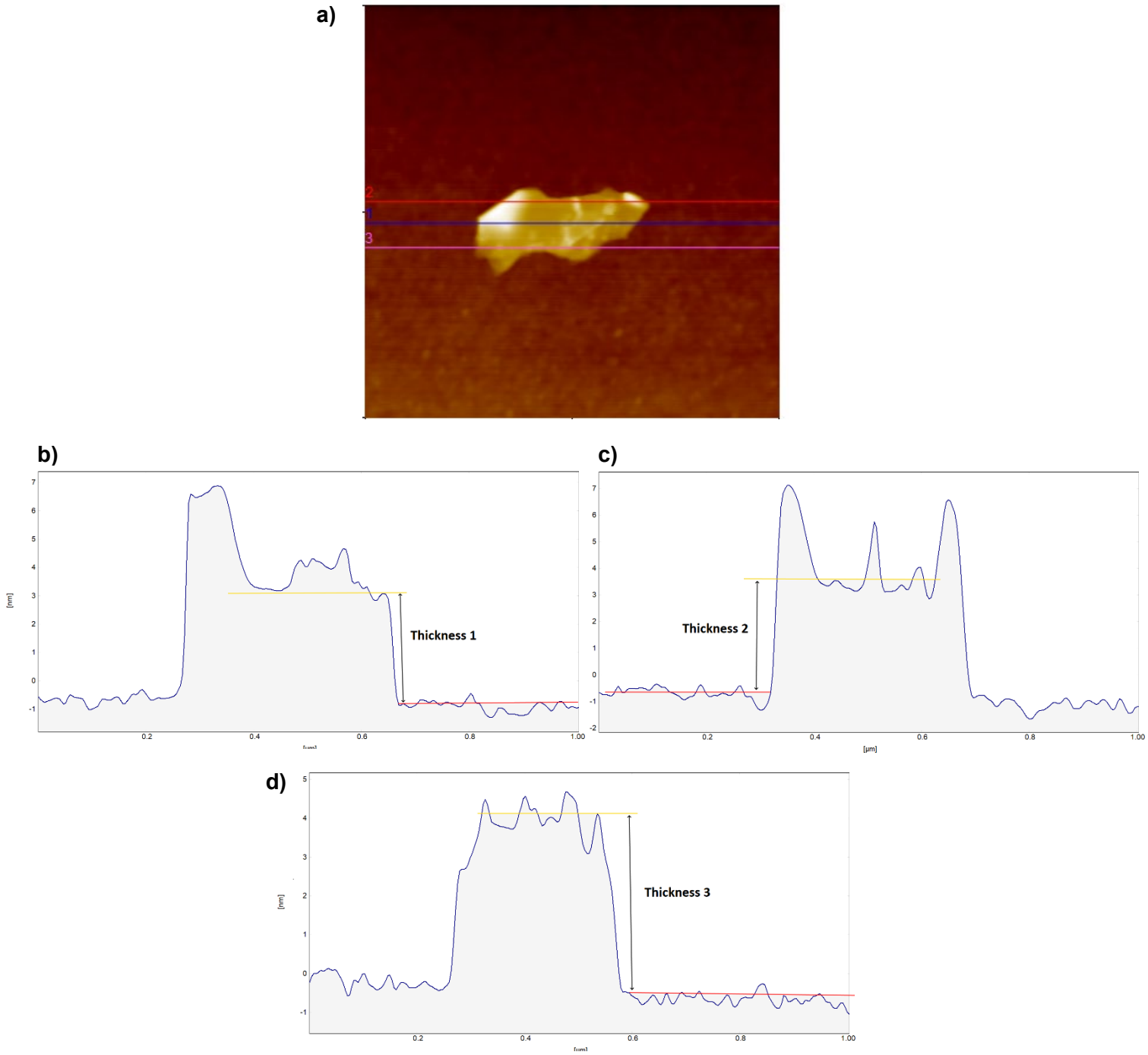


Figure 16 Example of AFM thickness measurements from a high resolution (1 µm × 1 µm) AFM height image of a sample from a different study. a) AFM topography image, with three profile lines detailed in b), c) and d) showing the two-line positions (yellow and red lines) for determining the three flake thickness measurements.

The standard deviation of the three values of thickness measured for each flake should be used as the estimated associated uncertainty. The root-mean-square (RMS) roughness of the substrate shall also be calculated for each AFM topography image used for measurements and reported in the AFM Measurement report Excel spreadsheet. The RMS value of the substrate can be calculated in the AFM software (see reference [3]).

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 17. The scatter plot should include error bars corresponding to the uncertainties detailed above, along with the best-fit linear correlation line and corresponding Pearson's R-squared value.

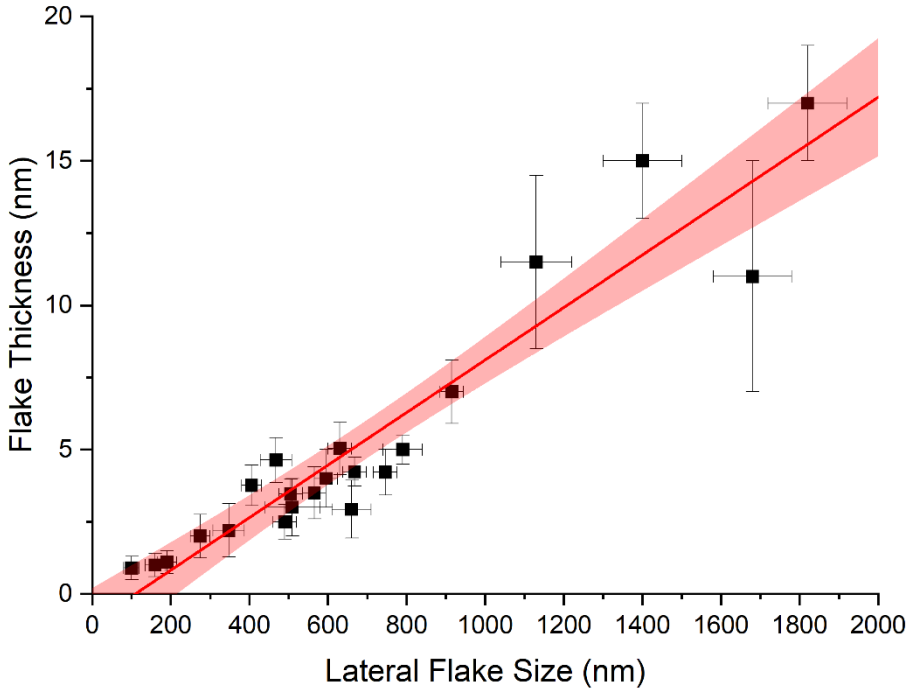


Figure 17 Flake thickness versus lateral flake size from AFM measurement data for individual flakes for more than 20 flakes within a sample, spanning the range of lateral flake sizes found in initial SEM measurements (note that these data are not from the sample used in this study) A linear fit is shown, along with 95 % confidence interval bands.

5.4 REPORTING THE AFM RESULTS

Results should be reported in the supplied AFM Measurement report Excel spreadsheet, where full details of the experimental conditions must be given, including:

- Instrument used.
- Environmental conditions (temperature and humidity).
- Cantilever type with spring constant, resonant frequency and tip radius.
- Imaging mode (i.e., tapping mode, peak force tapping or other similar mode).
- Image scan size and raster speed.
- Resolution (pixels).

- Closed loop used during imaging?
- Details of calibration procedure.
- Location on the substrate (numbered area) from which the measurements were obtained.

Full details of all data analysis steps must also be given, including:

- Image processing applied for images in report.
- Number of flakes measured.
- Maximum and minimum length and width measurements.
- Average mean lateral size measured, with uncertainty.
- Lateral pitch and mean height of reference grid on wafer, with uncertainties.

All raw AFM images must be returned to the lead participant, along with the AFM Measurement report Excel spreadsheet and the analysis, including the scatter plot (Figure 17).

6. SENDING THE RESULTS

A SharePoint folder has been created for each participant to collect the results of this study. All results shall be placed in the SharePoint folder for the specific participant. The lead participant shall than be informed that the data is uploaded by email (Dr Kostas Despotelis, kostas.despotelis@npl.co.uk).

The results should consist of a folder containing:

- SEM images (as tiff or jpg files with associated scale bar and working distance (WD) marked on the images).
- The SEM Measurement report Excel spreadsheet.
- The SEM lateral size histogram as shown in Figure 7.
- AFM images (in instrument's native format, and not image processed if possible).
- The AFM Measurement report Excel spreadsheet.
- The AFM scatter plot as shown in Figure 17.

7. FURTHER QUESTIONS AND TROUBLESHOOTING

If you are unsure of anything or need advice please contact Charles Clifford, (charles.clifford@npl.co.uk) or Kostas Despotelis (kostas.despotelis@npl.co.uk).

SUMMARY

Overall, this protocol aims to provide participants with a detailed methodology on how to handle the samples, locate the areas deemed as suitable for SEM and AFM measurements, obtain data, perform the analysis, and finally report back the results through the supplied Measurement report Excel spreadsheets. It is also expected that the provided samples will be sent back to the lead participant (NPL).

REFERENCES

[1] ISO/TS 21356-1:2021 Nanotechnologies — Structural characterization of graphene — Part 1: Graphene from powders and dispersions

[2] Raman Spectroscopy Measurement Protocol, A. Pollard and P. Turner, Surface Technology Group, National Physical Laboratory

[3] SPIP online manual: www.imagemet.com/WebHelp6/Default.htm