



POLITECNICO
MILANO 1863

**Blister in Intercalated HOPG:
Mechanical Properties of Individual
Structures Studied by Combined
High-Resolution Microscopy and
Spectroscopy**

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Cycle XXXVIII

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Academic year: **2024-2025**

Abstract

In recent years, significant attention has been devoted to the mechanical behaviour of thin crystalline membranes in layered van der Waals materials, motivated by their extreme mechanical compliance and by the strong coupling between deformation, electronic structure, and vibrational properties. Within this broad context, the formation of bubbles and blisters has emerged as a recurrent phenomenon whenever atomically thin or few-layer membranes are brought into contact with substrates or subjected to intercalation processes. Although long regarded as unavoidable morphological defects associated with imperfect interfaces or processing-induced contamination, blisters are now increasingly recognised as physically meaningful systems governed by general principles of membrane mechanics.

In two-dimensional materials, such as graphene and other van der Waals crystals, blisters have been extensively investigated as pressurised membranes enabling controlled strain engineering and the exploration of coupled mechanical, electronic, and vibrational phenomena. In contrast, blisters formed in bulk layered materials, and in particular in electrochemically intercalated highly oriented pyrolytic graphite (HOPG), have received comparatively limited attention as individual physical entities, with most studies focusing on surface morphology or electrochemical signatures rather than on their mechanical behaviour and internal strain state.

This thesis addresses this gap by investigating blisters formed in electrochemically intercalated HOPG as confined and mechanically active systems embedded within a multilayer graphite crystal. A multiscale experimental approach combining atomic force microscopy (AFM), correlative AFM–Raman spectroscopy, and tip-enhanced Raman spectroscopy (TERS) is employed to probe blister mechanics across different length scales, from their global elastic response to nanoscale strain heterogeneity.

AFM-based nanoindentation and mechanical manipulation experiments demonstrate that blistered regions behave as compliant pressurised membranes with an effective mechanical response that is markedly different from that of the surrounding basal plane. Correlative AFM–Raman measurements reveal that blister formation is associated with a well-defined strain state, which can be quantitatively

accessed through strain-induced shifts of the G and 2D Raman bands. By adopting a reference framework based on few-layer graphene, the spectroscopic response of individual blisters is interpreted in terms of finite-thickness membranes subjected to internal pressure.

The application of TERS provides direct access to the local vibrational response within single blisters, revealing pronounced spatial variations of strain that cannot be resolved by far-field spectroscopy. These measurements confirm that blister swelling does not act as a source of lattice defects and demonstrate that strain is heterogeneously distributed across the blistered membrane, with distinct behaviour at the centre and near the perimeter.

Taken together, the results presented in this thesis establish a coherent multiscale description of blisters in intercalated HOPG as confined mechanical systems. Beyond refining existing models of blister formation, this work redefines the role of blisters in bulk layered materials and highlights their relevance as model systems for studying strain, confinement, and membrane mechanics beyond strictly two-dimensional crystals.

Sinossi

Negli ultimi anni, notevole attenzione è stata dedicata al comportamento meccanico delle membrane cristalline sottili nei materiali stratificati di tipo van der Waals, motivata dalla loro estrema cedevolezza meccanica e dal forte accoppiamento tra deformazione, struttura elettronica e proprietà vibrazionali. In questo ampio contesto, la formazione di bolle e blister è emersa come un fenomeno ricorrente ogniqualvolta membrane atomiche o a pochi strati vengono messe a contatto con substrati o sottoposte a processi di intercalazione. Sebbene a lungo considerati come difetti morfologici inevitabili associati a interfacce imperfette o a contaminazioni indotte dai processi di fabbricazione, i blister sono oggi sempre più riconosciuti come sistemi fisicamente significativi, governati da principi generali della meccanica delle membrane.

Nei materiali bidimensionali, come il grafene e altri cristalli di van der Waals, i blister sono stati ampiamente studiati come membrane pressurizzate che consentono un'ingegneria controllata della deformazione e l'esplorazione di fenomeni accoppiati di natura meccanica, elettronica e vibrazionale. Al contrario, i blister formati in materiali stratificati bulk, e in particolare nella grafite altamente orientata pirolitica (HOPG) intercalata elettrochimicamente, hanno ricevuto un'attenzione relativamente limitata come entità fisiche individuali, con la maggior parte degli studi focalizzati sulla morfologia superficiale o sulle firme elettrochimiche, piuttosto che sul loro comportamento meccanico e sullo stato di deformazione interno.

Questa tesi affronta tale lacuna studiando i blister formati in HOPG intercalata elettrochimicamente come sistemi confinati e meccanicamente attivi, incorporati all'interno di un cristallo di grafite multilayer. Viene adottato un approccio sperimentale multiscala che combina microscopia a forza atomica (AFM), spettroscopia Raman correlata AFM-Raman e spettroscopia Raman esaltata da punta (TERS), al fine di investigare la meccanica dei blister su diverse scale di lunghezza, dalla risposta elastica globale fino all'eterogeneità della deformazione su scala nanometrica.

Esperimenti di nanoindentazione e manipolazione meccanica basati su AFM dimostrano che le regioni contenenti blister si comportano come membrane pressurizzate cedevoli, con una risposta meccanica efficace marcatamente diversa rispetto al piano basale circostante. Le misure correlate AFM-Raman mostrano che la formazione dei blister è associata a uno stato di deformazione ben definito, accessibile quantitativamente attraverso gli spostamenti indotti dalla deformazione

delle bande Raman G e 2D. Adottando un quadro di riferimento basato sul grafene a pochi strati, la risposta spettroscopica dei singoli blister viene interpretata in termini di membrane a spessore finito soggette a pressione interna.

L'applicazione della TERS consente di accedere direttamente alla risposta vibrazionale locale all'interno dei singoli blister, rivelando marcate variazioni spaziali della deformazione che non possono essere risolte mediante spettroscopia in campo lontano. Tali misure confermano che il rigonfiamento dei blister non costituisce una sorgente di difetti del reticolo e dimostrano che la deformazione è distribuita in modo eterogeneo all'interno della membrana, con comportamenti distinti tra il centro e la regione prossima al perimetro.

Nel complesso, i risultati presentati in questa tesi stabiliscono una descrizione multiscala coerente dei blister in HOPG intercalata come sistemi meccanici confinati. Oltre a raffinare i modelli esistenti di formazione dei blister, questo lavoro ridefinisce il ruolo dei blister nei materiali stratificati bulk e ne evidenzia la rilevanza come sistemi modello per lo studio della deformazione, del confinamento e della meccanica delle membrane oltre il limite dei cristalli strettamente bidimensionali.

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Acronyms

AFM Atomic Force Microscopy

EC-AFM Electrochemical Atomic Force Microscopy

STM Scanning Tunneling Microscopy

EC-STM Electrochemical Scanning Tunneling Microscopy

SPM Scanning Probe Microscopy

TERS Tip-Enhanced Raman Spectroscopy

SERS Surface-Enhanced Raman Spectroscopy

HOPG Highly Oriented Pyrolytic Graphite

GIC Graphite Intercalation Compound

CV Cyclic Voltammetry

WE Working Electrode

CE Counter Electrode

RE Reference Electrode

CCD Charge-Coupled Device

EMCCD Electron-Multiplying Charge-Coupled Device

FEM Finite Element Method

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the blister that has a strain less then 0.25 %. G peak confidence interval slope $m = (- 39,3 \pm 0,2) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+1582 \pm 4) \text{ cm}^{-1}$153

1

Introduction

1.1 Blisters and bubbles in stratified materials: from morphological defects to physical systems

Blisters and bubbles are commonly observed in stratified and two-dimensional materials whenever thin crystalline membranes are brought into contact with substrates or other layered solids. Historically, these structures have been regarded as unavoidable morphological defects, originating from the trapping of foreign species such as gases, liquids, or contaminants—during growth, transfer, intercalation, or annealing processes. In early studies, their presence was primarily interpreted as a signature of imperfect interfaces, often correlated with degraded electronic transport, reduced device performance, or local mechanical instability [1].

In van der Waals materials, including graphene, hexagonal boron nitride, and transition metal dichalcogenides, blister formation arises naturally from the combination of extreme mechanical compliance of atomically thin layers and strong interfacial adhesion. During lamination onto atomically flat substrates, van der Waals forces tend to expel adsorbed molecules from the interface, which subsequently aggregate into confined pockets forming bubbles or blisters, while extended regions of intimate contact remain largely contamination-free [2]. Within this framework, blisters were long considered passive by-products of interface cleaning processes, serving mainly as qualitative indicators of adhesion.

Over the last decade, however, a growing body of experimental and theoretical work has demonstrated that blisters and bubbles cannot be adequately described as mere topographical irregularities. Instead, they must be regarded as mechanically active systems in which elastic deformation, interfacial adhesion, and confinement of matter are intrinsically coupled. Atomic force microscopy studies have shown that two-dimensional membranes forming bubbles adopt well-defined geometries characterized by universal aspect ratios, largely independent of their lateral size, indicating an equilibrium governed by the balance between elastic energy and van der Waals adhesion [2,3]. This evidence marked a fundamental conceptual

transition, shifting the interpretation of blisters from accidental morphological artifacts to physical objects governed by general principles of membrane mechanics.

Beyond their mechanical significance, blisters have emerged as efficient platforms for local strain engineering. Two-dimensional materials are known to tolerate exceptionally large elastic deformations, with graphene sustaining tensile strains up to approximately 25%. When incorporated into a blister geometry, this mechanical resilience allows the membrane to accommodate substantial out-of-plane deformation, resulting in highly inhomogeneous strain fields localized at the blister centre and perimeter[4,5] These strain fields have profound consequences for the electronic, vibrational, and optical properties of the material, enabling phenomena such as pseudo-magnetic fields, exciton funnelling, and localized quantum emission.

In this framework, the blister must be reinterpreted as an autonomous physical system rather than a passive morphological defect. Its behaviour is governed by the interplay between membrane elasticity, interfacial adhesion, and the thermodynamic and mechanical properties of the confined phase. This reinterpretation has been further reinforced by studies showing that blisters can be intentionally fabricated, stabilized, and even manipulated through controlled irradiation, pressure, electrochemical cycling, or mechanical actuation, thereby transforming an originally undesired feature into a functional nanostructure [1,5].

1.2 Mechanics and physics of pressurized membranes in two-dimensional systems

Once blisters and bubbles are recognized as physically meaningful objects rather than accidental morphological features, their description naturally falls within the framework of thin, pressurized membrane mechanics. In this context, the upper layers forming a blister can be treated, to first approximation, as a deformable membrane clamped to the surrounding substrate and inflated by a confined phase trapped underneath. This approach has proven effective in capturing the essential features of blister geometry and mechanical response in a variety of two-dimensional and layered systems [6].

A common starting point is the spherical cap approximation, in which the blister profile is described as a section of a sphere characterized by a well-defined height-to-radius ratio. Within this model, the equilibrium shape results from the competition between elastic deformation of the membrane and adhesive interactions

with the substrate. Depending on the relative magnitude of these contributions, the mechanical response may be dominated either by bending rigidity or by in-plane stretching. For sufficiently large blisters, stretching typically prevails, leading to scale-invariant geometries and universal aspect ratios that are largely independent of lateral size[2,7].

Adhesion plays a central role in this balance, as it sets the boundary conditions at the blister edge and determines the energetic cost of delamination. Strong interfacial adhesion favours well-confined blisters with sharply defined perimeters, while weaker adhesion allows for more gradual transitions between the swollen and flat regions. In multilayer systems, such as stratified graphite, the effective mechanical response is further influenced by the number of layers involved in the deformation and by their degree of mechanical coupling, leading to deviations from ideal monolayer behaviour [1,8].

The mechanical deformation associated with blister formation inevitably gives rise to non-uniform strain fields within the membrane. In two-dimensional materials, strain directly affects the lattice dynamics and electronic structure, resulting in measurable shifts and modifications of vibrational modes. As a consequence, spectroscopic techniques most notably Raman spectroscopy provide a powerful, indirect probe of the underlying mechanical state by linking local deformation to characteristic spectral signatures [4,9].

Within this framework, the description of blisters as pressurized membranes establishes a direct conceptual bridge between morphology, mechanics, and spectroscopy. This connection is essential for interpreting experimental observations, as it allows geometrical parameters extracted from scanning probe measurements to be correlated with strain-induced spectroscopic features. The models introduced here therefore provide the physical foundation for the combined AFM, Raman, and TERS analyses presented in the following chapters.

1.3 HOPG Blisters

A distinct class of blisters is represented by those formed in electrochemically intercalated highly oriented pyrolytic graphite (HOPG). In this system, blister formation arises from the combined effects of ion intercalation and subsequent gas evolution occurring beneath the surface during electrochemical polarisation in acidic electrolytes. In contrast to blisters observed in transferred or exfoliated two-dimensional materials, HOPG blisters originate from processes taking place within

a bulk layered crystal, in which a finite number of graphitic layers locally delaminate and swell while remaining mechanically connected to the surrounding lattice [10].

Historically, blisters in intercalated graphite have been investigated predominantly from a morphological and kinetic perspective [11–13]. Both early and more recent studies have focused on their surface appearance, growth dynamics, and correlation with electrochemical parameters, often interpreting them as macroscopic manifestations of intercalation-induced degradation or as detrimental by-products of electrochemical exfoliation processes [14]. Within this framework, blisters were rarely treated as individual physical entities, but rather as collective surface features reflecting the progression of oxidation, gas evolution, or crystal swelling.

With respect to the case of two-dimensional materials, blisters formed in intercalated graphite exhibit several distinctive features that render them particularly suitable for systematic investigation. Notably, multiple studies have demonstrated that, once formed, these blisters are remarkably stable over time, persisting for extended periods both in liquid electrolytes and after removal into ambient conditions [15,16].

An additional advantage arises from the fact that HOPG blisters are generated directly on a single, continuous crystalline substrate. This intrinsic compatibility eliminates issues related to transfer-induced contamination or substrate variability and enables direct comparison between blistered regions and the surrounding pristine basal plane. Despite these favourable characteristics, the physical properties of individual HOPG blisters such as their local mechanical stiffness, strain distribution, defect landscape, and spectroscopic response have remained largely unexplored.

1.4 HOPG: a complex multilayer system

Since the isolation of graphene and the subsequent recognition of its physical properties, graphite can be conveniently described as a bulk crystal composed of stacked graphene layers. Within this framework, graphene represents the fundamental structural unit of graphite, consisting of a two-dimensional honeycomb lattice of carbon atoms arranged in a hexagonal geometry.

In crystalline graphite, graphene layers can adopt different stacking sequences. The most common arrangement corresponds to hexagonal stacking, typically described by an AB or ABA sequence, while a rhombohedral stacking (ABC

sequence) may also occur, although it is energetically less stable and therefore less frequently observed. The stacking order has important implications for the electronic properties of graphite, whereas the interlayer spacing remains approximately constant, with a value of about 0.35 nm in both configurations [17,18]. The corresponding crystal structures, schematically illustrated in Figure 1.1, consist of carbon honeycomb planes stacked along the crystallographic c-axis, with a lateral displacement of approximately 0.25 nm between adjacent layers in the AB configuration [18].

Within each graphene layer, carbon atoms are sp^2 -hybridized and bonded to three nearest neighbours through strong covalent σ bonds, with a bond length of approximately 0.14 nm [19]. These in-plane bonds are responsible for the high mechanical stiffness and thermal conductivity characteristic of both graphene and graphite. In contrast, the interlayer interaction is governed by the overlap of the out-of-plane 2pz orbitals, which gives rise to relatively weak π bonding along the stacking direction. This pronounced anisotropy between strong in-plane covalent bonding and weak out-of-plane van der Waals interactions underlies the layered nature of graphite and plays a central role in its mechanical response, particularly under conditions that promote interlayer decoupling or delamination.

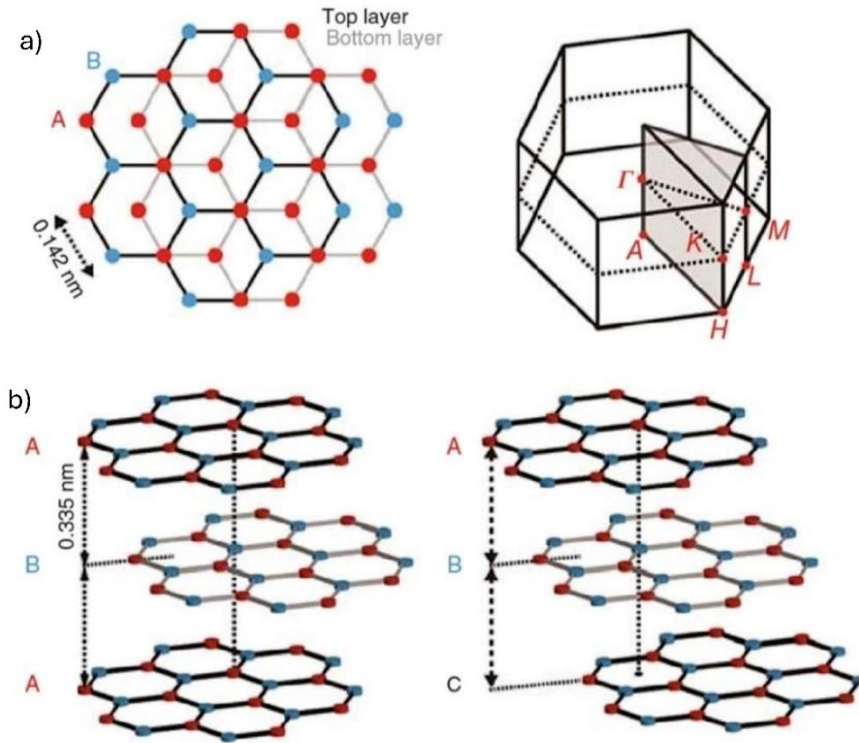


Figure 1.1: HOPG crystal lattice [20].

Highly Oriented Pyrolytic Graphite (HOPG) represents a widely used form of artificial graphite that enables the intrinsic properties of the hexagonal layered structure to be investigated under well-controlled conditions. HOPG is produced through the thermal decomposition of carbonaceous gases, typically methane, at temperatures exceeding 1200 °C, followed by a high-temperature, high-pressure annealing process that promotes a high degree of crystallographic alignment [21]. The resulting material is polycrystalline in nature, consisting of numerous graphite crystallites whose basal planes are preferentially oriented parallel to one another [22] .

The degree of alignment among individual crystallites is commonly quantified by the mosaic spread, which describes the angular distribution of the crystallographic c-axes. A smaller mosaic spread corresponds to higher structural quality and reduced inter-grain misorientation. Although HOPG is commercially classified into different grades on the basis of this parameter, its layered structure and anisotropic bonding are preserved across all grades. In particular, the combination of strong in-plane covalent bonding and weak interlayer van der Waals interactions gives rise to a pronounced mechanical anisotropy, which facilitates delamination along the out-of-plane direction [23–25].

This anisotropy renders HOPG especially suitable for the preparation of wide, atomically flat surfaces through mechanical exfoliation. The removal of the uppermost layers exposes fresh basal planes that remain largely uncontaminated over extended periods, owing to the weak interaction of the out-of-plane π orbitals with the surrounding environment. As a consequence, HOPG has become a reference substrate for surface-sensitive techniques, including scanning tunnelling microscopy and atomic force microscopy, where large, well-defined terraces are required for high-resolution investigations [26–29].

Beyond its relevance in surface science, the mechanical, thermal, and chemical stability of graphite also renders HOPG a model electrode material for electrochemical studies. Extensive research efforts have been devoted to elucidating the electrochemical behaviour of HOPG, with particular emphasis on the distinct roles played by basal planes and surface defects in redox processes occurring at the solid–liquid interface [30,31]. These investigations have further contributed to a broader comparison among different carbon allotropes, such as graphene, carbon nanotubes, and nanowires, whose electrochemical properties remain the subject of active research [32–34]. In this context, a detailed understanding of the electroactivity of HOPG surfaces is essential not only from a fundamental

standpoint, but also for assessing the suitability of graphite-based materials for energy-related applications, including ion-transfer and intercalation-based devices.

1.5 HOPG Intercalation according to Murray Model

Despite the generally good electrochemical stability of graphite when employed as an electrode material, specific electrolytes are capable of intercalating into the stratified structure of HOPG, partially screening the interlayer interactions and promoting local delamination in aqueous environments [27]. This behaviour has attracted considerable interest, as electrochemical intercalation represents an effective strategy for modifying the structural and physical properties of layered crystals. Results reported in the literature [35,36] have motivated the extension of analogous approaches to other van der Waals materials, such as MoS_2 and MnO_2 [37], although only a limited number of systems have been successfully investigated so far, primarily due to the difficulty in identifying suitable electrolytes.

Within this context, the investigation of the electroactivity of both the basal plane and surface defects in HOPG is of particular relevance, not only from a fundamental perspective but also in view of applications in electrochemical devices, including ion-transfer and intercalation-based batteries. When an electrochemical potential is applied to an HOPG electrode immersed in an electrolyte, ionic species can be driven into the interlayer galleries of the crystal, where they locally weaken the layer-layer coupling by occupying the interstitial spaces.

Electrochemical intercalation in HOPG is typically achieved by applying a controlled electrochemical potential to the graphite sample immersed in an aqueous acidic solution [38]. A key advantage of this approach lies in the possibility of precisely controlling both the onset and the kinetics of the intercalation process through the applied potential. Commonly employed electrolytes include sulfuric acid (H_2SO_4), used in this thesis, as well as perchloric (HClO_4) and nitric (HNO_3) acids. While these solutions efficiently promote ion intercalation, they are also associated with oxidation processes that may induce defects in the graphite lattice or in exfoliated graphene layers.

From a microscopic standpoint, the electrochemical intercalation process in graphite has been extensively investigated in the past [39–41]. At specific electrochemical potentials, acid ions penetrate into the interlayer gaps, leading to an expansion of the interlayer spacing and to charge transfer between the electrolyte species and the graphite host lattice. This behaviour can be rationalised in terms

of the electrochemical reactions reported in Eq. (1.1), which account for both cathodic and anodic contributions.



Here, M^+ and A^- denote cations and anions, respectively, while C_x represents a stoichiometric amount of graphite carbon atoms involved in the charge-transfer process.

The products of electrochemical intercalation are commonly referred to as graphite intercalation compounds (GICs), which primarily affect the uppermost layers of the graphite crystal. The structure of GICs is often described within a staged intercalation model, in which intercalant-filled layers alternate with pristine graphene layers in a statistically regular sequence. In a stage- n configuration, intercalated layers are separated by $(n-1)$ unintercalated graphene sheets. A schematic representation of several consecutive intercalation stages is shown in Figure 1.2.

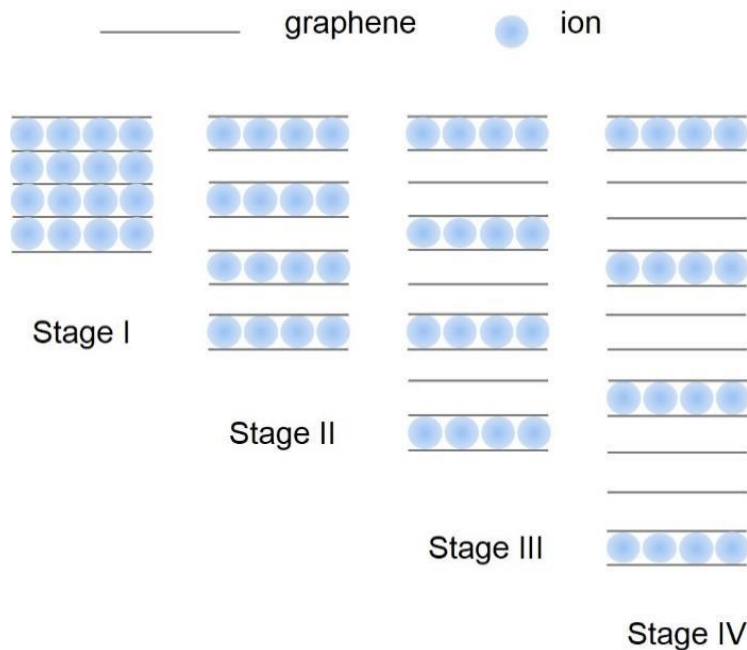


Figure 1.2: Schematic representation of the intercalation stage in four different GIC configurations. Grey line is the carbon layer and balls represent the intercalated ions. [20]

Experimental observations indicate that the intercalation process involves a set of phenomena and reactions that are significantly more complex and extensive than those schematically represented in Figure 1.2. Accordingly, Eq. (1.1) alone is

not sufficient to account for the experimentally observed behaviour. When graphite is immersed in an H_2SO_4 solution and a sufficiently high electrochemical potential is applied, the basal plane undergoes oxidation processes that may lead to carbon dissolution. In addition, it has been observed that the HOPG surface becomes swollen and that the internal graphite structure is progressively damaged under these conditions [13,14,42].

Within this complex experimental landscape, Murray and co-workers proposed a seminal interpretative model that rationalises the electrochemical intercalation of HOPG in diluted electrolytic solutions [39]. This framework provided, for the first time, a coherent physical picture linking ion intercalation, electrochemical oxidation, and surface blister formation. The key stages of the process namely the formation of electrochemical graphite oxide (EGO), the development of graphite intercalation compounds (GICs), and the subsequent evolution of blisters are schematically summarised in Figure 1.3.

The Murray model is based on two central assumptions. First, structural defects such as step edges, grain boundaries, vacancies, kinks, and other lattice irregularities act as preferential access points for anions entering the graphite structure. Second, the finite interlayer spacing of graphite enables the intercalation of solvated anions under suitable electrochemical conditions. When sufficiently high electrochemical potentials, close to the onset of the oxygen evolution reaction (OER), are applied to the graphite electrode, solvated anions intercalate and promote the oxidation of carbon in the near-surface region. This oxidation process leads to the formation of gaseous species, including CO , CO_2 , and O_2 , which accumulate beneath the uppermost graphene layers. The resulting gas confinement induces local swelling of the graphite surface and drives the nucleation of blisters, as illustrated in Figure 1.3c. The subsequent growth of these blisters is ultimately limited by the elastic response of the overlying graphene layers, whose in-plane strain stabilises the final surface morphology. In this context, the blistered region can be effectively described as a pressurised multilayer membrane, although this representation should be regarded as a simplified mechanical approximation of a system that also involves intercalation processes, chemical modification of graphite, and gas evolution, as shown in Figure 1.3d.

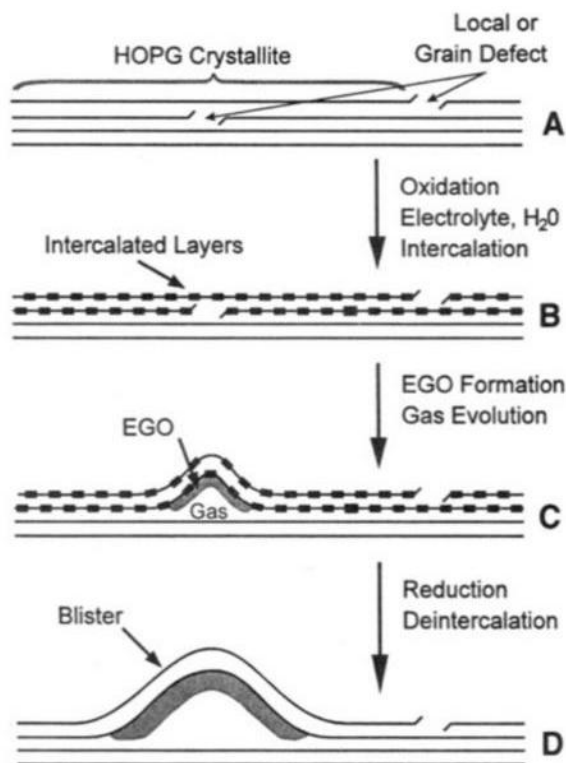


Figure 1.3: Model of blister formation and growth: **A)** cross section of the basal HOPG plane before the oxidation. Lines correspond to HOPG layers (graphene). Surface and bulk defect sites (e.g. crystalline grain boundaries, step edges, etc.) are also depicted. **B)** when HOPG is positively biased, carbon oxidation takes place. Dashed lines indicate areas where solvated anion intercalation occurred. **C)** initial blister growth. The shaded area represents graphite oxide. **D)** final and stable blister structure. [13]

Already in their original work, Murray and co-workers emphasised that this model should be regarded as a starting point rather than as a complete description of the electrochemical intercalation process [13]. In the years that followed, several of the open questions raised by this framework were addressed, leading to significant refinements of the original picture. De Rosa and co-workers, by combining electrochemical treatments with time-of-flight secondary ion mass spectrometry (TOF-SIMS), demonstrated that intercalation can proceed along grain boundaries to depths of several hundreds of nanometres, thereby highlighting the strongly three-dimensional character of the process [15]. Complementary insight into the dynamics of blister formation was provided by Yivlialin and collaborators, who quantified the characteristic timescale of blister nucleation and showed that, at an applied potential of 1.2 V, blister formation occurs on the order of a few seconds [12].

More recently, Jagadeesh et al. demonstrated that blister formation is not an unavoidable fingerprint of intercalation itself. By combining atomic force

microscopy with X-ray photoemission spectroscopy, they showed that when cyclic voltammetry is performed at sufficiently high scan rates, exceeding 600 mV s^{-1} , intercalation can still occur in the absence of surface blistering [43]. This result clearly decouples ionic intercalation from surface swelling, indicating that blister formation depends not only on the chemical modification of the graphite lattice, but also on the kinetics of gas evolution and on mechanical relaxation processes.

Taken together, these results provide a refined picture of electrochemical intercalation and blister formation in HOPG, clarifying the roles of defects, ion transport pathways, and kinetic effects. At the same time, they highlight an important limitation of existing descriptions: while the conditions leading to blister nucleation and growth are increasingly well understood, the physical properties of the resulting blisters remain largely unexplored. In particular, the mechanical response, internal strain state, and local heterogeneity of individual blisters cannot be inferred solely from electrochemical parameters or from morphological observations. This gap calls for an experimental perspective in which blisters are no longer treated as secondary surface features, but rather as confined mechanical systems whose properties must be directly probed.

1.6 Probing the mechanical properties of HOPG blisters: beyond the conventional approaches

The growing recognition of blisters in intercalated HOPG as physically meaningful objects raises a fundamental question concerning their experimental investigation: how can their mechanical properties be accessed in a reliable and comprehensive manner? Addressing this issue requires moving beyond the conventional use of surface-sensitive techniques as purely descriptive tools and adopting a framework in which blisters are treated as confined mechanical systems, whose response must be probed across different length scales and degrees of localization [44].

Historically, the study of HOPG blisters has relied predominantly on morphological observations, primarily obtained by atomic force microscopy. Such measurements have been instrumental in identifying blister formation, tracking their temporal evolution, and establishing correlations with electrochemical parameters [45–50]. However, while morphology provides essential information on blister geometry, it does not uniquely define the underlying mechanical state. Blisters with similar height and lateral extension may differ substantially in

stiffness, internal pressure, interlayer coupling, or strain distribution, particularly in a multilayer system such as HOPG.

A comparable limitation emerges when spectroscopic techniques are employed in isolation. Raman spectroscopy is intrinsically sensitive to strain, defects, and lattice perturbations in graphitic systems and has been extensively used to monitor intercalation-induced modifications in graphite [51–53]. Nevertheless, in the absence of a direct spatial correlation with surface morphology, Raman measurements collected on blistered HOPG inevitably average over multiple regions, including pristine basal planes, blister borders, and swollen areas. As a result, the specific spectroscopic signature associated with an individual blister cannot be unambiguously identified without precise morphological guidance.

These considerations indicate that the central challenge in blister studies is not the lack of experimental tools, but rather the way in which they are conventionally employed. In the context of this thesis, scanning probe microscopy techniques are not treated merely as imaging methods, but are instead exploited as active probes of mechanical behaviour. Atomic force microscopy, for instance, can be used not only to reconstruct blister geometry, but also to induce controlled deformations, perform local indentation, and probe the elastic response of the blistered membrane [54,55]. In this way, AFM becomes a means to directly access mechanical properties such as stiffness, compliance, and deformation pathways, rather than serving solely as a topographical mapping tool.

Within this conceptual framework, the correlation between AFM and Raman spectroscopy assumes a fundamentally different role. Rather than representing a simple superposition of two techniques, their combination enables a direct link between mechanical deformation and vibrational response. Morphological and mechanical information obtained by AFM provides the necessary reference to localize Raman measurements on specific regions of the blister, thereby allowing spectroscopic features to be interpreted in terms of well-defined mechanical states. Without this correlation, the Raman response of HOPG blisters would remain intrinsically ambiguous, particularly given the strong contribution of the underlying bulk crystal.

At even smaller length scales, the limitations of spatial averaging become increasingly severe, as strain and mechanical properties are expected to vary significantly across the blister, from its centre to its perimeter. In this regime, advanced scanning probe-based spectroscopic techniques, such as tip-enhanced Raman spectroscopy, offer access to the local vibrational response with nanometric

resolution, enabling the investigation of strain heterogeneity and interlayer coupling at the scale where continuum descriptions begin to break down [56,57]. Importantly, such approaches are not introduced as isolated methodological refinements, but as a natural extension of a multiscale strategy aimed at resolving the mechanical complexity of the blistered system.

Taken together, these considerations motivate an experimental approach in which different scanning probe-based techniques are combined to probe complementary aspects of blister mechanics. By integrating direct mechanical manipulation, strain-sensitive spectroscopy, and local vibrational analysis, it becomes possible to construct a coherent description of HOPG blisters as confined mechanical systems. This multiscale perspective provides the conceptual foundation for the experimental investigations presented in the following chapters, where mechanical response, strain distribution, and local strain variations are systematically explored.

1.7 Objectives of the thesis and structure of the manuscript

The aim of this thesis is to establish a coherent mechanical framework for blisters formed in electrochemically intercalated highly oriented pyrolytic graphite, treating these structures as confined and mechanically active systems. The work is based on the idea that blister mechanics can be accessed only by combining different scanning probe-based approaches, allowing the mechanical response and the associated strain distribution to be resolved from the micrometre scale down to the nanoscale.

Chapter 2 describes the experimental techniques employed in this work. The electrochemical configuration used for graphite intercalation is first introduced, followed by the operating principles of atomic force microscopy, scanning tunnelling microscopy, and Raman spectroscopy. The combined AFM–Raman setup and the Tip Enhanced Raman Spectroscopy configuration are also presented, establishing the methodological framework for the multiscale mechanical and spectroscopic investigations developed in the subsequent chapters.

Chapter 3 presents the experimental background and interpretative framework for blister formation in intercalated HOPG. The electrochemical response of graphite and the characteristic Raman features of pristine and intercalated material are discussed, together with the morphological evidence of blistered regions. On this basis, a physically grounded model of blister formation and evolution is introduced,

providing the conceptual reference for the mechanical and strain analyses developed in the following chapters.

Chapter 4 focuses on the direct mechanical characterization of individual HOPG blisters by means of atomic force microscopy. Through nanoindentation experiments and controlled mechanical manipulation, the elastic response, deformability, and stability of blistered regions are investigated. This chapter establishes a quantitative mechanical description of blisters and provides the reference framework for the subsequent strain-based analyses.

Chapter 5 addresses the relationship between mechanical deformation and vibrational response exploiting AFM–Raman spectroscopy correlation. In this chapter, large-area AFM–Raman maps acquired on intercalated HOPG are used to improve the interpretation of the Raman signal in the presence of intercalation-induced structural and mechanical modifications. This approach provides insights that had not previously been observed in intercalated graphite. Building on this framework, the analysis is extended to blistered areas in order to extract the characteristic Raman response associated with individual blisters and to relate it to their mechanical state.

Chapter 6 investigates strain at the local scale, where spatial variations are expected to play a dominant role. By exploiting Tip Enhanced Raman Spectroscopy, this chapter explores the nanoscale heterogeneity of strain within single blisters, revealing differences between distinct regions of the blistered membrane that cannot be captured by spatially averaged measurements.

Overall, the thesis presents a coherent experimental strategy in which mechanical probing, strain analysis, and local spectroscopic investigation are integrated to provide a multiscale description of the mechanical properties of HOPG blisters. This approach allows blisters to be studied as individual mechanical systems and establishes a framework that can be extended to other confined and strain-engineered structures in layered materials.

2

Experimental Methods

Two complementary experimental setups were employed in this work. All atomic force microscopy (AFM) measurements, mechanical manipulation experiments, and AFM–Raman correlation analyses, both in air and in liquid environments, were carried out at the SoLINano Σ Laboratory of Politecnico di Milano using an NTEGRA Spectra system (NT-MDT) [51]. Scanning tunnelling microscopy (STM) imaging and tip-enhanced Raman spectroscopy (TERS) experiments were instead performed using a LabRAM Odyssey Nano system (HORIBA), available at the ERMES Laboratory at Sorbonne Université [58]. Both systems enable the coupling of scanning probe microscopy (SPM) techniques with Raman spectroscopy and electrochemical control. The measurements presented in this thesis were performed either *in situ* or *ex situ*, but not under *operando* conditions.

Accordingly, the description of the experimental methodology begins with the electrochemical techniques employed to prepare blistered HOPG surfaces. In the second part of this chapter, the scanning probe techniques used throughout this work namely atomic force microscopy and scanning tunnelling microscopy are described in detail, with particular emphasis on the preparation of home-made STM tips. The third part is devoted to Raman spectroscopy, while the fourth and final part addresses the combination of Raman spectroscopy with scanning probe techniques. Special attention is given to tip-enhanced Raman spectroscopy (TERS), which relies on the coupling between STM and Raman spectroscopy to achieve nanoscale vibrational characterization.

2.1 Electrochemistry

2.1.1 General details

Electrochemical techniques constitute fundamental tools for the investigation of oxidation and reduction processes and are widely employed to study the kinetics and thermodynamics of electron and ion transfer phenomena [59]. Beyond charge-

transfer reactions, electrochemical methods have also proven to be highly effective for the investigation of adsorption and crystallisation processes occurring at electrode surfaces [60]. Their broad applicability is largely attributable to the relatively compact instrumentation available today, the high sensitivity achievable over wide concentration ranges for both inorganic and organic species, and the possibility of performing rapid analytical scans [61].

Electrochemical techniques can be broadly classified into static methods, such as potentiometry, and dynamic methods, in which either the electrochemical potential or the faradaic current is actively controlled [62]. When the applied electrochemical potential is varied within a defined energy interval, the technique is referred to as voltammetry. In this case, the resulting current, I_{EC} , flowing through the electrode is measured as a function of the applied electrochemical potential, V_{EC} . A conventional experimental configuration for voltammetric measurements is the so-called three-electrode cell, schematically illustrated in Figure 2.1.

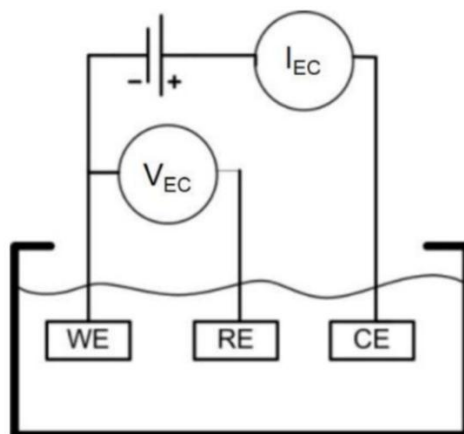


Figure 2.1: Schematic representation of an electrochemical cell [20]

A conventional three-electrode electrochemical cell consists of a working electrode (WE), a reference electrode (RE), and an auxiliary, or counter, electrode (CE). The oxidation or reduction processes of interest take place at the working electrode, while the complementary reduction or oxidation reactions occur at the counter electrode in order to maintain overall charge balance within the system. The reference electrode provides a stable and well-defined potential within the electrochemical environment, thereby serving as a reliable reference against which the potential of the working electrode is measured.

The working and counter electrodes are immersed directly in the electrolyte, whereas the reference electrode is typically placed in a separate compartment, in order to minimise contamination, and is electrically connected to the cell through

a salt bridge. For an electrode to function as a reference electrode, several criteria must be satisfied: (i) it must involve a reversible half-reaction, such that the electrode potential can be determined by the concentration of the species participating in the half-reaction, as described by the Nernst equation [63]; (ii) it must exhibit a constant potential over time, behaving as an almost ideally non-polarisable electrode whose potential is not perturbed by the electrochemical processes occurring within the cell; and (iii) it should be straightforward to assemble and maintain.

A variety of electrode materials and configurations, not discussed here, fulfil these requirements and are commonly employed as reference electrodes [64]. In all cases, the potential of the reference electrode is preferably reported with respect to the standard hydrogen electrode (SHE), which can be realised through the equilibrium established between molecular hydrogen and hydrogen ions at the surface of a platinum (Pt) electrode. In this configuration, the reference potential is defined by the electron-transfer reaction between neutral hydrogen molecules (H_2) and protons (H^+), as described by the following equation:



The EC potential can be calculated using the Nerst equation:

$$E = \frac{RT}{F} \ln \frac{a_{H^+}}{\sqrt{p_{H_2}/p_0}} \quad (2.2)$$

where a_{H^+} is the activity (effective concentration) of the hydrogen ions and p_{H_2} the gas pressure referred to atmosphere (p_0). At 25 °C, $E = 4.43$ V [65] but to be a term of comparison with all other electrode reactions, E is declared (convention) to be zero at all temperatures. A typical SHE design is shown schematically in Figure 2.2.

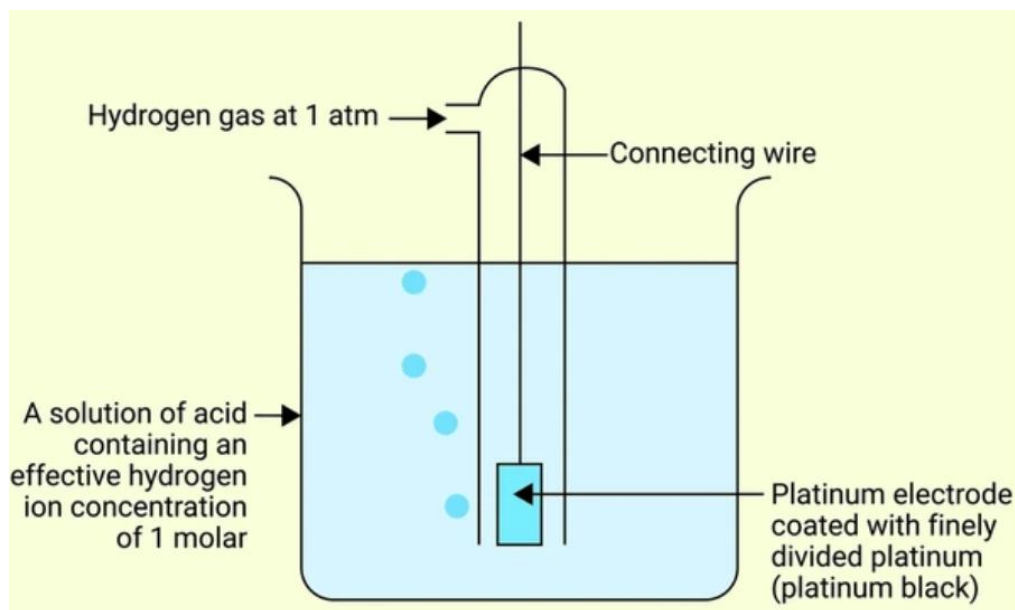


Figure 2.2: Standard hydrogen electrode scheme [66]

Unfortunately, SHE, as well as traditional RE (e.g. calomel and Ag/Ag chloride electrodes), have a limited range of applicability. With these electrodes the liquid junction is problematic and they cannot be used, either with wholly solid-state EC cells or for very high-temperature reactions. To overcome these problems, it was reported that a Pt wire, immersed inside an EC cell filled with different electrolytes, maintain a steady (within few mV) electrode potential [67]. Obviously, thermodynamic equilibrium cannot exist, since there is no common component (anion or cation) in the two adjacent phases (as H_2/H^+ in the SHE). To stress the differences with respect to a true RE, Pt wire is usually called a quasi-RE (QRef) [68]. Pt-QRef shows many advantages: it is very easy to be used; it shows a low impedance; it does not require a salt bridge; it does not contaminate the electrolyte solution; it is stable in many acids and does not disperse undesired ions in the EC solution.

For these reasons, it has been decided to use a Pt-QRef in all experiments involving electrochemistry in this thesis. The Pt wire is immersed in the electrolyte solution for 24 h before each experiment and then inserted in the EC cell. The Pt-QRef shows a defined energy shift of + 0.740 V vs the SHE, when used in diluted H_2SO_4 electrolytes, in agreement with the literature [69].

In voltammetry, an EC potential is applied to the WE with respect to the RE to alter the equilibrium conditions. In the presence of electroactive species (here, the diluted acid used as electrolyte solution) an ion diffusion (mass transport) from the bulk solution to the WE and CE starts. When ions are close to the electrodes

surface, they exchange electrons (redox process) and a faradaic current (I_{EC}) is measured between the WE and the CE:

$$I_{EC} = nAFj \quad (1.3)$$

where F is the Faraday constant, n the number of electrons per molecule involved in the electrochemical process and A is the WE area. Due to a diffusion process, the flux j is related to both the ion concentration (ϕ in mol/cm³) and the diffusivity (D in cm²/s) at the interface, by the well-known Fick's law [70]:

$$j = -\nabla(D\phi) \quad (1.4)$$

The features observed in the voltammograms (I_{EC} vs V_{EC} plots) are thus related to the type of the ions in the electrolyte solution, the EC cell temperature, the number of exchanged electrons, the electrodes geometry, the timescale of the measurement, as well as to capacitive effects at the electrode/liquid interface, mass transport and surface phenomena that can be involved in the EC process. This not simple picture is made even more difficult if other factors (e.g. the establishing of a local electric field, the ions migration, the presence of other charges, etc.), not contemplated in the Fick's law, are taken into consideration. Generally, these phenomena do not play a key role in voltammograms behaviour and will be neglected in the following discussions [71].

2.1.2 Cyclic Voltammetry

In cyclic voltammetry (CV), the EC potential applied to the WE is linearly swept starting from an initial value V_{EC}^i [72]. After reaching a maximum potential V_{EC}^f , the sweep is reversed and the potential returns to its initial value V_{EC}^i .

A typical CV voltammogram is reported in Figure 2.3. According to the IUPAC convention, the positive potentials are plotted in the positive abscissa direction, thus the anodic currents (due to oxidation) are positive, while the cathodic currents (due to reduction) are negative. The presence of current peaks ($I_{p,a}$, $I_{p,c}$) at different EC potentials ($V_{p,a}$ and $V_{p,c}$, respectively) indicates that an electroactive couple is in the electrolyte solution. The main CV parameter is the potential scan rate (v_{scan} rate = $\Delta V_{EC}/t$), since it controls the timescale of the voltammetric experiment. Typical scan rate values range from 1 to 1000 mV/s, accordingly to the studied EC process [64]. In fact, driving the electron transfer at the electrode-electrolyte interface, the scan rate can influence the reversibility of the overall EC process,

which is also influenced by the mass transport (see above). Consequently, if the scan rate is too fast with respect to the ion diffusion, the WE is polarized and an overpotential is required for the oxidation (or reduction) process. This alter the voltammograms, giving rise to unique CV profiles.

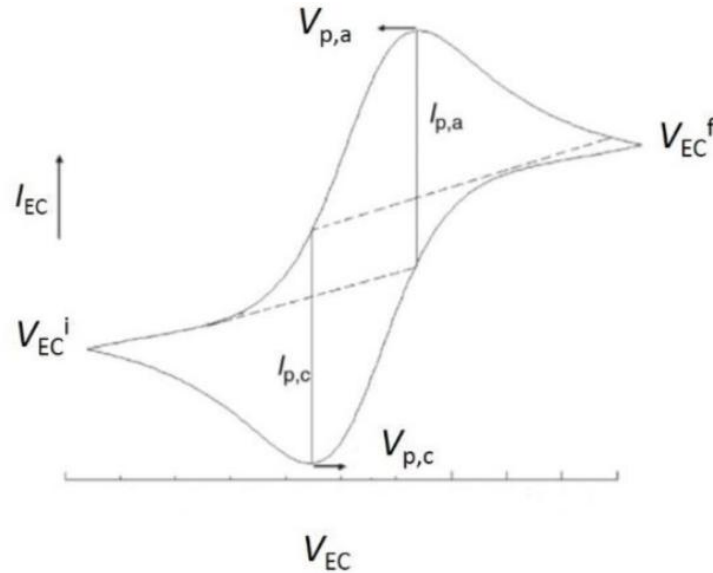


Figure 2.3: Cyclic voltammogram simulated for reversible charge transfer: $V_{p,c}$, cathodic peak potential; $V_{p,a}$, anodic peak potential; V_{EC}^i , initial potential; V_{EC}^f , switching potential; $I_{p,c}$, cathodic peak current; $I_{p,a}$, anodic peak current [20].

In the case of a reversible process, the intensity of the faradic current, I_p (both anodic and cathodic), is a root-square function of the scan rate and the ratio $I_{p,a}/I_{p,c}$ is equal to 1. The energy separation (ΔV_p) between the anodic and cathodic peaks, defined as $\Delta V_p = (V_{p,a} - V_{p,c})$, is relatively small and corresponds to a value of *ca.* $59/n$ mV (at 298 K) [60], regardless the used scan rate (as illustrated in Figure 2.4). If ΔV_p changes as a function of the scan rate, the redox process is quasi-reversible or irreversible.

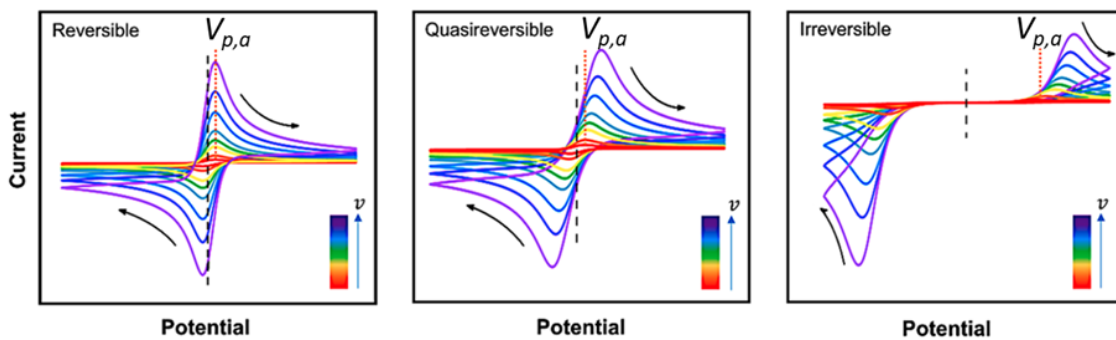


Figure 2.4: CV at different scan rates simulating reversible process ($\Delta V_p \sim 59/n$ mV); quasireversible ($\Delta V_p < 200/n$ mV); irreversible ($\Delta V_p > 200/n$ mV) [73].

During the redox reaction, the total charge exchanged between solution and electrode can be evaluated from the acquired voltammograms. Traditionally, a linear background is subtracted from the anodic or cathodic peak (see Figure 2.4) and the subtended area is calculated. The latter is proportional to the exchanged charge, as clarified by the following equation:

$$A_{a(c)} = \int_{V_{ECi}}^{V_{ECf}} i(V) dV = v_{scan\ rate} \int_{t_i}^{t_f} i(t) dt = v_{scan\ rate} Q_{a(c)} \quad (2.5)$$

The anodic curve gives rise to the oxidation charge (Q_a), while the cathodic feature to the reductive Q_c . Similarly to what reported above, the ratio $Q_a/Q_c = 1$, if the electrochemical process is reversible ($Q_a/Q_c > 1$, for quasi-reversible or irreversible process) [45].

2.2 Scanning probe techniques

The two scanning probe microscopy (SPM) techniques employed in this work are scanning tunnelling microscopy (STM) and atomic force microscopy (AFM), both of which emerged in the 1980s. Their introduction marked a paradigm shift in surface science, as these methods enabled unprecedented access to the structural, electronic, and mechanical properties of materials at the nanoscale. Owing to their versatility and precision, SPM techniques have often been described as providing “a laboratory on the tip”.

SPM techniques rely on the scanning of a sample surface by means of a probe with nanometric dimensions. Surface scanning is achieved through high-resolution piezoelectric actuators, which precisely control the relative displacement of the probe with respect to the specimen. For each pair of lateral coordinates (x, y), the interaction between the probe and the sample is recorded as a single measurement. The collection of these measurements over the scanned area yields the final SPM image. In this work, all SPM images were analysed using the open-source software Gwyddion.

In the following two subsections, atomic force microscopy and scanning tunnelling microscopy are described in detail.

2.2.1 Atomic force microscope

Atomic force microscope (AFM) was developed in 1986 by Gerd Binnig, Calvin F. Quate and Christopher Herber [29]. The AFM working principle is the

measurement of the interactive force between a tip and the sample surface using special probes made by an elastic cantilever with a sharp tip on the end. The interaction between the tip and the surface, results in bending the cantilever.

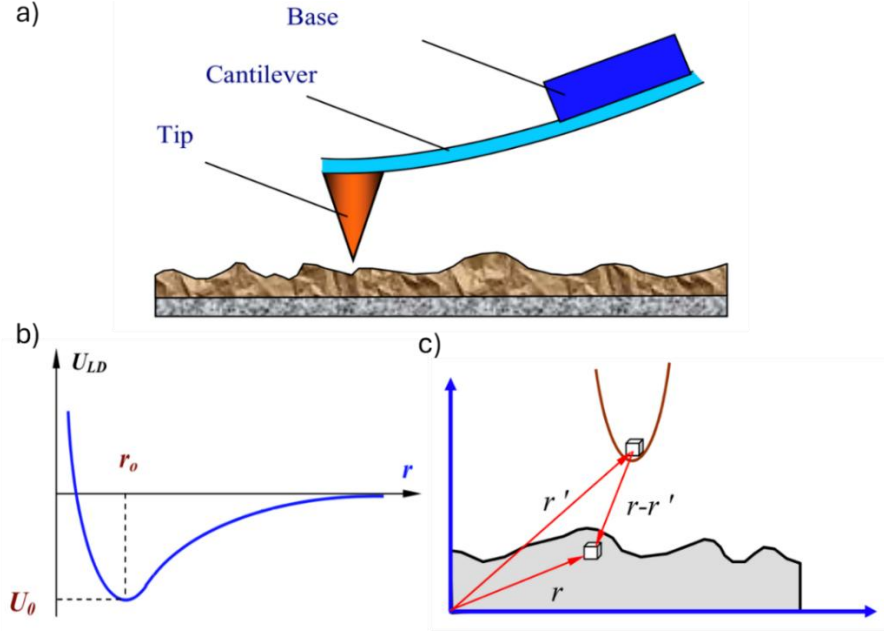


Figure 2.5: a) Schematic representation tip and surface. b) Lennard-Johnes potential respect the distance between two particles. c) Vectorial design of the interaction tip sample [74]

The interaction between the tip and the surface can be describe using the Van der Waals forces [75,76]. The Lennard-Jones potential (Figure 2.5 b) is useful for qualitatively describing the sample tip iteration. The analytical expression of the Lennard-Johnes potential is reported below:

$$U_{LD}(r) = U_0 \left\{ -2 \left(\frac{r_0}{r} \right)^6 + \left(\frac{r_0}{r} \right)^{12} \right\} \quad (2.6)$$

The expression depends on 2 exponential terms. The first one describes the attractive region between the sample and the tip, belongs to the weak long-range van der Waals interaction between dipoles, which follows a $\frac{1}{r^6}$ law.

The second term represents the repulsive region, which is dominated by a term that acts below 1 - 2 Å, (typical of a chemical bond length) and follows a $\frac{1}{r^{12}}$ law.

The energy of the tip-sample system can be derived, adding elementary interactions for all the tip and sample atoms.

For the energy interaction we get:

$$W_{PS} = \iint_{V_P V_S} U_{LD}(r - r') n_P(r') n_S(r) dV dV' \quad (2.7)$$

where $n_S(r)$ and $n_P(r')$ are the densities of atoms in the sample and in the tip. Accordingly, the force affecting the tip from a surface can be calculated as follow:

$$\vec{F}_{PS} = -\text{grad}(W_{PS}) \quad (2.8)$$

Generally, this force has both a component normal to the sample surface and a lateral component. However, a full description of the tip-sample interaction should take into account specific short-range forces (adhesion/contact and capillarity forces) arising from the surface, which are not considered in the Lennard-Jones approximation [74].

The analytical relations between the applied force and the resulting deformation, expressed by the stress (sigma) and strain (epsilon) tensors, can be obtained by using different theoretical models, which take into account (under several conditions) the surface energy contribution. According to different approximation degrees, the Bradley theory [77], the Derjaguin-Miller-Toporov (DMT) theory [78] and the Johnson-Kendall-Roberts (JKR) theory [79] have been developed in the past. Capillarity is instead an attractive force exercised by the liquid meniscus created in ambient condition between tip and sample; it follows from the adsorption of a thin water layer on a hydrophilic surface. The capillarity effects is not present in liquid, since the tip is immersed completely in the liquid.

The tip-sample interaction plays a key role in the kind of acquisition mode. Two are the main ones.

The first one is called contact (static) mode; in this case the tip approaches the sample at a distance where the repulsive force is acting, the force remains constant during the whole acquisition process.

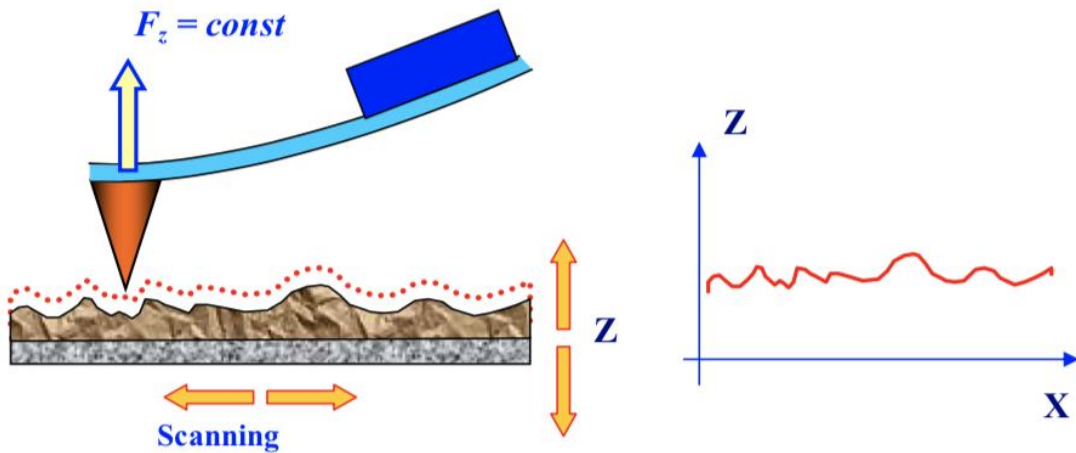


Figure 2.6: Schematic representation of how the system reconstruct the morphology from the interaction tip-surface.[74]

Conversely, in the non-contact mode, the cantilever oscillates and the AFM tip periodically reaches the surface at a distance where both attractive and repulsive forces act.

The non-contact mode has two sub-acquisition methods: amplitude- modulation (AM) and frequency-modulation (FM). In AM-AFM, the scanner actuator is driven by defined potential amplitude (V_i) and frequency (f_i), close to the resonance frequency (f_0) of the cantilever. When the tip approaches the sample (in the repulsive force region), elastic and inelastic interactions change both V and f with respect to the driving signal of the cantilever (V_i and f_i). These changes are used as feedback signals. In FM-AFM, the feedback signal is instead represented by changes of the eigenfrequency f_0 of the cantilever, being affected by the attractive tip-sample interactions during the scanning.

However, in order to detect changes in the amplitude and in the phase of cantilever oscillations in non-contact mode high sensitivity and stability of the feedback is required. In practice the so-called "semi contact mode" is used more frequently. In this technique the forced cantilever oscillations are excited near a resonance frequency with an amplitude about 10–100 nanometres. The cantilever is approached to the surface so that in the lower semi oscillation the tip gets in contact with the sample surface. During scanning, the changes of amplitude and phase of cantilever oscillations are recorded. The cantilever interaction with the surface in "semi-contact" mode consists in van der Waals forces plus the elastic force which is added during contact.

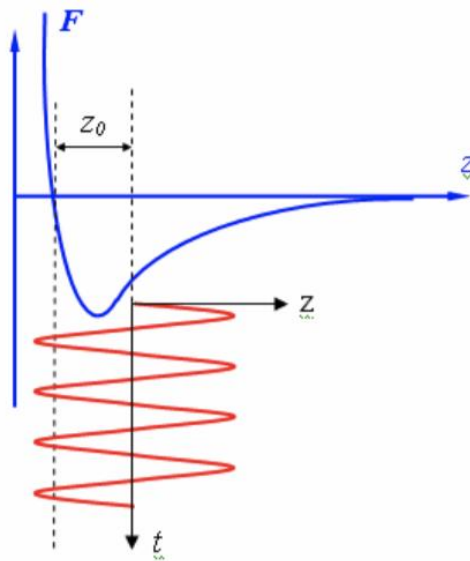


Figure 2.7: Representation of the tip oscillation during the semi-contact mode acquisition. [74]

The choice between the different AFM operating modes depends both on the characteristics of the sample surface and on the specific objectives of the measurement. For instance, semi-contact (tapping) mode is generally preferred when investigating particularly fragile or soft surfaces, as continuous contact between the tip and the sample may lead to surface damage.

In semi-contact mode, additional information can be obtained through phase-contrast imaging, which is sensitive to variations in local mechanical properties such as surface stiffness. In this operating mode, the cantilever is driven to oscillate at a selected frequency, and changes in the tip-sample interaction associated with variations in surface hardness induce shifts in the phase of the oscillation. These phase variations are detected by the AFM system and can be used to map relative differences in mechanical properties across the surface.

Contact mode, by contrast, is particularly suited for tribological measurements, as it allows the direct measurement of lateral forces acting on the tip while it is pressed against and scanned across the sample surface. In this work, all AFM images were acquired in semi-contact mode.

Topographic information is obtained by monitoring changes in cantilever deflection. To this end, a laser beam is focused onto the backside of the cantilever and reflected onto the centre of a position-sensitive photodetector, enabling precise detection of cantilever motion.

The photodetector is divided in 4 parts, from each part the incoming photon create a current, so the system converts the impinging laser beam in 4 currents. The alignment of the optical path consist in putting in the centre of the photodiode the laser spot reflected by the cantilever.

The deflection or the lateral torsion of the tip, changes the position of the reflected laser beam, and so change the current generated in each quadrant.

If photocurrent reference values are designated as I_{01} , I_{02} , I_{03} , I_{04} , and I_1 , I_2 , I_3 , I_4 are the current values after change of the cantilever position, differential currents from various sections of the photodiode $\Delta I_i = I_i - I_{0i}$ characterizes the value and the direction of the cantilever bending or torsion. We can derive the following current difference:

$$\Delta I_z = (\Delta I_1 + \Delta I_2) - (\Delta I_3 + \Delta I_4) \quad (2.9)$$

$$\Delta I_L = (\Delta I_1 + \Delta I_4) - (\Delta I_2 + \Delta I_3) \quad (2.10)$$

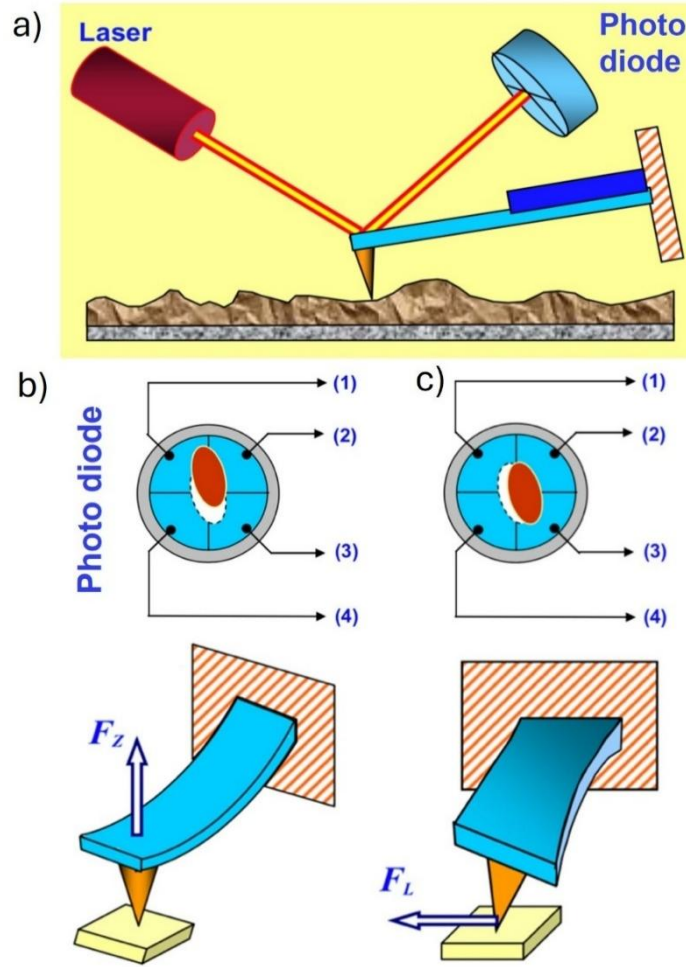


Figure 2.8: On the top schematic representation of the optical path of the laser beam. b,c) Representation on how the movement of the tip due the interaction with the surface change the position of the laser spot on the photodetector [74].

Where the first one is proportional to the cantilever bending due to a force normal to the sample surface, and the second one is proportional to the cantilever bending due to lateral forces, (Figure 2.8). The ΔIZ value is used as an input parameter in a feedback loop of the atomic force microscope Figure 2.9. The feedback system (FS) keeps ΔIZ constant with the help of a piezoelectric transducer (scanner), which controls the tip-sample distance in order to make the bending ΔZ equal to the value ΔZ_0 preset by the operator.

When scanning a sample in a $\Delta Z = \text{const}$ mode the tip moves along the surface, thus the voltage on the scanner Z-electrode is recorded in the computer memory as a surface topography $Z = f(x, y)$. The AFM lateral resolution is defined by the radius of curvature of the tip and by the sensitivity of the system in detecting the cantilever deviations. Currently the AFM are designed to allow obtaining atomic resolution.

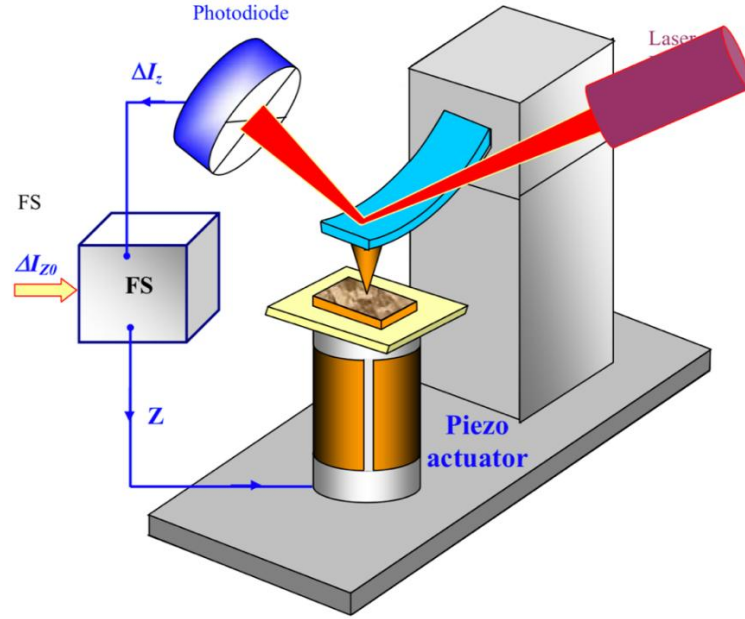


Figure 2.9: Representation of the AFM feedback loop working principle [74].

It is important to highlight several differences arising when atomic force microscopy is performed in liquid environments as opposed to vacuum or air. In liquid, cantilever alignment becomes more critical, as the change in refractive index at the liquid–air interface induces an additional deflection of the optical path of the detection laser, thereby requiring a corresponding adjustment of the photodiode position. Moreover, the choice of cantilever material is particularly important in liquid measurements in order to prevent surface matting or degradation, which would otherwise lead to a reduction in the intensity of the reflected detection laser. It is important to notice that the quality of the image between liquid and air is the same [80].

Although AFM imaging can be reliably performed both in air and in liquid, and comparable image quality can be achieved in the two environments, it is essential to recall that the topographic signal is always the outcome of a mediated measurement process. The recorded morphology results from the interplay between the probe, the interaction forces, and the feedback system that maintains the tip–sample separation.

For this reason, independently of the surrounding medium, the AFM image must be interpreted with caution, as its contrast may reflect not only intrinsic surface features but also the specific measurement conditions under which it was acquired.

Even though Atomic Force Microscopy provides a direct reconstruction of the surface morphology through the tip–sample interaction, the measured “topography”

is never a purely intrinsic property of the sample, because it is always mediated by the finite geometry and the local mechanical/chemical state of the probe apex; for this reason, a relevant fraction of the apparent features in an AFM image may originate from tip convolution or from instrumental/feedback artefacts, rather than from real surface structures [81].

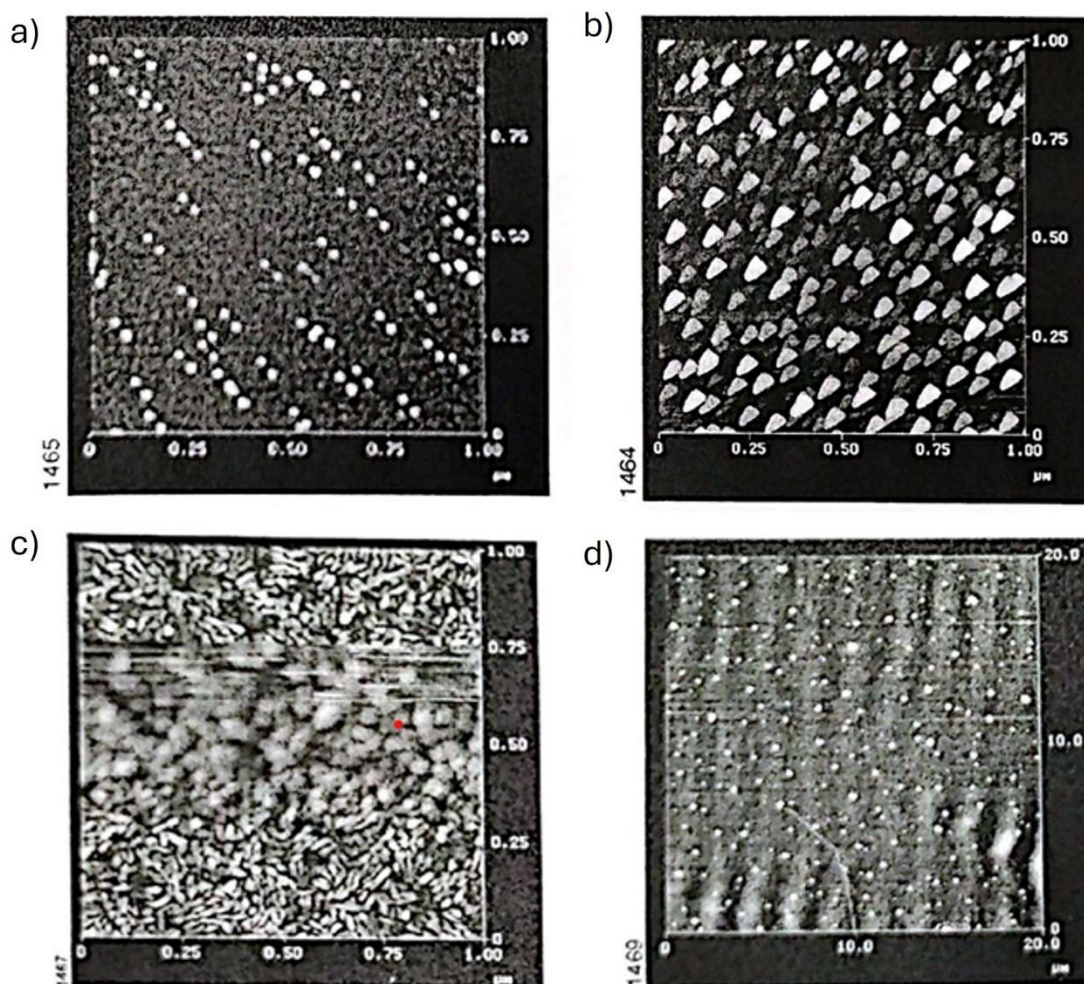


Figure 2.10: In the image are presented a) Dull/dirty tip creates triangular features artefacts b) Double tip artefacts. c) Contamination streaking artefacts. d) Optical interference artefacts.

In Figure 2.10 are reported some examples of tip artefacts that can occur during AFM image acquisition.

In practice, the most common source of misinterpretation is the evolution of the tip apex during scanning, either because the apex becomes blunt (progressive wear) or because it becomes contaminated by material picked up from the surface (or from the liquid environment, when operating in situ). In both cases, the effective interaction area increases, the lateral resolution decreases, and nanoscale objects can appear artificially enlarged or merged. A particularly clear signature of a dirty or damaged tip is the appearance of repeated triangular features (or “triangle-like”

objects) across the image: this pattern does not reflect a triangular sample morphology, but rather the fact that the image is being dominated by the multifaceted geometry of the contaminated apex, which replicates itself on every sharp surface feature [81]. Similarly, when the probe effectively behaves as a double tip (or multiple tip), the same object is imaged twice with a fixed lateral offset, producing “ghost” replicas of surface features; this artefact is especially insidious on periodic or step-rich surfaces because it can be mistakenly interpreted as a real secondary lattice, a duplicated step edge, or a second nanostructure population [81].

Beyond tip-shape effects, the AFM image can be distorted by artefacts that are generated by the dynamic response of the feedback loop. If the feedback parameters are not optimised for the chosen scan speed and for the local height gradients, the system may fail to track the surface in real time; in this condition, steep edges can be followed by artificial tails (edge “smearing” along the fast scan axis), and sharp protrusions can become elongated or asymmetric depending on the scan direction [81]. Conversely, excessively aggressive feedback may introduce oscillatory behaviour (“ringing”), which can create periodic ripples or repeated patterns that do not correspond to real topographic corrugations. Additional artefacts may arise from the specific optical detection scheme: optical interference between reflected beams can generate spurious contrast modulations, which are correlated with scan geometry and alignment rather than with the true surface morphology [81]. Finally, at larger scan sizes, non-ideal scanner motion and piezo nonlinearities can result in bowing or global curvature of the image background; although these distortions can often be reduced by plane subtraction or flattening procedures, it is important to recognise them as instrumental in origin so that they are not interpreted as real long-range sample curvature [81].

From an operational point of view, these limitations can be managed by adopting a few systematic checks: (i) repeating the scan after rotating the scan direction or flipping fast/slow axes, because real features remain invariant while feedback-related “tails” typically follow the fast scan direction; (ii) acquiring the same area at different scan rates and feedback gains, because true morphology is robust while tracking artefacts strongly depend on bandwidth; (iii) verifying tip condition by imaging a calibration grating or a well-known reference area, and replacing/cleaning the cantilever whenever double-tip patterns or triangular contamination signatures appear; and (iv) comparing topography with phase (or other complementary channels), because certain artefacts are channel-dependent and may be diagnosed by inconsistencies between channels [81].

Beyond the qualitative distortions introduced by tip degradation or feedback instabilities, it is equally important to consider the intrinsic spatial resolution of the technique. Even in the absence of evident artefacts, the ability of AFM to faithfully reproduce nanoscale features is fundamentally limited by the probe geometry and by the detection sensitivity of the system. For this reason, a brief discussion of the effective lateral and vertical resolution is provided below.

For completeness, the effective spatial resolution of AFM measurements and the main factors limiting it in the present experimental configuration are briefly discussed in the following. With respect to spatial resolution, Atomic Force Microscopy does not provide a fixed intrinsic resolution, as the effective lateral and vertical resolution depend on a combination of instrumental parameters, tip geometry, and operating conditions. In the present work, AFM measurements were performed using sharp silicon or gold-coated probes with nominal tip radii below 10 nm; however, the effective lateral resolution is primarily limited by tip-sample convolution effects and by the local surface morphology. Under typical imaging conditions, the practical lateral resolution can therefore be estimated to be on the order of 10–20 nm. As a consequence, lateral features smaller than the effective tip radius cannot be reliably resolved, and sharp edges or steep height gradients may appear broadened in AFM images [74].

The vertical resolution of AFM is generally much higher than the lateral one and is determined by the sensitivity of the cantilever deflection detection system and by the noise level of the feedback loop. Under typical operating conditions, vertical displacements on the order of a fraction of a nanometer can be resolved. In the context of this work, this resolution is sufficient to accurately detect monoatomic steps on the HOPG surface, which have a characteristic height of approximately 0.335 nm, as well as to quantify blister heights and indentation-induced deformations [81].

It is important to note that the effective resolution may vary during prolonged scanning, as the condition of the tip apex can evolve due to wear or contamination. Tip degradation can lead to a progressive loss of lateral resolution or to imaging artefacts, such as feature duplication or shape distortion. For this reason, AFM data were systematically cross-checked by repeated scans, variation of scan direction and imaging parameters, and, when necessary, replacement of the cantilever, in order to ensure the reliability of the acquired topographic and mechanical information [81].

While the previous discussion has focused on AFM as an imaging technique and on the spatial limits governing morphological reconstruction, the same instrumental

framework also allows direct investigation of local interaction forces. In this respect, AFM is not restricted to topographic mapping but can be operated in a spectroscopic mode, in which the cantilever response is monitored as a function of controlled tip-sample separation.

Finally, Atomic force microscopy enables the detailed investigation of local force interactions, providing quantitative information on the surface properties of the sample. To this end, so-called force-distance curves are acquired by monitoring the cantilever response during controlled approach to, and retraction from, the sample surface. These curves describe the dependence of the cantilever deflection, ΔZ , and therefore of the interaction force, on the vertical coordinate z , corresponding to the probe-sample separation. A representative force-distance curve is shown in Figure 2.11.

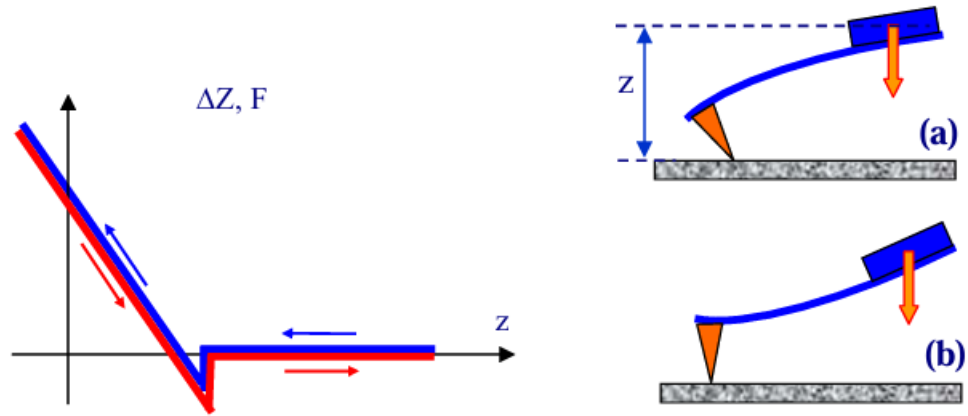


Figure 2.11: Schematic picture of the cantilever deflection ΔZ (proportional to the applied force F) versus the probe-sample distance z . Blue: approaching. Red: retracting. [74]

During approach to the surface the tip gets in the reach of attractive forces. This causes a cantilever bend toward the surface (Fig. 74 , insert (a)). The jump of the tip to the surface is due to the large gradient of the attractive force near the sample surface. For a Lennard-Jones type potential the range Z^* of the attractive force, where the gradient $F'(z)$ is high, is about 1 nanometre.

During further approach of the probe to the sample, the tip starts to experience a repulsive force, and the cantilever bends in the opposite direction (Figure 2.11, panel (b)). The slope of the curve $\Delta Z = f(z)$ in this region is determined by the elastic properties of both sample and cantilever. If interaction is perfectly elastic, the dependence of the bend on the distance, recorded during reverse motion, coincides with the dependence obtained during forward motion Figure 2.11. For soft (plastic) samples, such as films of organic materials, biological structures, etc.,

and also for samples with adsorbed layers of various materials, the shape of the $\Delta Z = f(z)$ curves is more complex [74].

Thus, by the shape of the $\Delta Z = f(z)$ curve it is possible to obtain information on the tip-surface interaction, to study the local stiffness of the sample and the distribution of the adhesion forces.

2.2.2 Scanning tunnelling Microscopy (STM)

The invention of the STM by Binnig and Rohrer in 1982 [82,83] paved the way for the investigation of surfaces on the nanometre scale [84–86]. Before 1986, the STM was usually used for measurements in Ultra-High-Vacuum (UHV) or in air, but since Sonnenfeld and co-workers [87] demonstrated that STM can operate in deionized (type 1) water, much progress has been made to explore the potential of this technique to work at the solid/liquid interface [88,89]. The first application of STM even in conductive solutions is attributed to K. Itaya, who developed the first electronic circuit (bi-potentiostat) to pilot the STM tip in an electrolyte (see below), realizing the first so-called EC-STM [90,91]. The latter combines the three-electrode cell with a STM scanner.

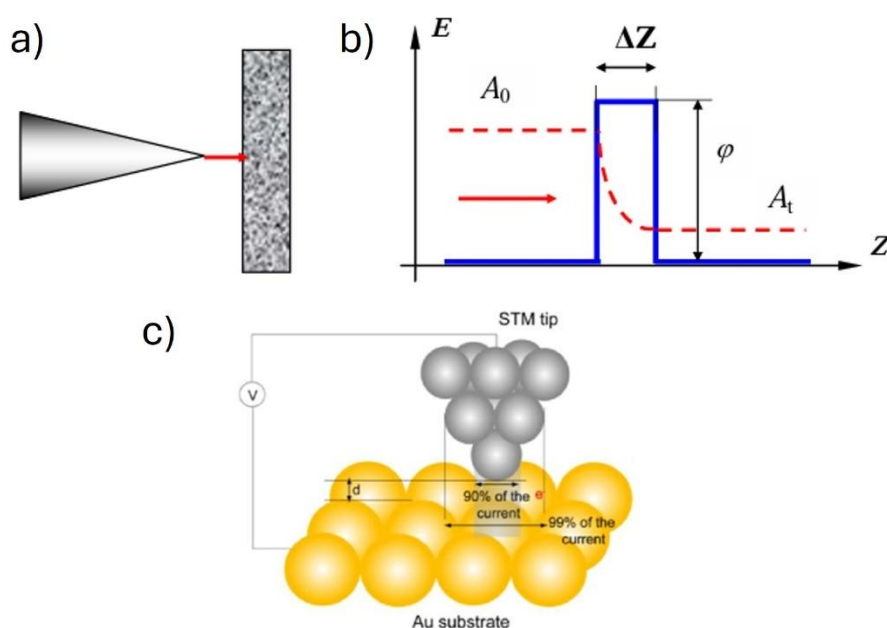


Figure 2.12: a, b) Scheme of electrons tunnelling through a potential barrier in STM. c) Schematic diagram of scanning tunnelling microscopy depicts the tip sample interaction. [74,92]

The real-space visualization of surfaces with an atomic-resolution is one of the most important characteristics of STM [93]. The working principle of STM is based on the phenomenon of electrons tunnelling through a narrow potential barrier between a metal tip and a conducting sample in an external electric field. The STM

tip approaches the sample surface to distances of several Ångströms. This forms a tunnel transparent barrier, whose size is determined mainly by the values of the work function from the tip (φ_T) and from the sample (φ_S) see Figure 2.12. The barrier can be approximated by a rectangular shape with effective height equal to the average work function φ^* :

$$\varphi^* = \frac{1}{2} (\varphi_T + \varphi_S) \quad (2.11)$$

Tunnelling is a quantum-mechanical phenomenon in which an electron, with an energy E , can penetrate the potential barrier $\varphi^* > E$. The wave function ψ , describing the quantum state of the electron, decays exponentially in the classically forbidden region according to:

$$\psi(z) = \psi(0) \exp \frac{-z\sqrt{2m(\varphi^*-E)}}{\hbar} \quad (2.12)$$

Where m is the mass of the electron, z is the barrier height and $\hbar$ is the Planck constant, ($\hbar = 1.05 \times 10^{-34} Js$). When the STM tip approaches the sample surface, the electron wave functions of both the tip and the sample overlap within the insulating narrow barrier (e.g. vacuum, air, etc.). A tunneling current (I_T) flows between the tip and the sample as a function of the sign and value of the bias voltage applied to the tip, according to the following equation [94]:

$$I_T \propto \sum_{\mu\nu} f(E_\mu) [1 - f(E_\nu + eV_{bias})] \times |M_{\mu\nu}|^2 \delta(E_\mu - E_\nu) \quad (2.13)$$

Here, $f(E_{\mu(\nu)})$ is the Fermi function of the probe (surface), V_{bias} is the applied bias voltage to the tip, $M_{\mu\nu}$ is the tunnelling matrix element between state ψ_μ of the tip and state ψ_ν of the surface and E_μ is the energy of state ψ_μ in absence of tunnelling. According to the theoretical model proposed by Tersoff and Hamann [80] for the interpretation of the tunnelling current, the latter is proportional to the surface local density of states (LDOS) at the Fermi level:

$$I_T \propto V_{bias} e^{-kz\sqrt{\varphi^*}} \rho(z, E_z) \quad (2.14)$$

where the quantity $(\varphi^* - E)$ is approximated by the average working function φ^* and the LDOS is expressed by the function $\rho(z, EF)$. The tunnelling current exponentially decreases as a function of the separation z between tip and sample, giving an extremely highly vertical resolution to the STM: a tip-sample distance

enhancement of $1 \text{ \AA}$ produces a tunnelling current reduction of about one order of magnitude. The STM lateral resolution is also enhanced by the tunnelling effect (see Figure 2.12 panel c). It is possible to demonstrate that an atomic separation down to $2 \text{ \AA}$ is easily resolved [92,95].

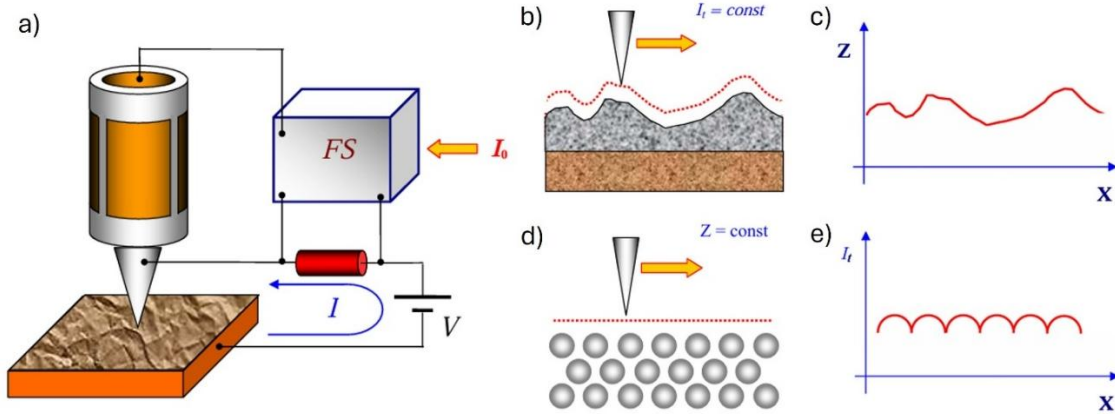


Figure 2.13: a) Simplified block-diagram of the feedback in STM b,c) Formation of STM images in the constant current mode d,e) Formation of STM images in the constant average distance mode [74].

The metal tip is placed into the scanner, i.e. a piezo tube containing three transducers (P_x , P_y , P_z), which converts electric signals V_x , V_y , V_z (with an amplitude ranging from 1 mV to 1 kV) into a tip mechanical motion, in a wide length range (from sub- $\text{\AA}$ to μm) [96]. Two operating modes can be chosen for the STM signal acquisition, namely the constant current mode and the constant height mode [97]. Constant-current mode: I_T and V_{bias} values are kept constant. To this end, the voltage applied to the P_z piezo is governed by a current feedback system, which adjusts the tip-sample distance (z) to keep the I_T constant during the surface scanning (see representative scheme in Figure 2.13 a).

In this acquisition mode, large and rough sample areas can be, in principle, imaged without any damage of the tip or of the sample surface (tip crash phenomena). A drawback is instead the limited scan rate, which must be kept relatively slow allowing the feedback system to fine control the z position of the tip. Constant-height mode: z and V_{bias} values are kept constant. A higher scan rate is allowed in this STM configuration and, hence, thermal drifts effecting a high resolution imaging can be largely eliminated. Conversely, being the feedback system switched off, scan sizes are limited and confined to flat areas in order to avoid “tip crash” phenomena [98].

For completeness, the effective spatial resolution of STM measurements and the main factors limiting it in the present experimental configuration are briefly

discussed in the following. With respect to spatial resolution, Scanning Tunnelling Microscopy does not provide a fixed intrinsic resolution, as the effective lateral and vertical resolution depend on the electronic properties of both the tip and the sample, as well as on the tunnelling parameters adopted during imaging. In the present work, STM measurements were not performed in the atomic-resolution regime, but were instead employed to investigate surface morphology at the nanometric scale, such as terraces, step edges, and extended surface features.

Under these conditions, the effective lateral resolution is limited by the finite extension of the tunnelling junction and by the convolution between the tip apex geometry and the local electronic density of states of the sample. In practice, the lateral resolution achieved in this work can be estimated to be on the order of several nanometres, which is sufficient to resolve step edges and mesoscale morphological features, but does not allow discrimination of individual atomic sites or atomic-scale corrugations.

The vertical resolution of STM is intrinsically very high due to the exponential dependence of the tunnelling current on the tip-sample separation. Even outside the atomic-resolution regime, sub-Ångström sensitivity to height variations can be achieved under stable tunnelling conditions. In the present work, this vertical sensitivity enables reliable detection of step heights on the HOPG surface and accurate tracking of relative height variations across terraces and blistered regions.

It should be noted that STM images are particularly sensitive to variations in tip condition and scanning geometry. Changes in the electronic structure of the tip apex or tip-induced interactions with surface atoms may lead to imaging artefacts, especially at step edges, such as apparent roughening or scan-direction-dependent contrast. For this reason, STM data were interpreted primarily in a morphological and comparative framework, and image reliability was assessed by varying tunnelling parameters and scan directions

As done for AFM, it is important to discuss the possible artefacts that can occur during STM image acquisition.

At atomic resolution, this intrinsic imaging mechanism gives rise to several well-known artefacts, including contrast inversion, apparent height variations driven by changes in the local density of states, and image duplication associated with multi-atom or double-tip apex configurations. In this regime, the STM image cannot be interpreted as a purely geometric topography, but rather as a convolution of electronic and structural information, strongly dependent on tip structure, bias voltage, and tunnelling conditions [99].

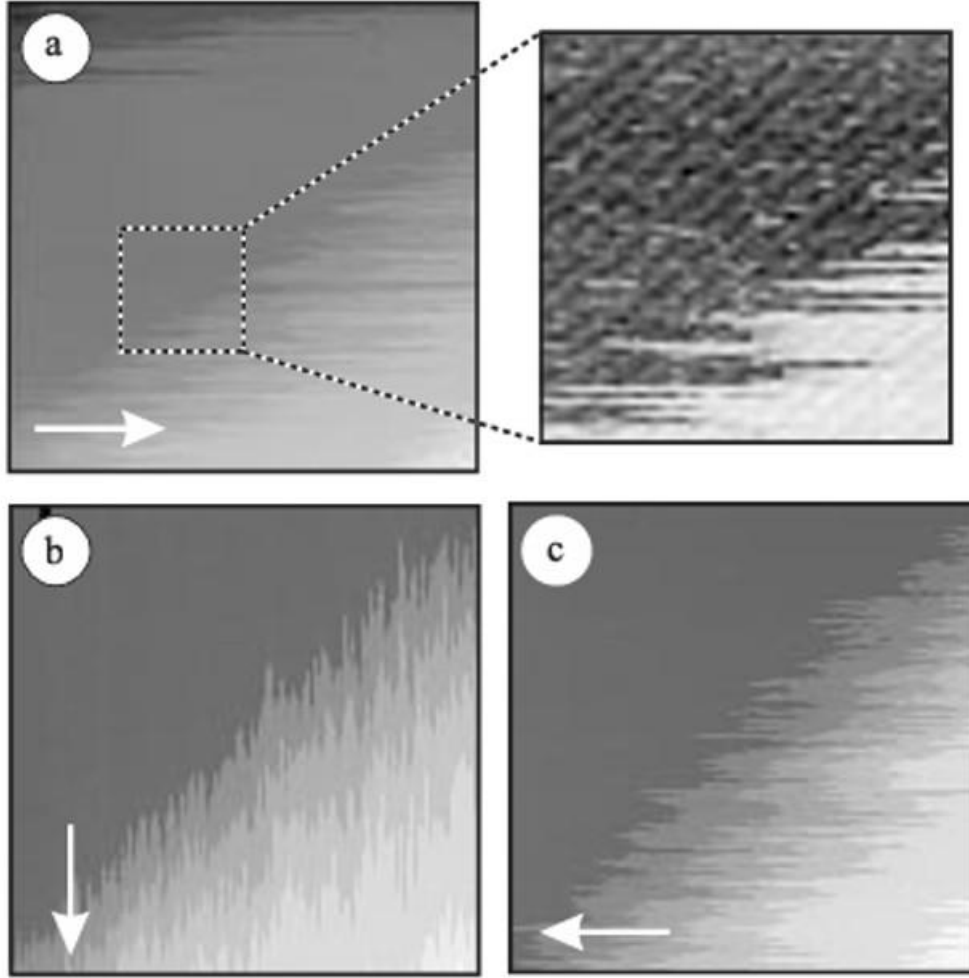


Figure 2.14: $(25 \times 25) \text{ nm}^2$ STM images of the same surface area on Ag(110) at 360 K and different fast scanning directions (indicated by arrows) revealing extended (110) terraces and fringed [110] steps which run parallel to the close-packed rows; panel **a**), right, shows the respective raw data at higher magnification; $U_T = -25 \text{ mV}$, $I_T = 1.8 \text{ nA}$, panels **b**) and **c**) shows the same step acquired changing the scanning direction, the direction is highlighted by the arrow in the panel [100].

In the present thesis, however, STM measurements were intentionally performed outside the atomic-resolution regime. The technique was primarily employed to investigate surface morphology at the nanometric to mesoscopic scale, such as terraces, step edges, and extended surface features. Although atomic-scale artefacts are therefore not directly relevant for the data discussed here, STM images acquired at lower spatial resolution remain sensitive to artefacts arising from the tip-surface interaction, feedback dynamics, and scanning geometry.

At the nanometric scale, a particularly important class of artefacts concerns the imaging of step edges. As demonstrated by Koch *et al.* in their systematic study of Ag(110), step edges that are intrinsically straight and thermodynamically stable may appear rough or “fringed” in STM images as a consequence of tip-induced atomic displacements, even under standard imaging conditions and at room

temperature [100]. In particular, when the fast scan direction is perpendicular to the step edge, the STM tip can transiently extract or displace atoms from the step, producing apparent fringes aligned with the scan direction. When the same area is scanned with the fast direction parallel to the step, the edges instead appear sharp and well defined, clearly indicating that the observed roughness is an imaging artefact rather than an intrinsic structural property of the surface.

These effects are especially relevant when STM is used to characterize step morphology without resolving individual atoms. In this regime, apparent step roughening, step broadening, or scan-direction-dependent contrast should not be interpreted as evidence of genuine step disorder or enhanced thermal roughness. Rather, they reflect the combined influence of local tip–surface interactions, effective tip geometry, and the dynamic response of the feedback loop. While the exponential decay of the tunnelling current often suppresses contributions from secondary tip asperities, effective multi-tip configurations and tip-induced atom manipulation can still influence the recorded topography at step edges and other high-gradient regions [99]

For these reasons, STM images presented in this work are interpreted primarily within a comparative and morphological framework. Step heights, terrace widths, and relative surface features are evaluated qualitatively and cross-checked by varying scan direction and imaging parameters.

In conclusion of this part on SPM technique, are shown as example two images acquired on pristine HOPG (Figure 2.15), acquired in two different regions. In panel a there is an AFM image acquired with the NTEGRA spectra system, silicon tips, In panel b, is shown an STM image, acquired with gold tips with the HR Evo Nano system from Horiba.

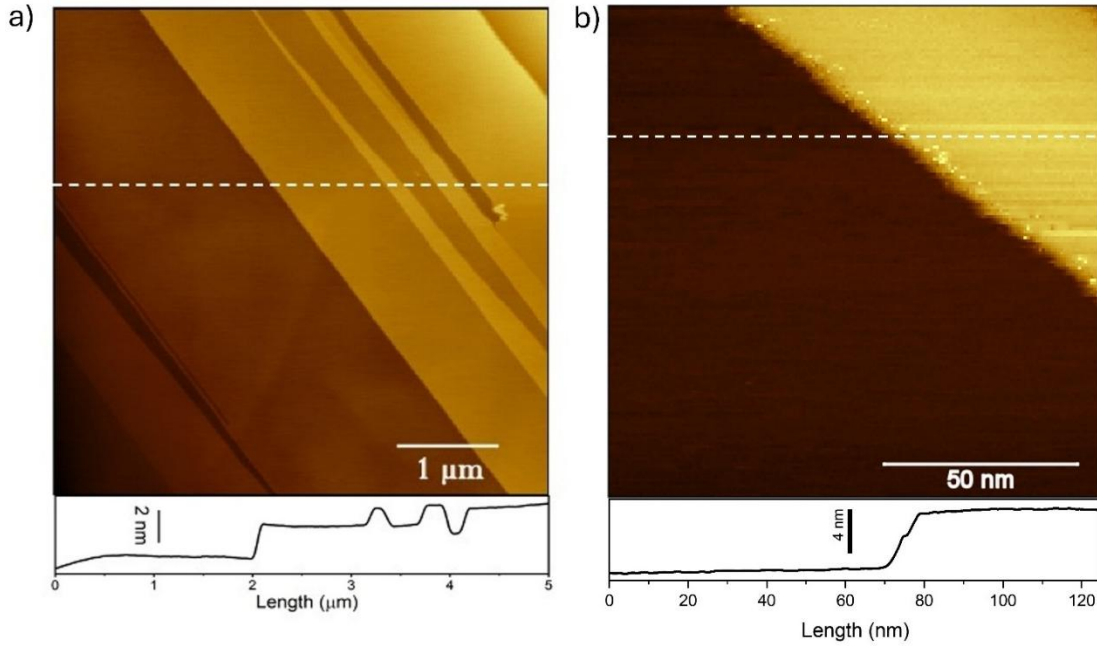


Figure 2.15: a) Pristine HOPG AFM image morphology b) Pristine HOPG STM image morphology, $V_{\text{bias}} = 0.1$, $I_T = 0.9$ nA.

2.3 Raman Spectroscopy

The inelastic scattering of light was theoretically predicted by Adolf Smekal in 1923 [101] and subsequently observed experimentally in 1928. The effect was first reported in organic liquids by C. V. Raman and K. S. Krishnan and, independently, in inorganic crystals by Grigory Landsberg and Leonid Mandelstam [102]. For his contribution to the discovery and interpretation of this phenomenon, Raman was awarded the Nobel Prize in Physics in 1930, and the inelastic scattering of light became known as the Raman effect.

In the original experiments, Raman employed a mercury vapour lamp with an excitation wavelength of $\lambda_{\text{exc}} = 485$ nm; in modern Raman spectroscopy, however, laser sources are predominantly used owing to their high monochromaticity, intensity, and stability.

The scattered radiation is collected and analysed by a spectrometer. Raman measurements can be performed in either reflection or transmission geometry, depending on the optical properties of the sample, such as reflectivity and transparency at the excitation wavelength.

When monochromatic light interacts with matter, the vast majority of the scattered radiation retains the same frequency as the incident light, giving rise to elastic Rayleigh scattering. A much smaller fraction of the scattered photons, however, exhibits a frequency shift with respect to the incident radiation,

corresponding to inelastic scattering. This phenomenon, known as the Raman effect, originates from the interaction between the incident electromagnetic field and the molecular dipoles, which oscillate upon excitation and subsequently emit radiation at shifted frequencies. The induced molecular dipole moment can be expressed as follows:

$$\vec{M} = \vec{M}_0 + \vec{\alpha}\vec{E} \quad (2.15)$$

The first term indicates the intrinsic dipole, the second term is indicated by an electric field and weighted by the sample polarizability tensor.

The Raman lines show up pairwise. The dominant Stokes lines have a lower frequency (longer wavelength) than the initial radiation, whereas the weaker (often nondetectable, because less probable) anti-Stokes lines have a higher frequency (shorter wavelength) [103]. The frequency shifts are virtually independent of the excitation wavelength and are characteristic of the particular substance/molecule. Usually one only records the relatively strong Stokes lines, which therefore are attributed a positive frequency shift. Such spectral coordinate is called Raman shift and it is measured in wavenumbers (cm^{-1}).

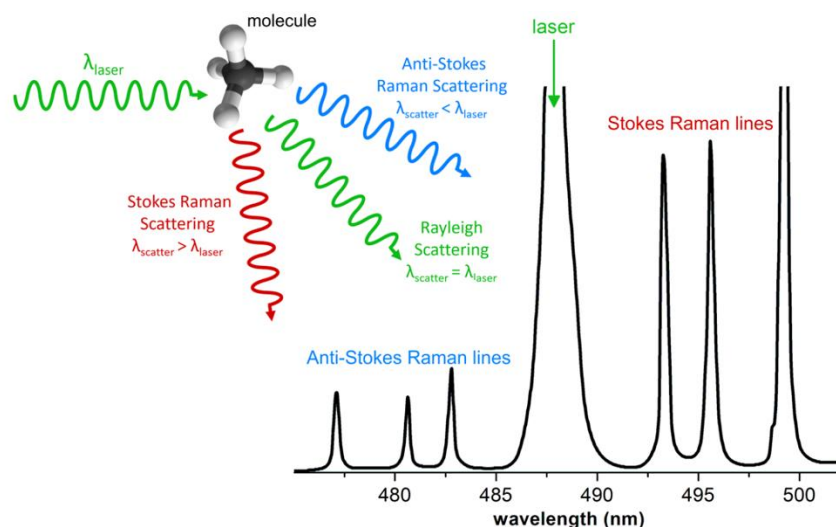


Figure 2.16: Schematic representation of the Raman effect.

The Raman spectrum is essentially a vibrational one. In particular a vibration is Raman-active (i.e. revealed as a spectral line in the Raman spectrum), if the polarizability of the molecule changes during the vibration. It often happens that Raman active vibrations are inactive in the IR, and vice versa. Therefore, Raman spectra often provide complementary information to IR spectra [103,104].

2.3.1 Raman spectroscopy physical explanation

As a first approximation, the system can be assumed to be initially in its vibrational ground state, corresponding to the quantum number $v = 0$. When a photon with energy $\hbar\omega_l$ interacts with the system, an oscillating dipole moment is induced and the system becomes polarised. Under this excitation, the system is promoted to a higher-energy configuration commonly referred to as a virtual energy level, which does not correspond to a true stationary eigenstate, but rather to a coherent superposition of allowed energy states.

Since the virtual level is not a stationary state, the system undergoes an almost instantaneous relaxation toward a stable vibrational state. If the relaxation occurs back to the original vibrational ground state, the emitted radiation has the same energy as the incident photon, and the process corresponds to elastic (Rayleigh) scattering. If, instead, the system relaxes to a vibrational state different from the initial one, the energy of the emitted photon differs from that of the incident radiation and is given by:

$$E = \hbar\omega_l - \hbar\omega_{vib} \quad (2.16)$$

The latter term represents the energy difference between the initial and final states of the system. In this regime, the incident photon interacts with the sample and may either lose or gain energy, depending on the vibrational transition involved [105]. The energy-level scheme shown in Figure 2.17 provides a useful framework for rationalising the origin of anti-Stokes scattering.

In the anti-Stokes process, an exchange of energy occurs between matter and radiation in which the system is initially in an excited vibrational state, with an energy higher than that of the vibrational ground state. As a consequence, the virtual energy level accessed during the interaction lies at a higher energy than in the Stokes case, since the incident photon carries the same energy while the initial vibrational energy of the system is increased.

Upon relaxation, the system transitions to a final vibrational state of lower energy than the initial one, resulting in the emission of a photon with higher energy than that of the incident radiation. The corresponding spectral feature observed in the Raman spectrum is referred to as an anti-Stokes peak. The intensity of anti-Stokes scattering follows Bose–Einstein statistics, as the population of vibrationally excited states is governed by thermal occupation and therefore depends on the temperature of the system [103].

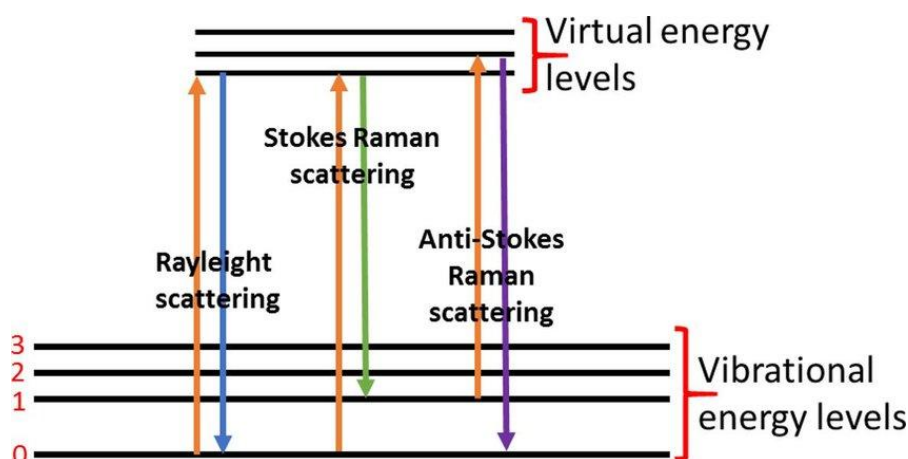


Figure 2.17: Representation of the vibrational energy levels and the possible energy transition.

2.3.2 Micro-Raman Spectrometer

The micro-Raman spectrometer, often referred to as a Raman microscope, represents the most common configuration of bench-top Raman instrumentation. In this setup, a high-resolution spectrometer is coupled with one or more laser sources through an optical microscope. The microscope objective is used both to focus the excitation beam onto the sample and to collect the scattered radiation in a backscattering geometry. Objectives with a high numerical aperture (NA) provide simultaneously high spatial resolution and high collection efficiency.

In order to achieve sub-micrometre spatial resolution, an infinity-corrected confocal optical configuration is employed in the Confotec NR500 system. This configuration enables both efficient illumination of the sample surface using different laser wavelengths and effective collection of the scattered radiation. High spectral resolution is ensured by the spectrometer, operated in a Czerny–Turner optical configuration, allowing spectral resolutions on the order of tenths of an Ångström. A schematic representation of a typical confocal Raman spectroscopy setup is shown in Figure 2.18.

One of the key components of the confocal configuration is the pinhole aperture, which selectively blocks scattered light originating from out-of-focus regions of the sample. By suppressing these unwanted contributions, the pinhole aperture enhances both the lateral and axial spatial resolution and improves the overall contrast of the Raman signal.

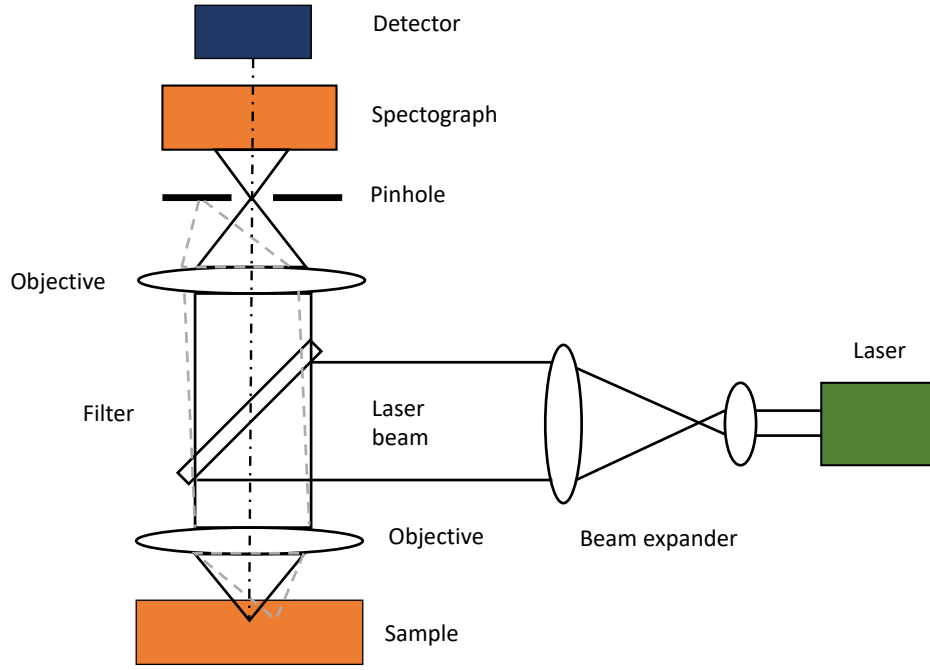


Figure 2.18: Schematic representation of a Confocal Raman setup.

2.3.3 Spatial resolution in confocal microscopy

In optics, the Airy disk is description of the best focused spot of light that a perfect lens with circular aperture can make, limited the diffraction of light. The diameter of the Airy disk has the following expression:

$$d = \frac{1.22 \cdot \lambda}{NA} \quad (2.17)$$

Where λ is the radiation wavelength, and NA is the objective numerical aperture.

If the NA is increase, the Airy disk diameter is reduce and consequently. The Rayleigh criterion establishes a reference to characterize the spatial resolution of an optical device: the minimum resolvable distance between two points before they become indistinguishable due to the light diffraction. The criterion is satisfied when the central maximum of the Airy disk of one point image falls below the first minimum of the second point, is classified in Figure 2.19. The resolution is determined by the following formula:

$$R_{lateral} = \frac{0.61 \cdot \lambda}{NA} \quad (2.18)$$

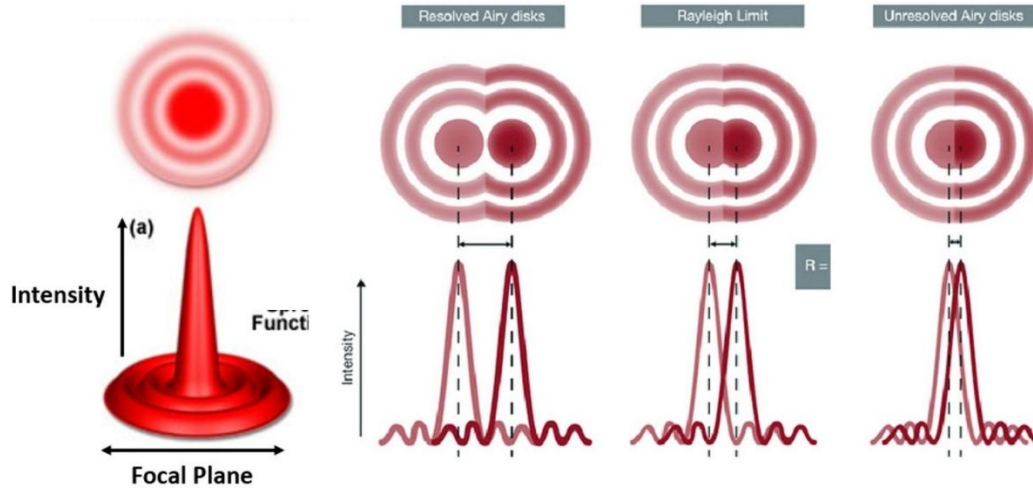


Figure 2.19: Different Airy disk representation and Image explanation of the lateral resolution criterium.

If the distance between two objects is smaller than $R_{lateral}$ they will be imaged as a single object. From a technical point of view, the size of the confocal pinhole, PH (μm), is related to $R_{lateral}$:

$$PH = \frac{1.22 \cdot \lambda}{NA} \cdot M \quad (2.19)$$

Where λ (μm) is the laser wavelength, NA is the objective lens numerical aperture, M is the optical system magnification. Another important technical parameter is the Axial resolution, or longitudinal resolution, i.e. the resolution in the Z direction. If the pinhole size is bigger than 1 AU, it can resolve two points in the axial direction when they are separated more than:

$$R_{axial} = \frac{0.88 \cdot \lambda}{n - \sqrt{n^2 - (NA)^2}} \quad (2.20)$$

Where n is the medium refractive index.

The lateral spatial resolution of Raman measurements is set by the diffraction-limited performance of the confocal optical microscope and can therefore be estimated using the Rayleigh criterion (Eq. 2.18).

In the axial direction, the confocal system provides a finite longitudinal (Z) resolution, which depends on the objective numerical aperture, the refractive index of the medium, and the pinhole size (Eq. 2.20). As a consequence, the optical configuration (NA, refractive index, and pinhole setting in AU) determines the instrument-limited axial discrimination between two planes along the optical axis.

In absorbing materials such as graphite, however, the effective depth from which the Raman signal originates is further limited by optical attenuation, so that the practical “depth selectivity” is governed by the minimum between the confocal axial resolution and the optical penetration depth. A simple estimate can be obtained using a Beer–Lambert description of attenuation: the excitation intensity at depth z decays as:

$$I_{\text{exc}}(z) = I_0 e^{-\alpha_{\text{exc}} z} \quad (2.21)$$

while the Raman photons generated at depth z are also attenuated when propagating back to the surface, leading to an additional factor $e^{-\alpha_{\text{R}} z}$. Therefore, the relative contribution of a slice at depth z to the detected Raman signal can be expressed as

$$W(z) \propto e^{-(\alpha_{\text{exc}} + \alpha_{\text{R}})z} \quad (2.22)$$

For visible excitation and small Raman shifts, it is often reasonable to assume $\alpha_{\text{exc}} \approx \alpha_{\text{R}} \equiv \alpha$, which yields

$$W(z) \propto e^{-2\alpha z} \quad (2.23)$$

The absorption coefficient α can be related to the extinction coefficient k through

$$\alpha = \frac{4\pi k}{\lambda} \quad (2.24)$$

so that a characteristic optical penetration depth (defined at $1/e$ for the excitation field) is

$$\delta_{\text{opt}} = \frac{1}{\alpha} = \frac{\lambda}{4\pi k} \quad (2.25)$$

Using $\lambda = 532$ nm and a representative visible-range extinction coefficient for graphite of $k \approx 1.3$, one obtains $\delta_{\text{opt}} \approx 33$ nm, corresponding to roughly ~ 100 graphene layers in HOPG [106]. Depending on the specific optical constants adopted for HOPG at 532 nm, values on the order of a few $\times 10$ nm (e.g. ~ 50 nm) are also reported, which is consistent with the conclusion that far-field Raman at 532 nm is intrinsically biased toward the near-surface region of graphite [107].

As final remark, it is presented the Raman spectrum of the pristine HOPG in Figure 2.20, with the characteristic peaks the G and the 2D peak. The details of this peak will be shown later in this work.

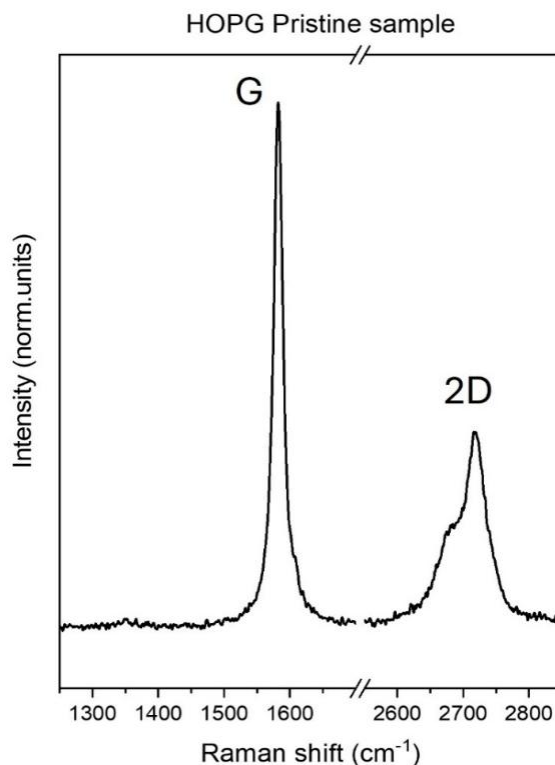


Figure 2.20: Pristine HOPG Raman spectrum. Laser wavelength 532 nm.

2.4 EC - AFM-Raman set-up

The NTEGRA Spectra system enables the correlation of electrochemical atomic force microscopy (EC-AFM) with Raman spectroscopy. The platform consists of two microscopes: an optical microscope, which is not discussed in this work, and an atomic force microscope. These two microscopes are integrated with a Raman spectrometer, allowing simultaneous or correlated AFM and Raman measurements. In the following, the system configuration and the coupling between EC-AFM and Raman spectroscopy are described [51].

The electrochemical cell, shown in Figure 2.21a, features a circular geometry in order to ensure an isotropic electrochemical environment. The cell is mounted on a magnetic plate, which allows precise positioning on top of the AFM scanner. A Teflon disc containing a central cavity is used to accommodate the sample, which acts as the working electrode (WE). Electrical contact is ensured by a copper wire placed at the bottom of the sample. An O-ring is positioned on top of the Teflon disc to provide sealing, followed by an additional Teflon ring. The entire assembly is fixed to a metal support by means of plastic screws.

The upper Teflon ring is equipped with a circumferential groove and several apertures. The groove is used to position the counter electrode (CE), while the

apertures allow electrical connection of both the reference electrode (RE) and the counter electrode to the external circuitry. This configuration preserves the isotropy of the electrochemical system, thereby avoiding preferential directions for charge transfer.

The AFM operates in a sample-scanning configuration. Both the sample and the electrochemical cell are positioned on the scanner through magnetic coupling. As illustrated in Figure 2.21b, the scanner consists of an elongated arm terminating in a magnetic disc on which the sample is mounted.

Scanner motion is controlled by piezoelectric actuators along the three spatial directions (X, Y, and Z), as shown in Figure 2.21c. This configuration enables scanning over relatively large areas, with a maximum scan range of approximately $100 \times 100 \times 12 \mu\text{m}^3$. Owing to the geometric configuration of the scanner, large scan sizes may introduce image distortions; however, these artefacts can be readily corrected through software-based post-processing. Coarse positioning of the scanner in the X and Y directions is achieved by micrometric screws, while motion along the Z direction is controlled by a stepper motor combined with a feedback loop for precise sample positioning.



Figure 2.21: a) Electrochemical cell. b) photo of the scanner AFM, 1) scanner protection; 2) points where the screw act in order to move the scanner along the x direction; 3) scanner magnetic disk. c) schematic representation of the scanner: 1) piezoelectric that controls the movements in the 3 directions; 2) support; 3) scanner magnetic disk [108,109].

In order to perform the different measurements, the system allows the exchange of the so-called AFM head. The head comprises the detection and registration components of the AFM, including the laser for cantilever deflection detection, the cantilever holder, and the photodetector. For coupling AFM with Raman spectroscopy, dedicated heads equipped with optical objectives are required. Two specific configurations are available: one designed for AFM–Raman measurements in air and a second one optimised for measurements in liquid or acidic environments.

The AFM–Raman head for measurements in air is equipped with a 100 $\times$ objective with a numerical aperture (NA) of 0.75, whereas the head designed for operation in liquid environments incorporates a 60 $\times$ objective with NA = 1. In the latter case, the terminal part of the objective is coated with Teflon to allow immersion in strongly acidic solutions. The instrumentation further includes an illumination lamp and a CCD camera, which are used for sample observation and for coarse laser alignment.

In order to geometrically couple the AFM detection system with the optical objective, the instrument adopts a specific optical layout, schematically illustrated in Figure 2.22a,b. In both air and liquid configurations, Raman measurements are performed in an axial geometry, with the AFM tip positioned beneath the objective. In air, the AFM detection laser impinges on the cantilever at a large angle ($>70^\circ$) with respect to the vertical axis. This configuration cannot be adopted in liquid environments; instead, the optical objective is used to focus the AFM detection laser onto the cantilever. This configuration introduces a trade-off, as moving the focal plane from the cantilever to the sample in order to acquire Raman spectra leads to a partial degradation of AFM image quality .

Combined AFM–Raman measurements require the use of cantilevers with a specific tip geometry, as schematically shown in Figure 2.22c,d. The tip apex is inclined with respect to the cantilever axis, which allows the Raman laser to be focused in close proximity to the tip apex [110]. The cantilevers used in this work are gold-coated probes, with an average resonance frequency of approximately 260 kHz, a nominal spring constant of about 25 N m $^{-1}$, and a nominal tip radius smaller than 10 nm.

The characteristic geometry of the cantilever and tip can be directly observed using the optical microscope, which is employed for an initial manual alignment of the Raman laser, as shown in Figure 2.22. The terminal portion of the cantilever is not coated with gold and can therefore be used for Raman calibration, while the main body of the cantilever is coated with Au, as indicated by the yellow region. The AFM infrared detection laser appears as a purple shadow on the cantilever, allowing optical verification of its alignment. The Raman laser spot, shown in green, can be displaced by means of a piezoelectrically actuated mirror system and aligned with the tip apex. The tip position can be readily identified, as scattered light produces a characteristic shadow on the cantilever, as visible in Figure 2.22.

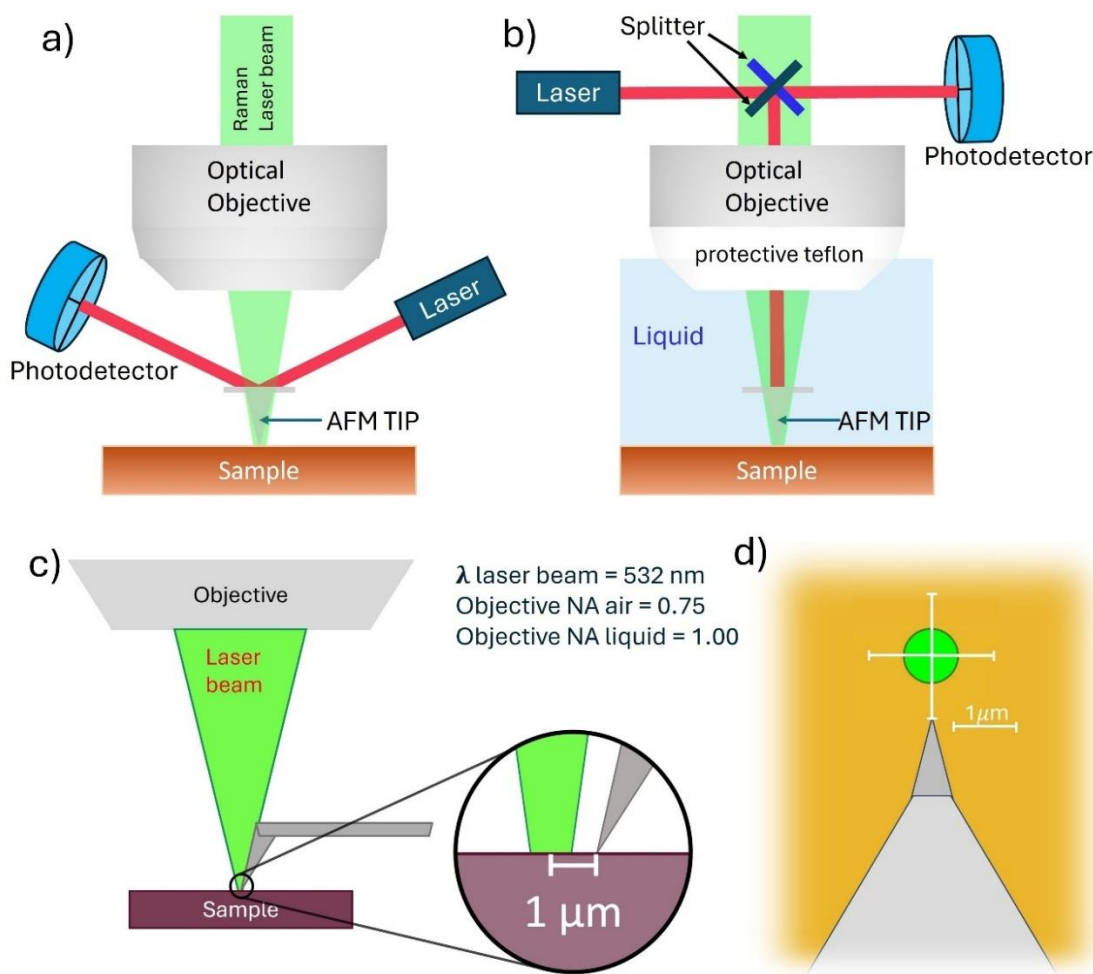


Figure 2.22: a) AFM-Raman sketch for air measurements. b) AFM-Raman sketch for liquid measurements. c,d) Side and top view of the laser beam-AFM tip alignment.

The mirror system can also be used for scanning, enabling two distinct Raman mapping modes: mirror-driven mapping, in which the laser position is scanned, and scanner-driven mapping, in which the sample is displaced. After coarse optical alignment, fine alignment is achieved by performing a rapid mirror-driven scan of a region where the tip apex is expected. The apex is identified as a local hotspot in the silicon Raman peak region, after which the laser is positioned approximately 2–3 μm below the tip apex in order to avoid contributions from silicon to the Raman spectrum. This positioning is achieved with a precision of approximately 1 μm , corresponding to the diffraction limit of the laser, as shown in Figure 2.23e. Subsequent Raman maps are acquired in scanner-driven mode, ensuring that the relative alignment between the tip and the laser is preserved throughout the measurement.

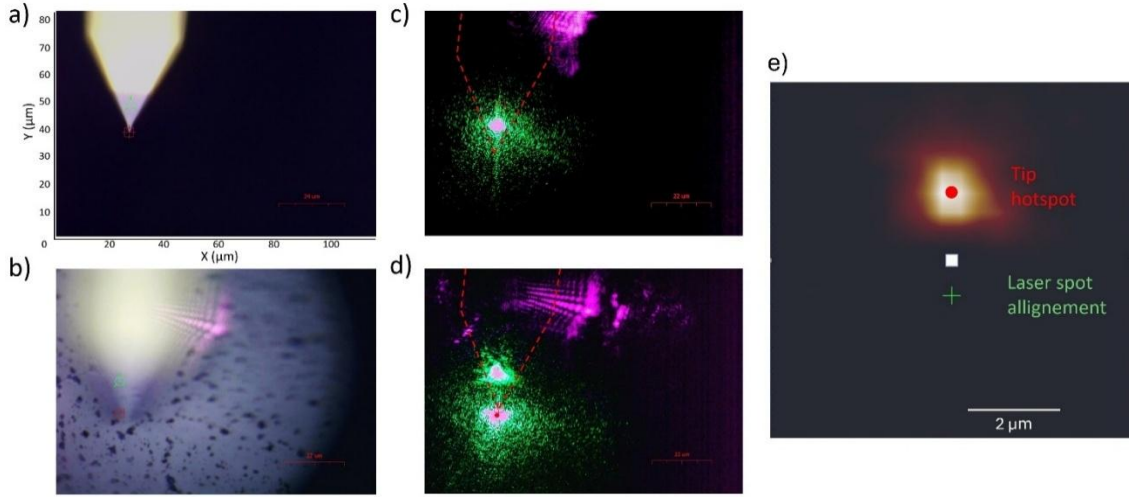


Figure 2.23: Optical microscope images of **a)** focus on the tip far from the sample **b)** focus on an intercalated HOPG sample **c)** laser spot on the silicon part of the tip (used for calibration) **d)** laser spot on HOPG aligned on the tip apex. **e)** 8x8 μm mirror-driven Raman map of the Si peak at 520.7 cm^{-1} . [111]

In addition to the instrumental configuration described above, the operational workflow of correlated AFM–Raman mapping is outlined in the following. Combined AFM–Raman measurements follow a well-defined procedure that enables direct correlation between surface morphology and local vibrational response within the same area of the sample. The workflow can be schematically divided into four main steps.

In the first step, the surface morphology of the region of interest is acquired by AFM. The AFM image provides the spatial reference for all subsequent spectroscopic measurements and allows the identification of relevant surface features, such as terraces, steps, or blistered regions. The scanned AFM area defines the maximum region over which correlated Raman mapping can be performed.

After AFM acquisition, Raman measurements are performed on the same area by defining a user-selected spatial grid. The grid parameters, including the number of points and the spacing between adjacent pixels, are chosen by the user and must be compatible with the spatial resolution of the Raman probe. In practice, a slight oversampling is typically adopted in order to ensure adequate spatial continuity of the Raman map. For instance, when the diameter of the Airy disk is of the order of 800 nm, the distance between the centres of adjacent pixels is commonly set to approximately 500 nm. This procedure results in a virtual discretization of the AFM image into pixels, each associated with a specific Raman acquisition point.

For each pixel of the defined grid, the system acquires a full Raman spectrum over the selected spectral range. The resulting raw dataset therefore consists of a collection of complete Raman spectra, each spatially associated with a well-defined

position within the AFM image. At this stage, no spectral selection or integration is performed, and all vibrational information is retained for subsequent analysis.

During the analysis stage, the acquired dataset can be exploited in two complementary ways. Individual Raman spectra corresponding to specific pixels can be extracted and analysed in order to investigate the local vibrational response of selected regions of the sample. Alternatively, Raman intensity maps can be reconstructed by selecting a specific Raman band or spectral feature of interest. In this case, a two-dimensional colour map is generated, in which the colour intensity associated with each pixel is proportional to the intensity of the selected Raman feature at the corresponding spatial position. This approach enables a direct visualization of spatial variations in Raman response and facilitates correlation with the underlying AFM morphology.

An illustrative example of the correlated AFM–Raman mapping procedure is shown in Figure 2.24, referring to pristine HOPG measured in air. Panel (a) reports the AFM topographic image of the selected area, which serves as the spatial reference for the spectroscopic measurements. Panel (b) shows the corresponding user-defined grid employed for Raman map acquisition, in which the AFM area is discretized into pixels associated with individual Raman measurement points. Panel (c) displays a representative full Raman spectrum acquired at a single pixel of the grid, highlighting the characteristic vibrational features of pristine graphite. Finally, panel (d) presents a Raman intensity map obtained by integrating the G-band intensity over the entire mapped area, providing a two-dimensional visualization of the spatial distribution of the selected Raman feature.

Although the AFM–Raman correlation approach provides a powerful framework for linking surface morphology and vibrational response, its main limitation arises from the lateral spatial resolution of Raman spectroscopy, which is fundamentally constrained by optical diffraction.

As a consequence, the method can be reliably applied only to structures whose characteristic dimensions are larger than the lateral resolution of the Raman probe (i.e. the Airy disk diameter), while smaller features cannot be spectroscopically resolved as independent objects.

In addition, the spatial alignment between AFM and Raman measurements is affected by an intrinsic uncertainty on the order of the Raman spatial resolution, which limits the accuracy of point-by-point correlation between local morphology and spectroscopic response. These aspects must be taken into account when interpreting the results, particularly in the context of quantitative correlations.

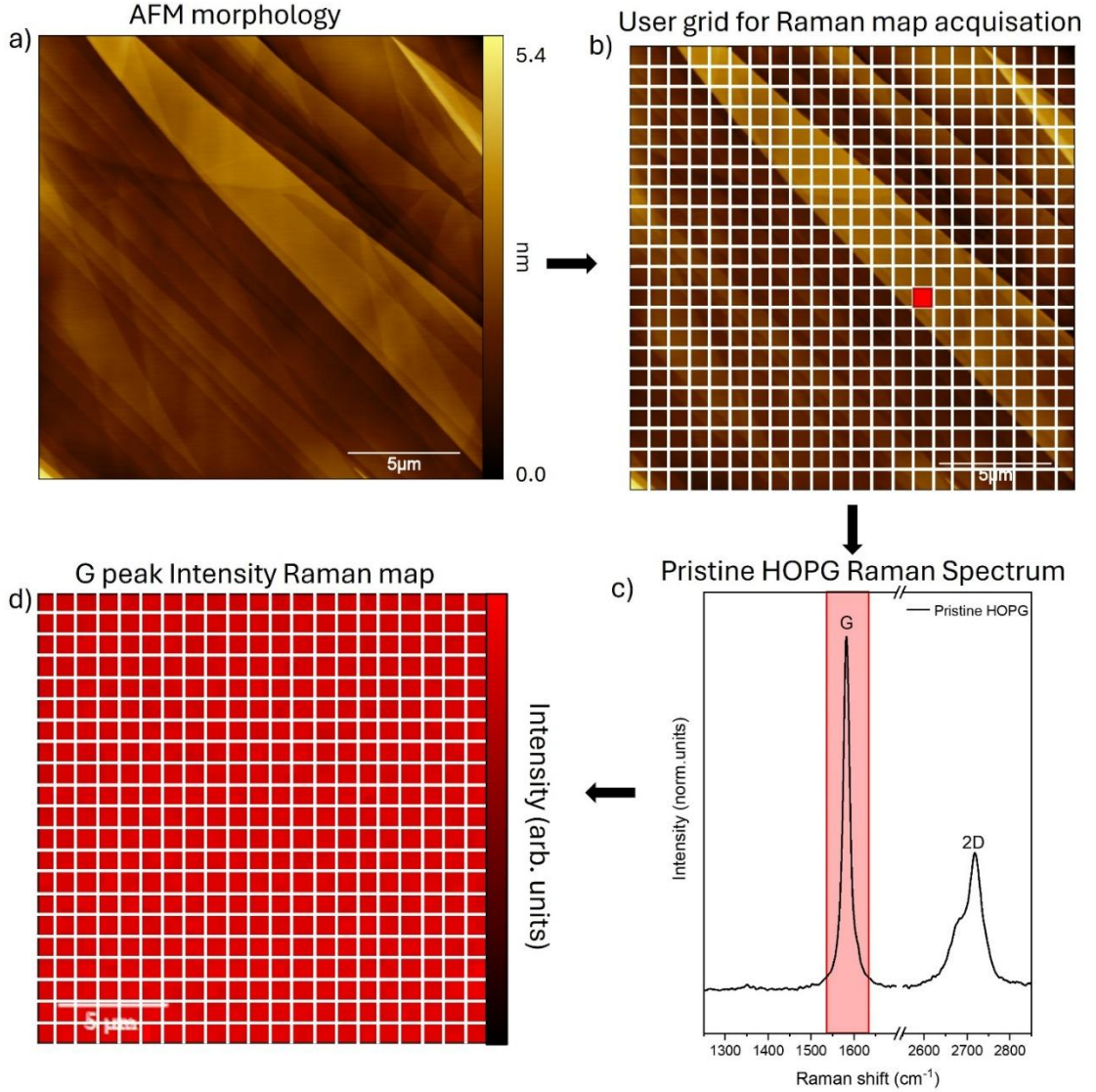


Figure 2.24: a) AFM topographic image of pristine HOPG acquired in air. b) Raman mapping grid obtained by discretization of the AFM scan area into pixels corresponding to individual Raman acquisition points. c) Full Raman spectrum acquired at a single pixel of the grid. d) Raman intensity map of the G band.

2.5 Tip-Enhanced Raman Spectroscopy (TERS)

2.5.1 Principle of the technique

Tip-Enhanced Raman Spectroscopy (TERS) represents a natural extension of conventional Raman spectroscopy, conceived with the aim of overcoming the optical diffraction limit that constrains its spatial resolution to values on the order of several hundreds of nanometres. As discussed in the previous sections, in a standard confocal Raman configuration the collected signal is averaged over the entire area illuminated by the laser beam, whose minimum size is determined by the

wavelength of the incident radiation and by the numerical aperture of the objective. This results in a loss of local information when heterogeneous systems at the nanometric scale are investigated [56].

TERS makes it possible to circumvent this limitation by coupling a scanning probe microscopy technique (Scanning Probe Microscopy, SPM) with Raman spectroscopy. The fundamental principle of the technique relies on the use of a sharp tip made of a noble metal, typically gold or silver, which is approached to the sample surface through the feedback system of the SPM technique employed for the coupling [112,113]. Under these conditions, the interaction between the incident radiation and the metallic tip induces the excitation of localized plasmonic resonances at the tip apex, giving rise to an electromagnetic field that is strongly confined in space and significantly enhanced with respect to the incident field. When AFM is employed for TERS measurements, silicon tips coated with gold or silver are typically used [114], whereas in the case of STM-based TERS the tip is entirely made of gold or silver [112,115].

The interaction volume responsible for the enhanced Raman signal is therefore no longer determined by the optical characteristics of the system, but essentially by the radius of curvature of the tip apex and by the tip-sample distance [115]. In this way, the spatial resolution of TERS measurements becomes independent of the diffraction limit and can reach values of a few nanometres in optimized configurations.

The first experimental work that unequivocally demonstrated this principle dates back to the year 2000 [56], when Stockle and co-workers showed that a metallic tip, used as a probe in an AFM system coupled to a Raman spectrometer, was able to produce a significant increase in the Raman intensity originating from a molecular film, localized exclusively in the region close to the tip apex. This result marked the formal birth of TERS as a high-spatial-resolution spectroscopic technique.

The Raman signal enhancement in TERS is dominated by electromagnetic mechanisms, analogous to those known in Surface-Enhanced Raman Spectroscopy (SERS) [116], but confined to a single, spatially controlled hotspot. In particular, the excitation of localized surface plasmon resonances (LSPR) of the metallic tip leads to an intense concentration of the electric field at the apex, often referred to as the lightning-rod effect, which is further reinforced by the plasmonic resonance itself.

From a quantitative point of view, the intensity of the enhanced Raman signal can be approximated as being proportional to the fourth power of the local electric field amplitude, according to the relation:

$$I_{\text{TERS}} \propto |E_{\text{loc}}|^4 \quad (2.26)$$

where E_{loc} represents the local electric field in the tip-sample interaction region. This dependence highlights how even a moderate increase in the local field can result in a very significant enhancement of the measured Raman intensity [117].

In addition to the plasmonic effect, the tip geometry and the polarization of the incident radiation also contribute to the overall enhancement. In particular, an electric field component parallel to the tip axis favours charge accumulation at the apex, thereby maximizing field confinement [118]. In configurations in which the tip is positioned above a metallic substrate (i.e., gold, silver, platinum, or copper), as in the so-called gap-mode TERS, the interaction between the dipole induced in the tip and its image charge in the substrate leads to a further enhancement and to an even more extreme confinement of the electromagnetic field. When the tip and the substrate are made of the same metal, the enhancement is optimized.

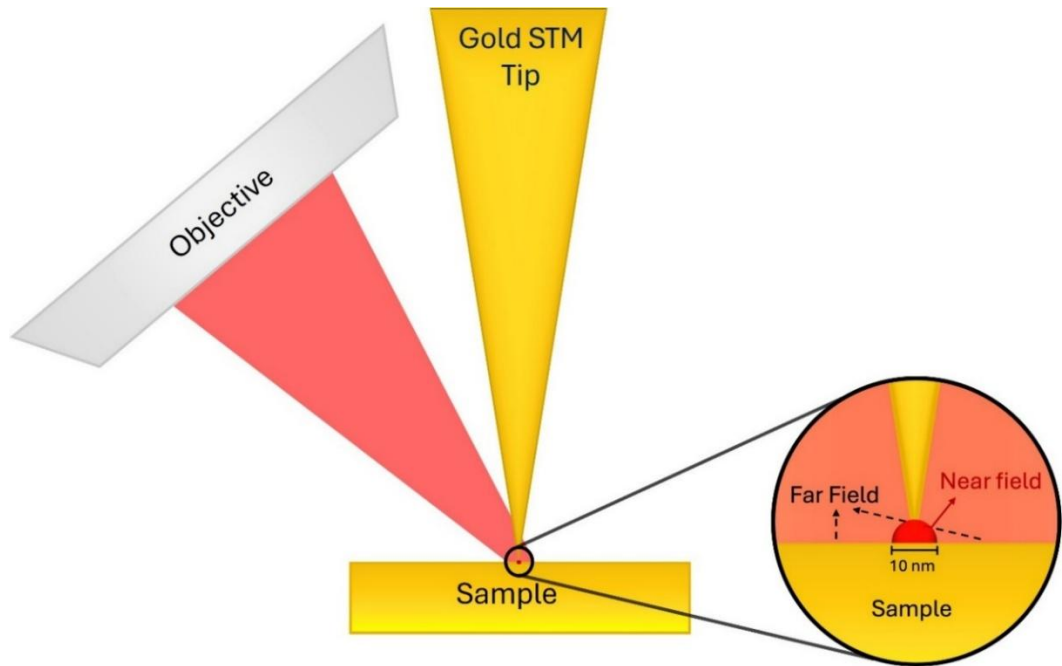


Figure 2.25: Sketch of the STM-TERS

The contribution of chemical mechanisms, associated with charge-transfer processes between the tip and the adsorbed molecules, is generally considered secondary in TERS and relevant only at extremely short distances. In most

experimental cases, the observed behaviour is consistent with a dominant electromagnetic enhancement mechanism[119].

From an experimental point of view, TERS can be implemented using different scanning probe microscopy modes, in particular AFM-TERS and STM-TERS. In the case of STM-TERS, which is also employed in the present work, the tip-sample distance is controlled through the tunnelling current feedback signal, ensuring an extremely precise positioning of the metallic apex with respect to the conductive sample surface [120].

One of the main advantages of STM-TERS lies in the possibility of using uncoated, sharply etched metallic tips, obtained by electrochemical etching, which exhibit a very small radius of curvature and excellent plasmonic properties. This allows a higher spatial resolution to be achieved compared to many AFM-TERS configurations, albeit at the expense of the ability to analyse non-conductive samples.

In tip-enhanced Raman spectroscopy, the spatial resolution is not determined by optical diffraction, but by the spatial confinement of the enhanced electromagnetic near field at the apex of the metallic tip. As a consequence, the effective lateral resolution of TERS is primarily governed by the radius of curvature of the tip apex, the tip-sample distance, and the efficiency of plasmonic field confinement. In the present work, gold-coated AFM probes with nominal tip radii below 10 nm were employed, resulting in an effective lateral resolution on the order of a few tens of nanometres. This resolution is sufficient to resolve spatial variations in vibrational response within individual blisters, while remaining well above the atomic scale [57,58].

Along the vertical direction, the spatial selectivity of TERS is dictated by the evanescent nature of the enhanced near field, which decays exponentially away from the tip apex. As a result, the Raman signal enhancement is strongly localized within the first few nanometres from the sample surface, effectively confining the probed volume to the uppermost layers of the material. In layered systems such as HOPG, this intrinsic near-field confinement makes TERS particularly sensitive to the graphene layers forming the blister skin, while largely suppressing contributions from deeper regions of the bulk.

It should be emphasized that the effective TERS resolution is not a fixed instrumental parameter, but may vary during the experiment due to changes in tip condition, tip-sample coupling, and plasmonic efficiency. Tip wear, contamination, or modifications of the apex geometry can lead to a reduction of spatial confinement or to fluctuations in enhancement factor, thereby affecting both resolution and

signal reproducibility. For this reason, TERS data in the present work were interpreted in a comparative and spatially relative framework, and the consistency of the measurements was assessed through repeated scans and correlation with AFM morphology, rather than by relying on absolute spatial resolution claims.

On the basis of calibration measurements performed on reference systems and considering the specific probes employed in this work, the effective lateral spatial resolution of the TERS measurements can be estimated to be on the order of 10–15 nm, while the effective vertical confinement of the enhanced near field is not expected to exceed approximately 2 nm.

2.5.2 Preparation of TERS tips

The quality and reliability of TERS measurements critically depend on the characteristics of the tip employed. An effective TERS tip must simultaneously fulfil mechanical, electrical, and optical requirements: it must be sufficiently sharp to ensure high spatial resolution, exhibit a smooth surface in order to avoid spurious signals, and possess suitable plasmonic properties in the spectral region of interest.

In the present work, TERS tips were prepared following the procedure developed by Ren, Picardi, and Pettinger, based on direct-current electrochemical etching of gold wires in a solution of concentrated hydrochloric acid and ethanol [121]. This method allows the reproducible fabrication of tips with a radius of curvature below 10 nm and a relatively smooth surface, which are essential characteristics for TERS applications.

The electrochemical etching process exploits the anodic dissolution of gold in a chloride environment, with an applied voltage typically in the range of 2.2–2.4 V. The addition of ethanol to the HCl solution plays a key role in reducing gas bubble formation during the etching process, thereby improving the surface quality of the tip. The etching rate is higher in the liquid–air meniscus region, where a progressive necking of the wire occurs until the lower part breaks off, marking the end of the process.

The choice of gold as the tip material is motivated not only by its favourable plasmonic properties in the visible range, but also by its high chemical stability, which allows repeated measurements to be performed without rapid degradation of the tip performance.

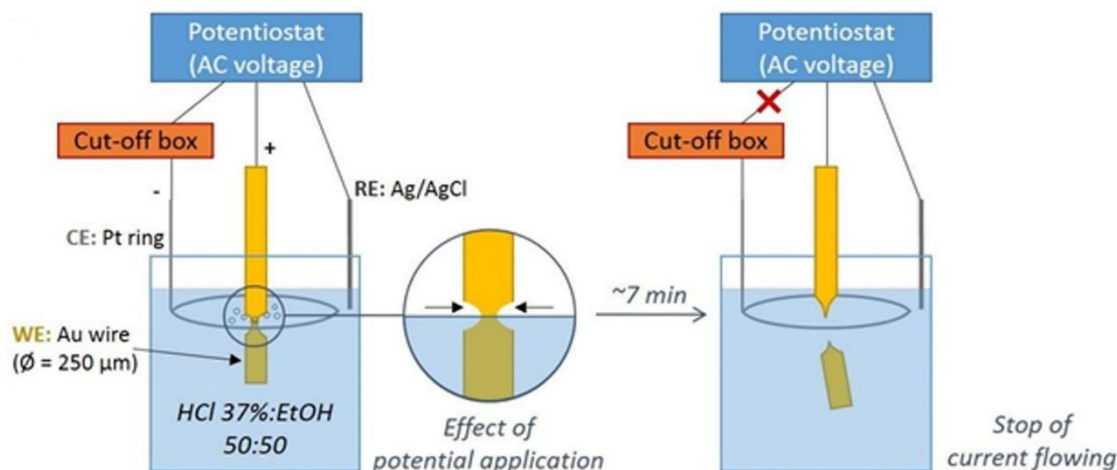


Figure 2.26: Sketch of the Gold tip preparation [122].

2.5.3 HORIBA experimental system for TERS measurements

TERS measurements were carried out using a commercial HORIBA system that integrates a scanning tunnelling microscope with a confocal Raman spectrometer. The instrumental configuration is analogous to that described in the thesis of Alice Fiocco, since the same instrument was employed [58,119,122].

The system is based on a high-resolution Raman spectrometer coupled to an STM module mounted on an antivibration platform, in order to ensure the mechanical stability required to maintain proper tip-sample alignment during spectral acquisition. Sample illumination is performed in a side-illumination configuration, in which the laser beam is focused onto the tip apex by means of a medium numerical aperture objective, compatible with the geometry of the STM microscope.

The Raman-scattered radiation is collected by the same objective and directed towards the spectrometer, where it is dispersed by a diffraction grating and detected by a cooled CCD. This configuration enables the acquisition of Raman spectra both in the far-field regime, with the tip retracted, and in the near-field regime, with the tip in close proximity to the sample, allowing a direct comparison between conventional Raman and TERS signals.

The possibility of simultaneously correlating topographic and spectroscopic information represents one of the main strengths of the technique, as it enables local variations in the Raman signal to be unambiguously associated with specific morphological features of the analysed surface.

2.5.4 Acquisition of TERS spectra

In order to verify the correct operation of the TERS setup and to optimize the experimental alignment, a preliminary set of measurements was performed on a calibration sample consisting of a self-assembled azobenzene monolayer deposited on a polycrystalline gold film grown on mica. This configuration allows operation in coupling mode, where both the metallic tip and the substrate contribute to the enhancement of the local electromagnetic field. Under these conditions, the interaction between the laser beam and the tip apex is maximized, providing optimal experimental conditions for observing the TERS effect [123].

The experimental setup enables precise positioning of the objective along the three spatial directions (x, y, and z), allowing fine alignment between the laser focus and the tip apex. A Raman intensity map acquired by laterally scanning the objective in the x-y plane shows localized regions of increased signal intensity, corresponding to effective coupling between the laser beam and the metallic tip. When the incident laser power is set to approximately 0.1 mW, a detectable Raman signal is observed exclusively at the tip position, while no signal is detected in the surrounding areas. This behaviour confirms the correct laser-tip alignment and the successful establishment of the coupling condition, which is a necessary prerequisite for subsequent TERS measurements.

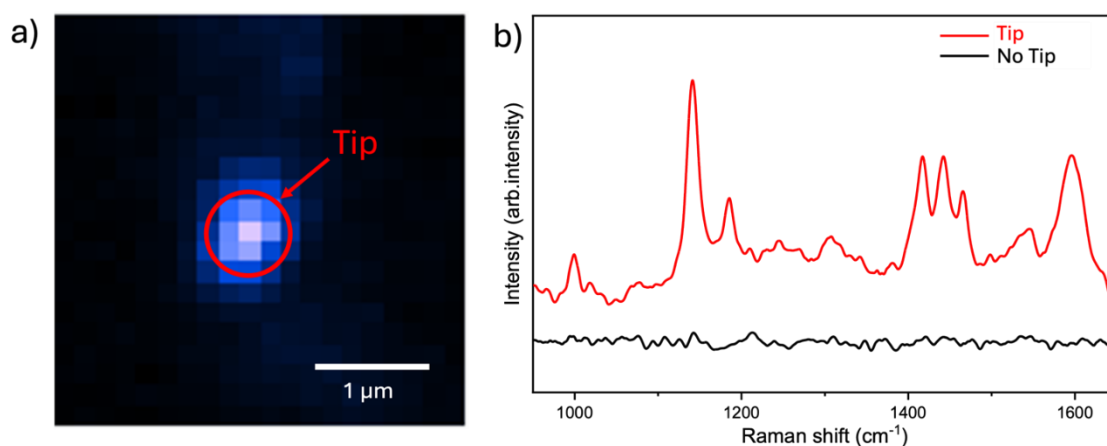


Figure 2.27: **a)** Raman map obtained moving the optical objective in X and Y direction, in the red circle is highlighted the presence of the tip. **b)** Two Raman spectra on azo-benzene, in red where the tip is present, in black where there is no tip.

Once the alignment procedure is completed, the calibration sample is replaced by the HOPG substrate. In this case, since the substrate is not metallic, coupling mode cannot be achieved. As a consequence, the alignment becomes more critical, because the additional enhancement provided by the metal-metal cavity is no

longer available. For this reason, the alignment is systematically performed using the calibration sample prior to each measurement session, and only afterward the HOPG sample is introduced, ensuring that the laser focus remains as close as possible to the optimal position.

Under these experimental conditions, the system exploits only the local electromagnetic enhancement generated by the metallic tip, and the far-field Raman contribution is not fully suppressed. In particular, for the laser power used during HOPG measurements (approximately 1 mW), the far-field signal remains detectable. Therefore, different acquisition strategies were adopted depending on the type of information required.

For extended surface investigations, TERS maps were acquired with the tip continuously in contact with the sample surface. In this configuration, both near-field and far-field contributions are collected simultaneously. This approach is particularly suitable for studying HOPG surfaces, especially in their intercalated form, which exhibit significant spatial variations in their spectroscopic response. A representative example is the behaviour of the D peak, which is directly related to the local defect density: it is absent in defect-free regions and becomes clearly visible in areas characterized by a higher concentration of structural defects. In these cases, conventional TERS mapping proved sufficient to obtain detailed spatial information with a resolution unattainable by standard Raman spectroscopy.

The investigation of blistered regions required a different approach. In order to improve the signal-to-noise ratio and to isolate the near-field contribution more accurately, single-point TERS measurements were performed in addition to mapping. For each selected position, two spectra were acquired: one with the tip in contact with the surface and one with the tip retracted by approximately 50 nm. The contact-tip spectrum contains both near-field and far-field contributions, whereas the retracted-tip spectrum represents the far-field signal only. By subtracting the latter from the former, it is possible to isolate the pure near-field Raman contribution associated with the TERS effect.

This subtraction procedure allows a clear separation between far-field and near-field components and provides an accurate representation of the local Raman response of the blister surface. While the far-field spectrum already indicates partial structural damage of the HOPG, as evidenced by a non-zero D peak intensity, the near-field contribution reveals a significantly stronger D peak when the tip is positioned over regions characterized by increased structural disorder. In the pure

near-field spectrum, the D peak intensity exceeds that of the G peak, demonstrating the capability of TERS to selectively enhance defect-related vibrational modes.

These results confirm the high spatial sensitivity of TERS and its effectiveness in revealing local structural modifications that cannot be resolved by conventional far-field Raman spectroscopy.

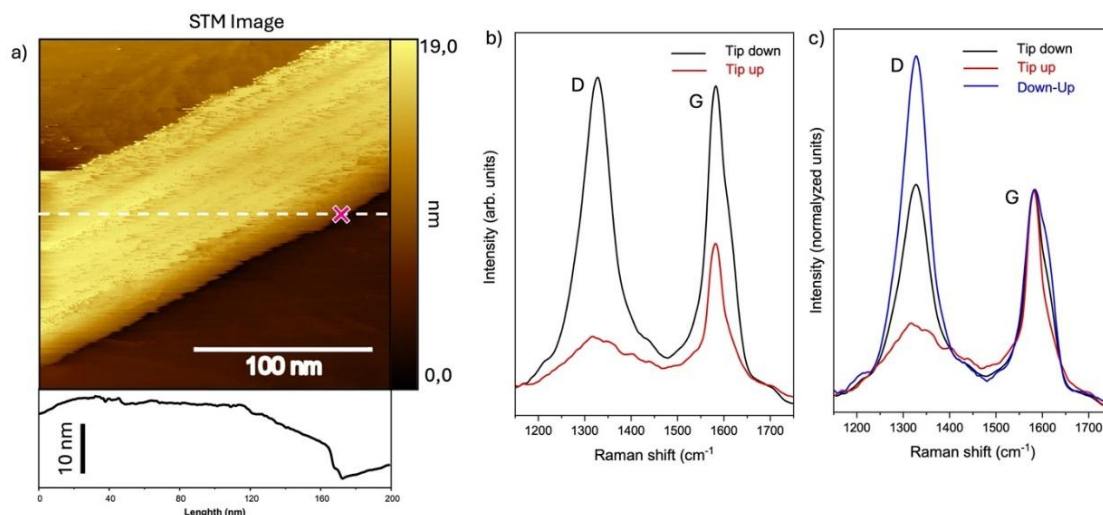


Figure 2.28: a) STM image acquired on STM step, the red cross highlight the analysed position. b) Raman spectrum acquired with the tip in contact with the sample, tip down, and 50 nm distant from the sample (tip up). c) Normalized spectra, tip down (black line), tip up (red light) and the blue one is the result of the difference between the tip down and the tip up spectrum.

2.6 Selection of Characterization Techniques

The choice to employ AFM, far-field Raman spectroscopy, and Tip-Enhanced Raman Spectroscopy (TERS) in this work was driven by the need to access complementary physical information across different spatial scales during the investigation of blistered graphitic systems.

The structures analysed in this thesis exhibit characteristic lateral dimensions in the micrometric range and vertical dimensions of several tens of nanometres. These geometrical scales are particularly well suited for AFM characterization, which provides quantitative topographic reconstruction with nanometric vertical resolution, enabling reliable morphological and mechanical assessment.

Raman spectroscopy was adopted as a complementary technique because it directly probes the vibrational response of the graphitic lattice. Beyond chemical identification, Raman measurements allow the evaluation of strain-induced spectral shifts and structural modifications of the carbon framework, thus providing access to lattice perturbations at the micrometric scale.

However, far-field Raman spectroscopy inherently averages the signal over the diffraction-limited laser spot. For this reason, TERS was employed in selected cases to access spectroscopic information at a sub-diffraction spatial scale. By combining localized electromagnetic enhancement with scanning probe positioning, TERS enables the investigation of vibrational properties with nanoscale lateral resolution, revealing local heterogeneities that remain spatially averaged in conventional Raman measurements.

Taken together, these techniques provide complementary insight into morphology, mechanical behaviour, and vibrational response, forming the experimental backbone of the present study.

2.7 Data Processing and Analysis Workflow

The experimental datasets presented in this work consist of correlated AFM topography images and spatially resolved Raman spectra (far-field Raman and TERS). In order to ensure consistency, reproducibility, and comparability across different measurements, a unified data processing and analysis workflow was adopted for all datasets.

The workflow can be divided into the following main steps.

1. AFM Pre-processing and Morphological Reference:

AFM images were first processed using standard topographic correction procedures. These include background subtraction (plane fitting or polynomial flattening) in order to remove sample tilt and scanner-related artifacts.

Regions characterized by abrupt morphological variations were carefully evaluated and, when necessary, masked to avoid artificial correlations during spectroscopic alignment. The processed AFM image serves as the morphological reference for subsequent spatial correlation with Raman maps.

2. Raman Spectral Pre-processing

For each pixel of the Raman dataset, the raw spectrum was individually processed.

A linear background subtraction was applied to each spectrum in order to remove fluorescence contributions and baseline offsets. This procedure ensures that peak intensities and positions are extracted from comparable spectral conditions across the entire mapped area. No smoothing or artificial filtering was introduced unless explicitly stated.

3. Peak Parameter Extraction

For each Raman band of interest, two main quantities were extracted at every spatial position:

- The peak intensity
- The peak position (energy shift)

Peak intensity was determined by identifying the maximum intensity within a predefined spectral window centered around the expected Raman shift of the selected band.

Simultaneously, the spectral position corresponding to this maximum was stored, allowing the construction of spatial maps of peak energy variation.

This procedure was applied systematically to all relevant Raman features investigated in this work.

4. Intensity Normalization

In order to compensate for local variations in overall signal intensity (e.g., due to slight defocusing, tip-sample distance variations, or local enhancement effects), peak intensities of all bands—except for the G band—were normalized to the corresponding G-band intensity at each spatial position.

The G band, being the most intense and structurally representative Raman feature of graphitic systems, was used as an internal reference.

Consequently:

- All peak intensity maps (except the G map) represent normalized intensity values.
- The G-band intensity map is reported without normalization.

This normalization procedure ensures that intensity variations reflect relative spectral changes rather than global signal fluctuations.

5. Spatial Map Construction

Based on the extracted parameters, two primary types of Raman maps were generated:

1. Normalized peak intensity maps, representing the spatial distribution of relative band intensities.
2. Peak position (energy shift) maps, representing the spatial variation of the maximum position of selected Raman bands.

These maps were constructed directly from the processed spectral volume and retain the original spatial discretization of the measurement grid.

6. AFM–Raman Spatial Alignment

The spatial alignment between AFM images and Raman maps was performed by considering the known geometrical offset between the laser focal spot and the probe apex, as well as by exploiting characteristic spectroscopic contrasts (e.g., defect-related D-band intensity variations) to refine the alignment.

This procedure enables direct morphological–spectroscopic correlation at the micrometric and sub-micrometric scale.

7. Region of Interest (ROI) Selection and Spectral Fitting

Regions of interest (ROIs) were defined based on combined morphological and spectroscopic criteria.

From these selected areas, individual spectra were extracted from the spectral volume for further detailed analysis.

Peak fitting procedures were subsequently performed (using standard fitting functions appropriate for graphitic systems) in order to:

- Accurately determine peak positions,
- Evaluate intensity ratios,
- Analyse peak component structures where necessary.

These quantitative parameters were then used for strain evaluation, defect assessment, and comparative analysis across different regions and experimental conditions.

8. General Applicability

The workflow described above was systematically applied to all AFM–Raman and TERS datasets presented in this thesis, ensuring methodological consistency throughout the manuscript

Automated spectral preprocessing and parameter extraction were performed using custom routines, while spatial alignment and ROI selection were performed semi-manually based on morphological–spectroscopic correlation.

3

Experimental background on blister formation and blister interpretative model

The formation of blisters in electrochemically intercalated HOPG emerges from a complex interplay between electrochemical processes, structural modifications, and mechanical relaxation. While the phenomenology of blister formation has been introduced in the introduction, a detailed understanding of the experimental conditions under which blisters nucleate and evolve is essential in order to interpret the mechanical and spectroscopic investigations presented in the following chapters.

This chapter provides the experimental background required to bridge electrochemical intercalation with the mechanical characterization of blisters. By combining cyclic voltammetry, atomic force microscopy, and Raman spectroscopy, the key processes leading to surface swelling and blister formation are introduced and discussed at a qualitative level. The goal is not to present an exhaustive analysis, but rather to establish the experimental and conceptual framework necessary for understanding the results presented in the following chapters.

In this context, the experimental observations reported in this chapter primarily serve to reproduce and validate, within the present instrumental framework, the main morphological and spectroscopic features of electrochemically induced blisters in HOPG that are already reported in the literature. This step is essential to demonstrate experimental control over the system and to define a reliable reference state, upon which the original analyses and interpretations developed in the subsequent chapters are built.

Building on the experimental observations introduced in the previous sections, this paragraph presents the interpretative model adopted in this work to describe the formation and mechanical nature of blisters in electrochemically intercalated HOPG.

3.1 Electrochemical response of HOPG during cyclic voltammetry

To induce and control anion intercalation in HOPG, cyclic voltammetry was employed as the electrochemical technique throughout this work. An illustrative cyclic voltammogram of an HOPG electrode immersed in a 1 M H_2SO_4 aqueous solution is shown in Figure 3.1. Cyclic voltammetry provides a direct means to define the electrochemical potential window in which intercalation processes become active and to control the extent of anion insertion into the graphite lattice [48].

Pristine HOPG surfaces are characterized by extended basal planes interrupted by atomic steps of different heights, corresponding to regions where a variable number of graphene layers terminate [47,50]. These step edges and structural defects play a central role in the intercalation process, as they act as preferential access points for anions entering the layered structure. During the anodic sweep of the applied electrochemical potential, intercalation is observed to occur within the potential range between approximately 0.3 V and 1.3 V, as highlighted in the voltammogram reported in Figure 3.1 .

The arrangement of intercalated anions within the HOPG crystal depends on the intercalation stage reached during the anodic sweep. In the present work, the upper limit of the anodic potential was set to 1.3 V in order to reach the stage IV intercalation regime [48]. This stage is associated with the appearance of a characteristic shoulder at around 1.15 V in the voltammogram, which is commonly taken as an electrochemical signature of this intercalation state. An additional feature appearing around 0.6 V is attributed to the presence of adsorbed species at the working electrode surface and does not play a direct role in the intercalation process.

The intercalation process in HOPG under these conditions is generally regarded as quasi-reversible. Accordingly, during the reverse (cathodic) sweep of the potential, a negative feature centred at approximately 0.9 V is observed. The smaller area subtended by this cathodic contribution with respect to the anodic one

indicates a partial expulsion, or de-intercalation, of anions from the graphite lattice [42].

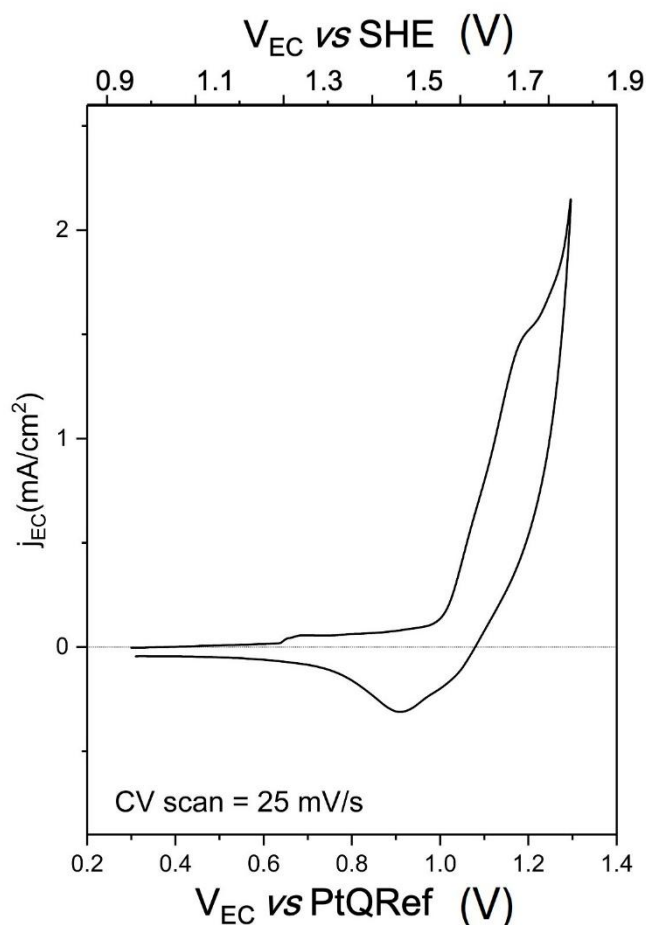


Figure 3.1: HOPG Cyclic voltammetry in 1M H₂SO₄ solution.

3.2 Raman spectra in pristine and in intercalated HOPG

The vibrational and chemical properties of the pristine and intercalated HOPG where deeply studied in the past. Figure 3.2 presents a comparison between the Raman spectra acquired on pristine HOPG and on electrochemically intercalated HOPG. Panel (a) shows the full spectral range, while panel (b) focuses on a detailed analysis of the 2D band.

The Raman spectrum of pristine HOPG is dominated by a prominent peak centred at 1582 cm⁻¹, with a full width at half maximum (FWHM) of approximately 20 cm⁻¹. This feature corresponds to the first-order Raman-active phonon mode with E_{2g} symmetry at the Γ point of the Brillouin zone and is commonly referred to as the G band [124]. Another characteristic feature of graphite is the so-called 2D band, located around 2700 cm⁻¹ [124,125]. This band arises from a second order,

double-resonance Raman process involving two phonons near the K point of the Brillouin zone and is composed of multiple components.

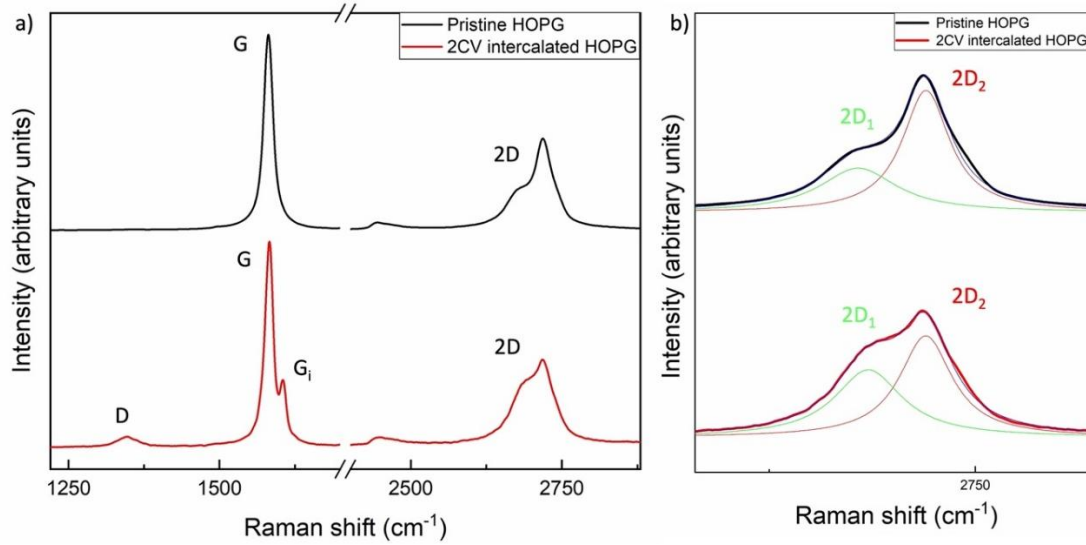


Figure 3.2: **a)** Normalized HOPG Raman spectra, black line pristine HOPG, red line intercalated HOPG. **b)** 2D band detail of spectra presented in panel a, 2 component fitting is performed in order to individuate the two components of the 2D peak, 2D₁ is the component at lower energy shift, 2D₂ is the component at higher energy shift.

For an excitation wavelength of 532 nm, the 2D band in pristine HOPG can be described by two main contributions: a dominant component at 2720 cm⁻¹ and a weaker one at 2680 cm⁻¹, whose intensity is approximately half that of the main peak. Since the 2D band is a resonant feature, its spectral position depends on the excitation wavelength. In the following, the components at 2680 cm⁻¹ and 2720 cm⁻¹ will be referred to as 2D₁ and 2D₂, respectively, with a characteristic intensity ratio 2D₁/2D₂ of about 0.25 in pristine graphite [126,127].

In high-quality, defect-free graphite, the D band, typically observed near 1350 cm⁻¹, ($\lambda = 532$ nm) is absent [128]. This band involves phonons near the K point that are forbidden by selection rules in a perfectly ordered crystal and becomes Raman active only in the presence of structural or chemical defects, such as edges or grain boundaries, which relax momentum conservation [26]. For this reason, the D band is commonly regarded as a fingerprint of disorder. In addition to the intense 2D-related features, the second-order Raman spectrum of graphite may also exhibit weaker bands, such as the signal observed around 2450 cm⁻¹ in the present measurements [53,125,129].

Upon electrochemical intercalation, the Raman spectrum of HOPG undergoes significant modifications. Two additional features emerge. The first is the appearance of the D band at approximately 1350 cm⁻¹, whose intensity relative to

the G band reflects an increased density of defects in the basal plane. After the first cyclic voltammetry cycle, the D band is detected across the entire scanned area, albeit with spatial variations in intensity [51]. A related feature is the D' band, observed near 1620 cm^{-1} , which originates from an intravalley double-resonance process and is likewise associated with defect-induced symmetry breaking [125,126]. In Figure 3.2, the D' band is not clearly visible because it is masked by the presence of the G_i component. However, in regions where the G_i peak is absent, the D' band can be observed, appearing as a shoulder on the high-energy side of the G peak [130].

The second additional feature is the so-called G_i band, appearing around 1605 cm^{-1} , which is commonly correlated with the presence of anions remaining intercalated within the graphite structure [45]. These intercalated species locally modify the interlayer spacing of the HOPG crystal, effectively introducing regions with altered vibrational properties that coexist with pristine graphite [52,124]. The relative intensity of the G_i band with respect to the G band varies across the scanned area, and in some regions the feature is barely detectable, in agreement with previous observations reported by Bussetti et al. [51].

A further notable change induced by intercalation concerns the shape of the 2D band. A fitting analysis of the 2D peak (Figure 3.2) reveals a modification of the relative intensities of its components, with an increase in the $2D_1/2D_2$ intensity ratio after intercalation. This behaviour is not straightforward to interpret, particularly given the pronounced spatial variability of the 2D band shape across the intercalated surface. One contribution to this effect may be linked to the increased intensity of the D band: previous studies have shown that regions with a higher defect density tend to exhibit a reduced intensity of one of the 2D components [131,132]. An additional contribution may arise from the presence of intercalated ions, which locally modify the interlayer spacing and, consequently, the electronic structure of the affected graphene planes, thereby influencing the resonant Raman response [133].

3.3 HOPG blister characterized by AFM

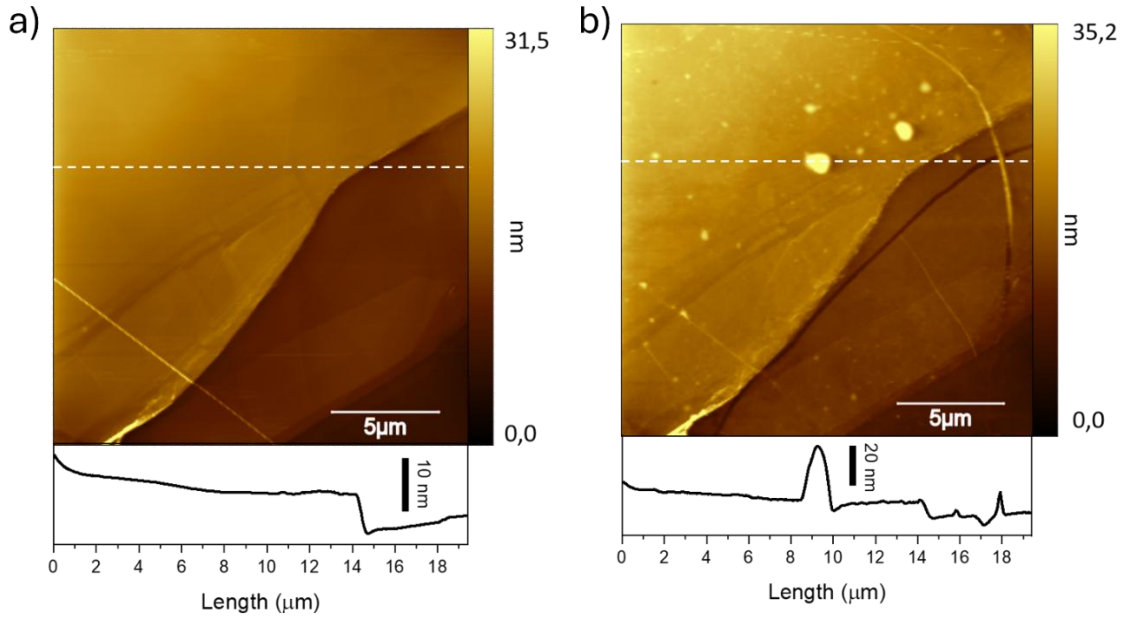


Figure 3.3: **a)** In situ AFM morphology of the pristine HOPG in diluted solution of sulphuric acid. **b)** In situ AFM morphology of the Intercalated HOPG in diluted solution of sulphuric acid, same area of the pristine HOPG.

Figure 3.3 illustrates the evolution of the HOPG surface upon electrochemical intercalation, as observed *in situ* by atomic force microscopy. Panel (a) shows the morphology of pristine HOPG, where the characteristic stepped structure of the basal plane is clearly visible. The prominent linear feature crossing the image near the lower left corner is likely associated with a locally swollen defect generated during the mechanical exfoliation process.

Panel (b) reports the morphology of the same surface region after the cyclic voltammetry procedure. In contrast to the pristine state, the surface now exhibits the appearance of rounded, swollen regions, whose presence is clearly evidenced by the corresponding height profile. These features are identified as blisters formed as a result of the intercalation process. In addition to blister formation, the image also reveals signs of surface degradation, attributable to acid-induced corrosion of the graphite, which leads to partial carbon dissolution and increased surface roughness [10]. Blisters formed under these conditions display a wide range of dimensions: their lateral diameter d typically spans from several hundreds of nanometres up to a few micrometres, while their height h can vary from a few nanometres to approximately 300 nm. In the present work, the analysis is restricted to blisters with diameters larger than 1 μm, which allow for reliable mechanical and spectroscopic characterization.

A comparison between non-swollen regions of the basal plane in panel (b) and the corresponding areas in panel (a) further highlights an increase in surface roughness following electrochemical treatment [47]. This observation indicates that, alongside blister formation, the HOPG surface undergoes additional degradation processes induced by the acidic environment. The image therefore emphasizes that multiple phenomena intercalation, gas evolution, surface swelling, and corrosion occur simultaneously and interact during the electrochemical process, contributing to the final surface morphology [13].

Focusing on the blister itself, Figure 3.4 shows an AFM topographic image of a representative blister with a diameter exceeding $2\ \mu\text{m}$, as determined from the corresponding line profile. Panel (b) presents a schematic cross-sectional view of the blister, analogous to that introduced in Figure 1.3 in the context of the Murray model. The sketch is not drawn to scale and serves only to illustrate the qualitative structural features of the blister. As evident from both panel a and b, blisters may appear isolated or in close proximity to other blisters, but in most of the cases, they partially overlap or coexist at different depths beneath the surface. It is important to notice that the AFM is not capable of detecting possible blister completely below bigger ones, something that led to increase the complexity of the system. In panel b are shown also some steps, characteristic of the pristine HOPG morphology; it is important to notice that steps are swelled together with the layers below them.

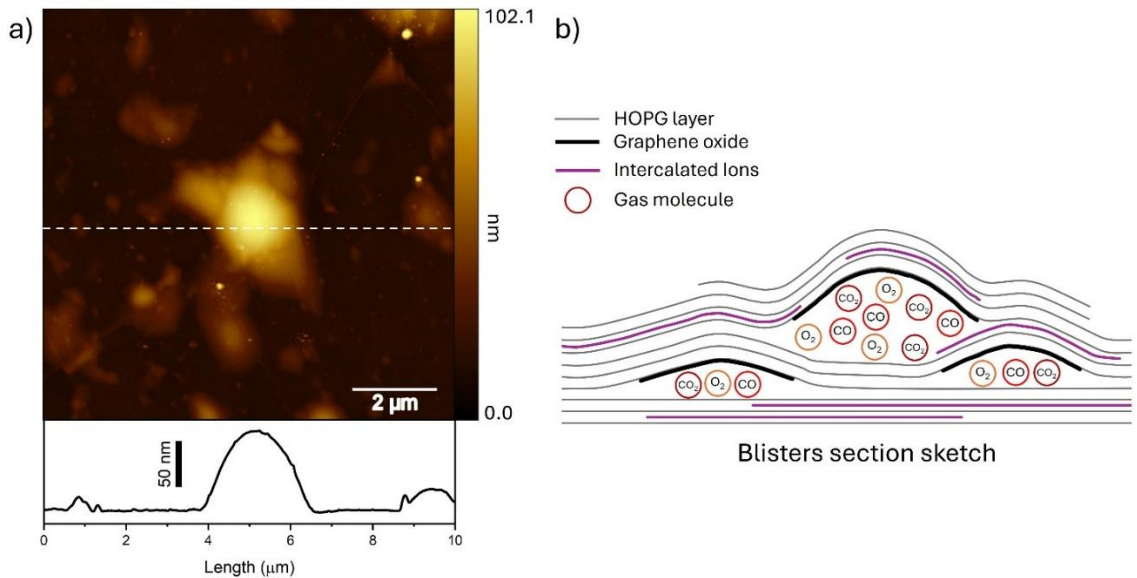


Figure 3.4: a) AFM image of a blister. b) Sketch of a blister section.

From spectroscopic information it is possible to suppose that there are anion intercalates between the swelled layers, together with the one below the blister. According to Murray’s model [13] just below the swelled layers there is formation of graphene oxide.

An important aspect of blister structure concerns the number of graphene layers involved in forming the swollen membrane. Experimental evidence indicates that the blister membrane consists of at least two layers [47], while the exact number of layers participating in the deformation of a single blister has not been unambiguously determined. Based on indirect considerations, Murray estimated that the blister membrane may involve between five and thirteen graphene layers [13]. Throughout this thesis, the term *blister skin* will be used to denote the stack of graphene layers that is lifted and deformed by the gas confined underneath.

3.4 Blister interpretative model

From the discussion presented in the previous section, it emerges that blisters formed in electrochemically intercalated HOPG represent structurally and chemically complex systems. Unlike blisters observed in transferred two-dimensional materials, where delamination typically originates from imperfect adhesion to an external substrate, electrochemically induced blisters arise within a bulk layered crystal as a consequence of intercalation reactions and gas generation between graphite layers. As a result, their structure may involve the coexistence of intercalated ions, locally oxidized regions, pressurised gaseous species, and possible interactions with crystallographic steps.

In order to provide a coherent framework for interpreting the experimental results presented in this thesis, a simplified interpretative model is introduced. The purpose of this model is not to capture the full chemical and structural complexity of the system, but rather to isolate the essential physical ingredients governing blister formation and mechanical response.

In particular, features such as the possible formation of graphene oxide regions or the presence of intercalated ionic species are not explicitly included in the simplified description. These contributions are not excluded from the physical system, but are instead incorporated in an effective manner into the model parameters, such as internal pressure and interlayer adhesion. The model should therefore be regarded as a reduced physical representation aimed at capturing the dominant mechanical behaviour, rather than as a complete chemical description of the blister structure.

Figure 3.5 illustrates this approach. Panel (a) schematically represents a qualitative cross-section of a realistic blister in electrochemically intercalated HOPG, where multiple contributions including intercalated ions, possible graphene oxide regions, and gas species may coexist. Panel (b) shows the simplified model adopted throughout this work. As a first approximation, interactions between neighbouring blisters, as well as the possible presence of underlying or stacked blisters beneath the one under investigation, are neglected. The system is therefore treated as a single, isolated swollen region embedded in an otherwise homogeneous graphite matrix.

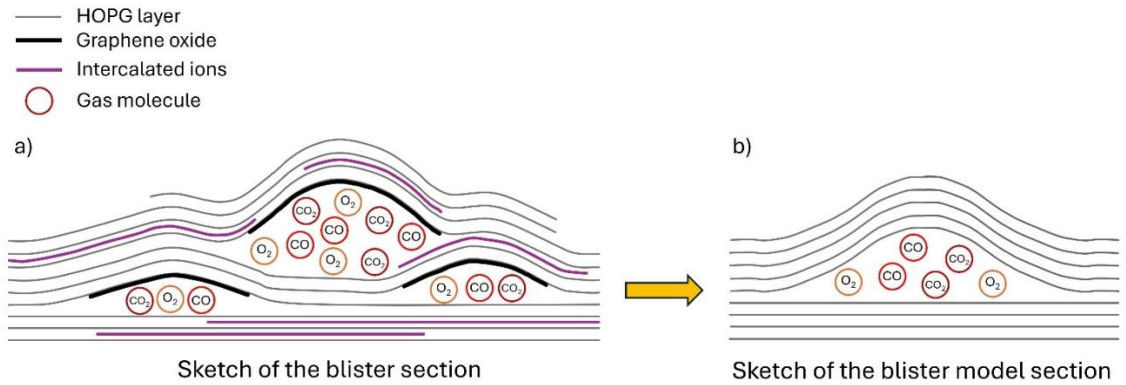


Figure 3.5: a) Sketch of the blister section. b) Sketch of the blister model section.

From a structural and mechanical standpoint, the blister is treated as a composite system consisting of a confined gaseous volume and a partially delaminated multilayer membrane. The latter, referred to hereafter as the *blister skin*, that consists of a finite number of graphene layers (equal to or greater than two), whose collective mechanical response can be described through an effective two-dimensional stiffness associated with their total thickness. The blister skin represents the mechanically active component of the system, capable of storing elastic energy through in-plane stretching and out-of-plane bending, while the bulk graphite beneath it acts as a rigid substrate. This local delamination effectively decouples the blister skin from the bulk mechanical response of graphite, allowing the system to behave as a confined multilayer membrane rather than as a bulk elastic solid.

The swelling of the blister is attributed to the presence of pressurised gaseous species confined beneath the blister skin and generated during the electrochemical intercalation process. The internal gas provides the pressure responsible for deforming the multilayer membrane. The equilibrium geometry of the blister — characterised by its radius and height — is therefore interpreted as the result of a balance between three main physical contributions: the elastic energy stored in the

deformed multilayer membrane, the work performed by the internal gas pressure, and the effective interlayer adhesion energy that tends to restore contact between the lifted layers and the underlying graphite. The geometrical parameters of the blister are thus not arbitrary morphological quantities, but reflect the equilibrium configuration resulting from this energetic balance.

In this perspective, blister nucleation corresponds to a local mechanical instability arising when the internal gas pressure overcomes the effective interlayer adhesion, triggering partial delamination and membrane-like behaviour. Once formed, the blister reaches a stable configuration in which elastic deformation, internal pressure, and adhesion are in mutual equilibrium. Although this energetic balance is not developed quantitatively at this stage, the adhesion energy constitutes a key physical parameter of the system. Its quantitative estimation, together with that of the internal pressure, will be addressed in the concluding part of Chapter 6, where the simplified framework introduced here is formalised within a membrane-based energetic model and compared with the geometrical and spectroscopic parameters determined experimentally.

From a chemical perspective, the simplified model neglects, in a first approximation, both the presence of intercalated ions within the layers forming the blister skin and the possible existence of graphene oxide layers immediately beneath it. Similarly, it is assumed that no crystallographic steps or extended surface defects intersect the blistered region. These assumptions are introduced to construct a physically transparent model that can be directly compared with blisters observed in two-dimensional materials, which provide a useful reference system for interpreting membrane-like behaviour in layered crystals. The validity and limitations of these assumptions will be critically evaluated in the light of the experimental evidence presented in the following chapters.

The assumptions underlying the simplified interpretative model can therefore be summarised as follows:

- the blister consists of a single, isolated swollen region with approximately circular geometry;
- the blister skin is a multilayer graphene membrane composed of two or more layers and mechanically decoupled from the bulk beneath it;
- swelling is driven by pressurised gaseous species confined below the blister skin;
- the equilibrium configuration results from the balance between elastic deformation, internal pressure, and interlayer adhesion;
- no crystallographic steps or extended defects intersect the blistered region;
- no graphene oxide is present beneath the blister skin;

- no intercalated ions are present between the layers forming the blister skin.

The simplified configuration is schematically represented in Figure 3.5b and constitutes the reference framework adopted throughout this thesis for the interpretation of the mechanical, morphological, and spectroscopic results discussed in the following chapters. In this sense, the model does not merely provide a schematic representation, but establishes the physical hypotheses that are subsequently tested and refined through the experimental investigations presented in this work.

3.5 Original contributions of the present work beyond the state of the art

Building upon the simplified interpretative framework introduced in §3.4, the present work advances beyond the existing literature by treating blisters in electrochemically intercalated HOPG not merely as morphological surface features, but as confined and mechanically active multilayer systems whose behaviour can be quantitatively probed across multiple length scales.

While previous studies have primarily focused on the electrochemical response or on the morphological appearance of blisters, this thesis establishes a direct and systematic connection between blister morphology, mechanical response, and vibrational properties. The original contributions of the present work can be summarised as follows:

- **Direct mechanical quantification of individual blisters.** Through AFM-based nanoindentation experiments, blisters are experimentally demonstrated to behave as pressurised multilayer elastic membranes. This approach enables the extraction of their effective mechanical response and provides, for the first time in this specific system, a quantitative mechanical description that goes beyond purely morphological observation.
- **Exploration of the mechanical stability beyond the linear elastic regime.**

By applying high normal pressures and controlled lateral displacements via the AFM tip, the present work probes the response of blisters under extreme mechanical loading conditions. These experiments reveal collapse phenomena, controlled incision processes, and deformation reversibility, thereby clarifying the elastic nature of blister swelling and the structural integrity of the underlying graphite lattice.

- **Multimodal spatially resolved characterization of intercalated HOPG.**

The combined use of AFM, Raman spectroscopy, and optical microscopy provides a correlated description of the morphological, optical, and spectroscopic modifications induced by electrochemical intercalation. This integrated approach establishes a robust experimental reference framework for interpreting the mechanical and vibrational behaviour associated with blister formation.

- **Quantitative correlation between local strain and Raman response.**

The present work demonstrates a direct relationship between the strain field generated by blister swelling and the frequency shifts and intensity variations of the strain-sensitive Raman bands, in particular the G and 2D modes. By correlating AFM-derived morphology with local spectral variations, Raman spectroscopy is established as a quantitative probe of the mechanical state of the graphene layers forming the blister skin.

- **Nanoscale investigation through tip-enhanced Raman spectroscopy (TERS).**

The application of TERS to HOPG blisters enables access to the nanoscale spatial distribution of strain and vibrational response, overcoming the spatial averaging intrinsic to far-field Raman spectroscopy. This approach reveals local heterogeneities within individual blisters that cannot be resolved by conventional optical techniques.

- **Experimental demonstration of the absence of defect generation during blister formation and manipulation.**

- Raman and TERS measurements consistently show the absence of defect-related bands following blister swelling and mechanical deformation. This finding clarifies that blisters represent mechanically deformed yet crystallographically intact regions, rather than defect-mediated structural damage.

- **Critical validation and refinement of the blister interpretative model.**

By quantitatively comparing the assumptions underlying the simplified model introduced in section 3.4 with the experimental results presented in the subsequent chapters, this thesis defines the validity range of the model and refines its physical interpretation, thereby providing a more rigorous description of blisters in bulk layered graphite.

4

Mechanical

Characterization by

Nanoindentation and

Controlled Manipulation

As discussed in the introductory chapter, the overarching objective of this thesis is to investigate and characterize HOPG blisters as individual micro- and nanostructures, with particular emphasis on their local mechanical behaviour. In order to achieve this goal, I employed atomic force microscopy (AFM) not solely as a tool for traditional morphological imaging, but rather as a multifaceted platform capable of probing the mechanical response of the blistered surface through complementary operational modes. All measurements presented in this chapter were performed in situ in 1M H_2SO_4 solution, corresponding to the electrochemical conditions under which blister formation occurs. In this way, it was possible to reveal mechanical properties that had not been previously quantified for these systems.

Although AFM is widely recognized for its capability to reconstruct surface topography with nanometric precision, the technique offers a much broader potential. Depending on the mode of operation, AFM can be exploited to measure a variety of mechanical descriptors or, alternatively, to mechanically perturb the sample surface in a controlled manner.

In particular, Force–Distance measurements (or force–indentation curves) provide access to a detailed description of the local mechanical response. By analysing the balance between attractive and repulsive interactions occurring at the tip–sample interface, one can extract parameters such as adhesion, elasticity (expressed via the Young’s modulus), hardness, and viscoelastic behaviour. These

measurements yield a true nanoscale “mechanical fingerprint” of the material, enabling the identification of subtle variations that would remain undetectable through conventional bulk-averaged techniques.

Conversely, by deliberately exploiting the mechanical interaction between the cantilever tip and the surface, AFM can also be used to modify the sample in a controlled fashion. This second approach allows one to observe, *in situ*, how the blistered graphite layers respond to mechanical stress, thereby providing insight into their stability, internal pressure, and ability to deform or fail under load.

In this chapter, I present the results obtained through these two complementary approaches—mechanical indentation and controlled manipulation—applied to the study of HOPG blisters. More specifically:

1. **Nanoindentation measurements** were carried out to determine the effective Young’s modulus of the blisters and to investigate whether a correlation exists between their average stiffness and the number of cyclic voltammetry (CV) cycles used to generate them.

2. **Mechanical manipulation experiments** were performed to perturb the blister surface by applying high normal forces and inducing relative motion between the tip and the sample. The resulting deformation, displacement, or rupture of the blister provided additional information about its mechanical integrity and internal structure.

Both sets of experiments were designed in the framework of the model presented in section 3.4, which describes HOPG blisters as locally delaminated graphite layers inflated by pressurized gas trapped beneath the surface. The results presented in this chapter not only corroborate the main assumptions of this model but also provide new insights into the elastic behaviour, structural stability, and mechanical evolution of blisters formed through electrochemical intercalation.

4.1 Nanoindentation measurements

As described in Chapter 2, section 2, AFM operated in contact mode relies on the continuous detection of cantilever deflection, which serves as the input parameter for the feedback loop regulating the scanner movement along the Z-axis. By treating the cantilever as an elastic spring, the measured deflection can be converted into an estimate of the tip–sample interaction force, with an overall uncertainty of approximately 30%, as previously discussed. This approach enables

the acquisition of quantitative nanoindentation data at selected locations across the investigated area.

Building on this principle, nanoindentation measurements can be performed on different regions of the sample in order to extract local mechanical properties. This methodology has already been applied to electrochemically intercalated HOPG, where it has proven particularly effective for evaluating the stiffness of blistered domains. Figure 4.1 illustrates, through a schematic representation, the interaction between the AFM tip and the blister surface during indentation. Panel (b) reports an example of a force-indentation curve acquired on a blister, which serves as the basis for the mechanical analysis presented in the following sections.

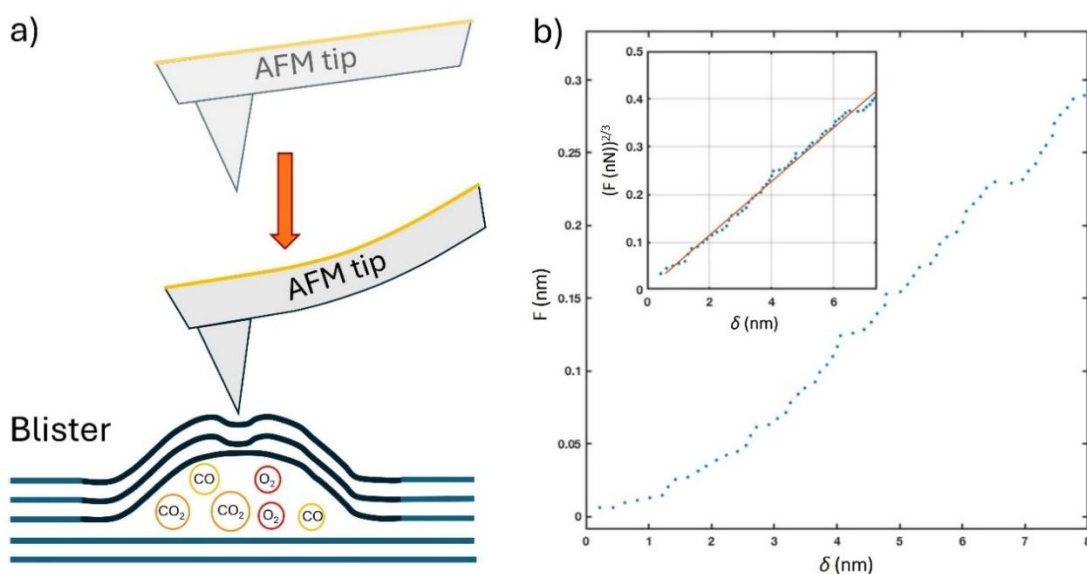


Figure 4.1: a) Scheme not in scale of the nanoindentation on blister, the cantilever is deflected respect is rest position, the blister surface undergoes a small indentation b) An example of the indentation curve on a blister surface [108].

Several hundred raw curves of cantilever deflection (expressed in volts) versus z -piezo displacement (in nanometres) were collected on different blisters, typically three to five for each analysed sample. An example of such a dataset is shown in panel (e). The blisters investigated in this study were identified and imaged *in situ* on the graphite surface during the cyclic voltammetry (CV) procedure, with each blister corresponding to a specific number of electrochemical cycles.

Following acquisition, the raw deflection–displacement curves were converted into force–indentation plots, expressed as force F (in nN) versus indentation depth δ (in nm), by applying the standard calibration protocols described in Ref. [20]. The resulting F – δ curves were subsequently fitted using the Hertz contact model [134–136], reported in equation 4.1, in order to determine the effective Young’s

modulus E of each blister. This modulus reflects not only the intrinsic elastic response of the graphitic shell but also the mechanical contribution arising from the internal gas pressure confined beneath the delaminated layers.

$$F = \frac{4}{3} \frac{\sqrt{RE}}{(1-\nu^2)} \delta^{3/2} \quad (4.1)$$

In Eq. 4.1, R is the tip radius and ν is the Poisson's ratio.

For all datasets, the tip radius R was fixed at 10 nm. This value was selected based on the nominal tip radius specified by the manufacturer (7 nm) and by accounting for a moderate increase due to tip wear occurring during repeated indentation and manipulation measurements. The adopted value therefore represents a realistic effective radius under experimental conditions. The Poisson ratio ν was set to 0.5, a value commonly adopted for materials exhibiting an incompressible elastic response. Finite-thickness corrections [134] were not included in the analysis, as the indentation depths involved were considerably smaller than the total blister height. Although this approximation may introduce a systematic deviation in the absolute value of the extracted Young's modulus estimated to be on the order of 20–40% it remains justified for the purpose of comparing different blisters under consistent experimental conditions. More sophisticated fitting approaches based on thin-shell mechanics [2] could in principle be employed; however, the Hertz model was preferred due to its analytical simplicity and its ability to provide a reliable effective modulus that reflects the overall elastic behaviour of the blister.

Using this framework, the effective Young's modulus of blisters formed after progressively increasing numbers of CV cycles was quantified. This modulus represents the resistance of the blister to normal compression and incorporates both the intrinsic stiffness of the graphitic shell and the contribution from the internal gas pressure trapped beneath the delaminated layers. The logarithmic distributions of the extracted moduli, corresponding to blisters generated after 1 to 16 CV cycles, are shown in Figure 4.2. Overall, the modulus values fall within the range of approximately 1 to 10 MPa. The distributions are rather broad, which can be attributed to height variations among blisters; since the shell curvature and its mechanical coupling with the underlying basal plane change with blister height, the measured stiffness is expected to vary accordingly.

A clear stiffening trend is observed for blisters formed between 1 and 4 CV cycles, as evidenced by the shift of the dominant peak toward higher modulus values. Blisters obtained after 2 CVs appear to represent a transitional regime in

which signatures of both low and high stiffness coexist. The distributions corresponding to 4 and 8 CVs are particularly broad, displaying extended tails toward higher modulus values, indicative of a growing heterogeneity in blister structure and internal pressure.

Previous studies have shown that blisters produced after only a few electrochemical cycles tend to be isolated, suggesting that the number of anions intercalated within the graphite galleries is initially low.

As the number of CV cycles increases, several phenomena occur simultaneously: (i) the faradaic current exhibits a slight decrease, implying that part of the intercalated anions remain confined within the graphite rather than being expelled during the quasi-reversible process [10]; (ii) the number of blisters rises, and the basal plane begins to show signs of structural degradation [10]; (iii) deeper graphite planes start to participate in the blistering process [15,16].

With further cycling, both the average blister size and the number of graphite layers involved increase, leading to a larger population of trapped anions within the structure. This, in turn, suggests that a greater amount of gas is produced through electrochemical reactions involving these confined species. The combined effect of increased intercalant retention, expanding blister volume, and deeper structural involvement provides a coherent explanation for the progressive stiffening observed in Figure 4.2. Finally, it is worth noting that the system appears to approach a saturation regime. After approximately 4 to 6 CV cycles, the stiffness distributions tend to stabilise between 2 and 4 MPa, indicating that the near-surface region reaches a mechanically steady swollen state in which additional cycling induces only modest variations in blister rigidity.

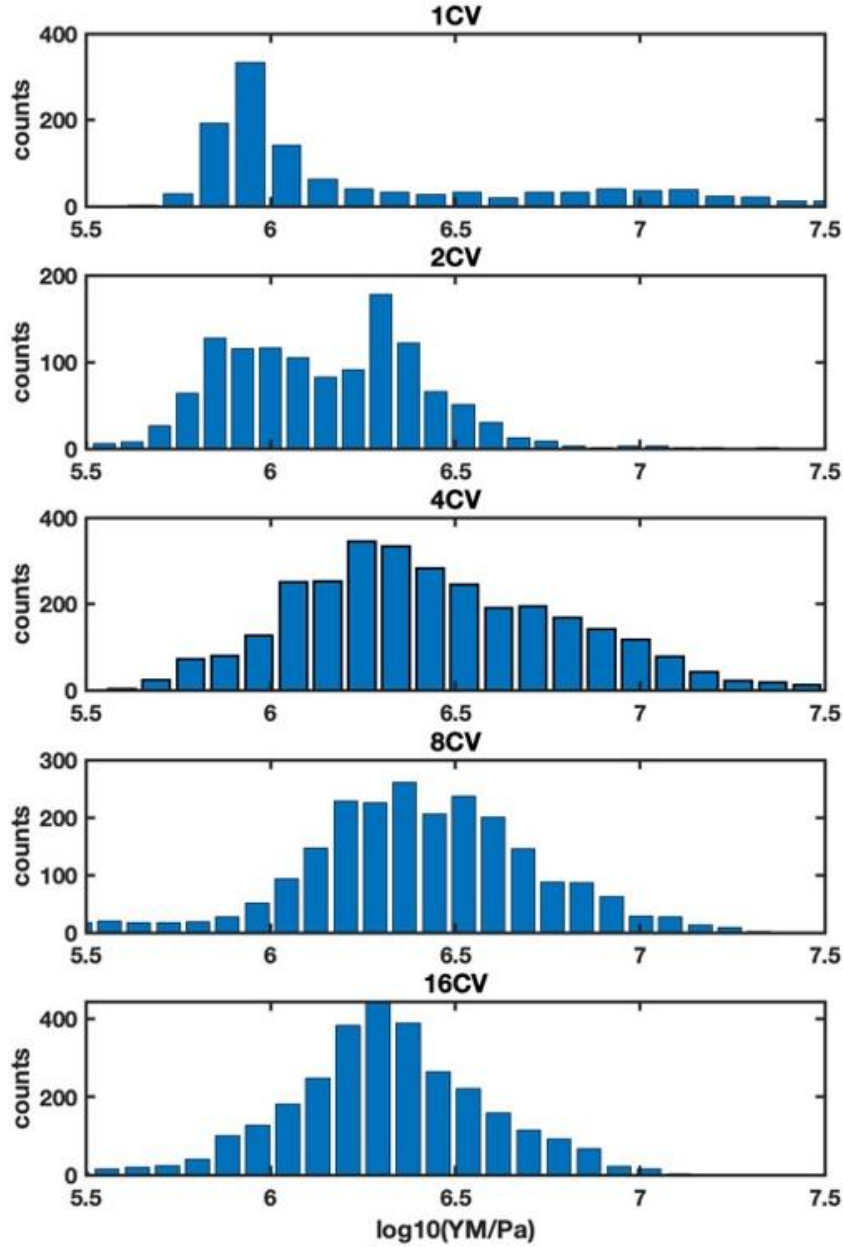


Figure 4.2: Distributions of Young's modulus values measured on blisters obtained after a different number of CVs. Measurements performed and analyzed by Lorenzo Marfori and Alessandro Podestà (Università Statale di Milano) [108].

4.2 Mechanical manipulation on Blisters

When a sufficiently high normal force is applied between the AFM tip and the basal plane, it becomes possible to mechanically manipulate the position or shape of a blister. The experimental procedure consists in scanning the tip across the blister from one side to the other while progressively increasing the applied load, as illustrated in Figure 4.3. Within the framework of Murray's model, blisters are interpreted as locally swollen regions of the graphite surface, produced by gas trapped beneath a delaminated graphitic shell. Under this interpretation, it is

reasonable to expect that such structures can be displaced, deformed, or otherwise perturbed through controlled mechanical interaction with the AFM tip.

A particularly striking outcome of this approach is that the blister can indeed be translated across the HOPG basal plane and, when subjected to even higher forces, can be mechanically ruptured. An intuitive analogy may help contextualize this behaviour: the manipulation resembles the way one might use a fingertip to push an air bubble trapped beneath a protective film on a phone screen, or how a blade can be used to intentionally puncture it. In an analogous manner, the AFM tip behaves either as a gentle pusher or as a cutting tool depending solely on the magnitude of the applied force.

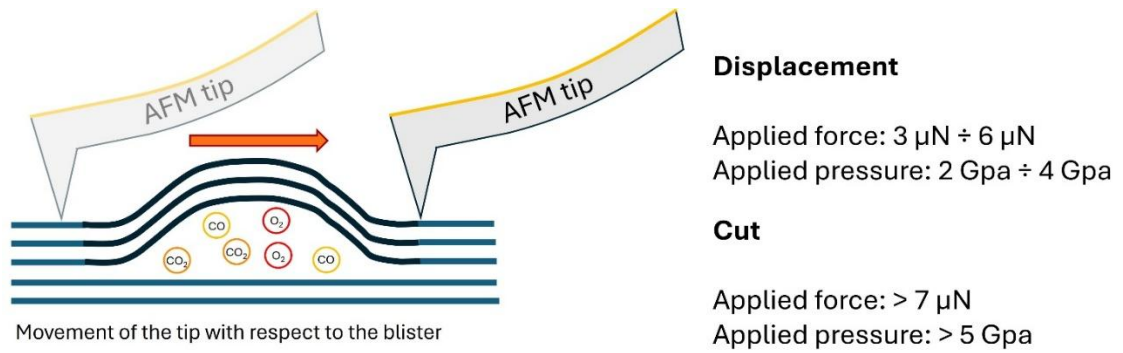


Figure 4.3: Scheme not in scale of the relative movement between the tip and the blister, in the right part the value of the force and the pressure between the tip and the basal plane before the relative movement between the tip and the blister.

To perform this measurement I used chips with an average elastic constant of 26 N/m , calibrate using thermal noise method. The shape of the tip respect the cantilever is not the common one but the tip is positioned at the apex of the cantilever, like shown in the scheme in Figure 4.3. Tip radius of the pristine tip is below 10 nm .

As done for the nanoindentation measurements, is it possible from the detection of the DFL signal to estimate the force between the tip and the sample. The estimate applied forces are:

- Between $3 \mu\text{N}$ and $6 \mu\text{N}$, for the displacement of the blister.
- Higher than $7 \mu\text{N}$, it is possible to obtain the cutting.

Knowing the force, is it possible to estimate the applied pressure by the tip. With a tip radius of 20 nm , is it possible to calculate the contact area between the tip and the sample. Dividing the force by the contact area we obtain the following applied pressure:

- Estimated applied pressure for the displacement 2 GPa and 4 GPa

- Estimated applied pressure for the cutting > 5 GPa.

To perform the mechanical manipulation experiments, the AFM was operated in contact mode with the feedback-loop gain set to zero. This configuration ensures that the instrument does not retract or compensate when the tip encounters the blister surface, thereby maintaining a constant and fully controlled mechanical interaction. Both for lateral displacement and for cutting, the tip movement was regulated by imposing a rectangular scan pattern with a width of approximately 10 nm and a length of about 10 μm (the latter adjusted according to the blister dimensions). The scan consisted of a single line with a resolution of 1×512 pixels, resulting in one forward and one backward pass directly across the blister. The scan direction was chosen to be parallel to the cantilever axis in order to maximize mechanical stability and ensure reproducible force application.

A scan rate of 10 Hz was selected to produce a relatively rapid relative motion between the tip and the blister. This high-speed interaction enhances the mechanical impact during each pass and facilitates both displacement and rupture when sufficiently high forces are applied.

The samples used for these experiments were prepared via two cyclic voltammetry (CV) cycles, performed between 300 mV and 1300 mV at a scan rate of 25 mV/s, in a 1 M H_2SO_4 electrolyte solution. These electrochemical conditions reliably generate a sufficient density of blisters on the HOPG basal plane, thus providing an adequate number of structures suitable for manipulation.

In Figure 4.4, the results obtained *in liquid* are presented as a sequence of images illustrating the mechanical displacement of two neighbouring blisters. Panel (a) shows the initial configuration of the double blister prior to manipulation. When the AFM tip is scanned forward and backward along the trajectory indicated by the white arrows, the entire swollen region is progressively displaced toward the right-hand side of the image, as clearly visible in panel (c). The dashed white lines serve as a reference to highlight the magnitude of the displacement. A second scan induces an additional shift (panel e), and after a third pass, reported in panel (g), the blisters reach their final position. In panels (e) and (g), it can also be observed that the larger blister incorporates the smaller one, suggesting that the two structures physically merge during the mechanical perturbation. Importantly, the smaller blister located in the upper-right corner remains unchanged throughout the process, demonstrating that the manipulation is highly localized and does not perturb the surrounding basal plane.

By comparing the blister volume before and after the manipulation, a value of approximately $(5.2 \pm 0.2) \mu\text{m}^3$ is obtained in both cases, within experimental error. This observation suggests that one blister may have been absorbed into the other during the manipulation, providing strong evidence for the mechanical mobility and fluidity of blister structures on the graphite surface. The evolution of the swollen region is clearly corroborated by the phase-contrast images shown in panels (b), (d), (f), and (h), which offer additional insight into how the basal plane responds during the mechanical event.

While blisters exhibit a distinct phase-contrast signature relative to the unperturbed basal plane, the graphite surface exposed after blister displacement shows a phase response indistinguishable from that of the pristine regions. This indicates that the graphitic layers forming the blister shell are able to relax and reattach to the basal plane without leaving detectable morphological or mechanical traces, further supporting the interpretation of blister deformation as a fully reversible elastic process.

As discussed previously, increasing the applied load does not merely allow the blister to be displaced but also enables its mechanical rupture. The experimental parameters used for cutting are identical to those employed during lateral manipulation, with the sole difference being the significantly higher normal pressure applied by the AFM tip. Figures 4.5 and 4.6 summarise the results obtained from cutting experiments conducted on three representative blisters.

In panels (a), (b), and (c), the cutting trajectory is indicated by a green dashed line, marking the precise path along which the tip was scanned with sufficient force to penetrate the blister shell. The effectiveness of the procedure is confirmed by the shallow incision visible in panels (d), (e), and (f). This trench appears as a narrow, elongated depression aligned with the tip trajectory. Minor irregularities along the cross-sectional profiles, shown at the bottom of each panel, likely stem from small fluctuations in the tip-sample interaction during the high-force scan.

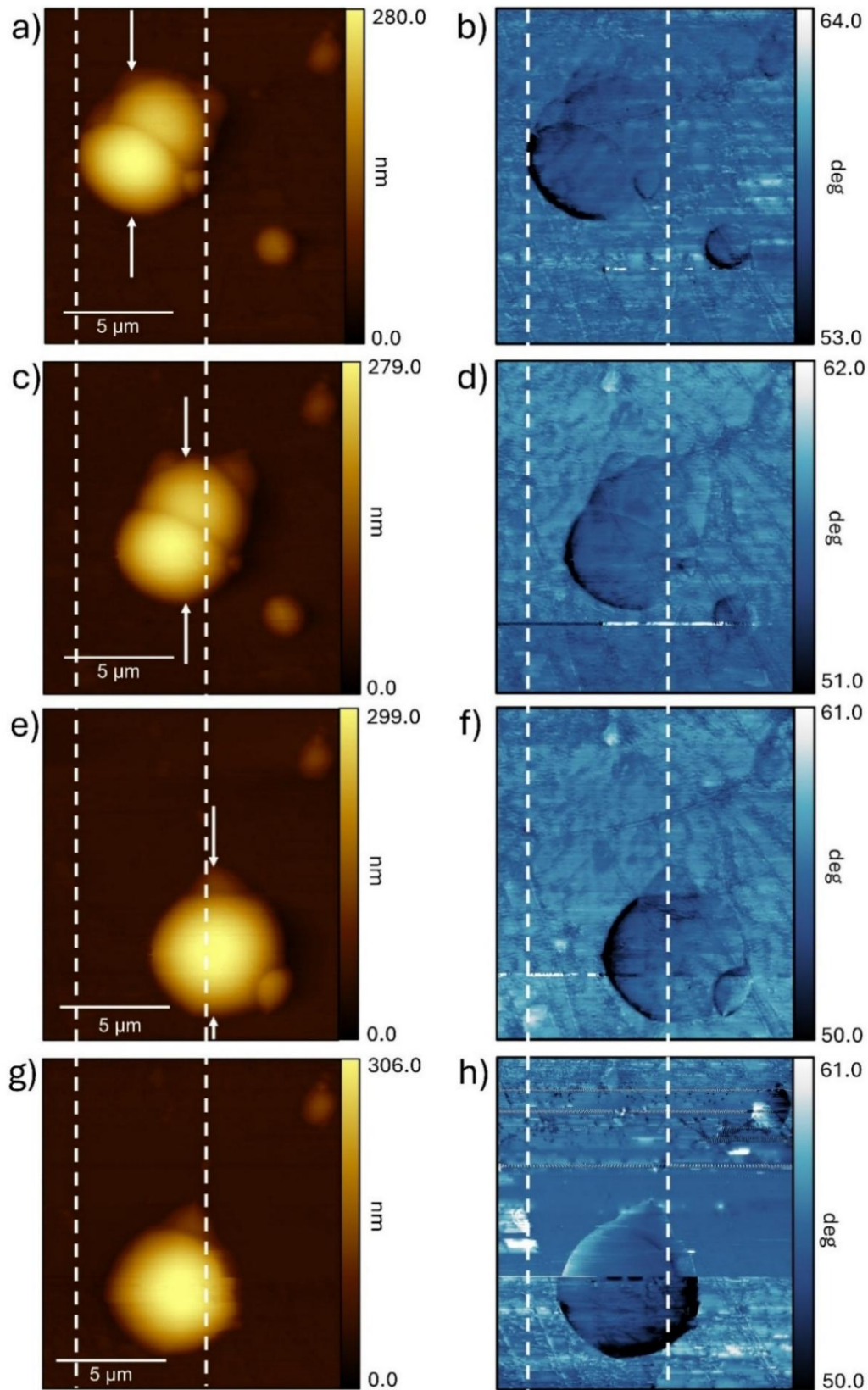


Figure 4.4: Subsequent topography images (panels **a**, **c**, **e**, **g**), acquired in situ and in non-contact mode, of a double blister, moved by the AFM tip. The white arrows highlight the AFM tip path used to obtain the mechanical movement of the blister. The white dash lines are a guide for the eyes to better observe the blister movement. The correspondent phase-contrast images are reported in panels **b**, **d**, **f**, **h**.

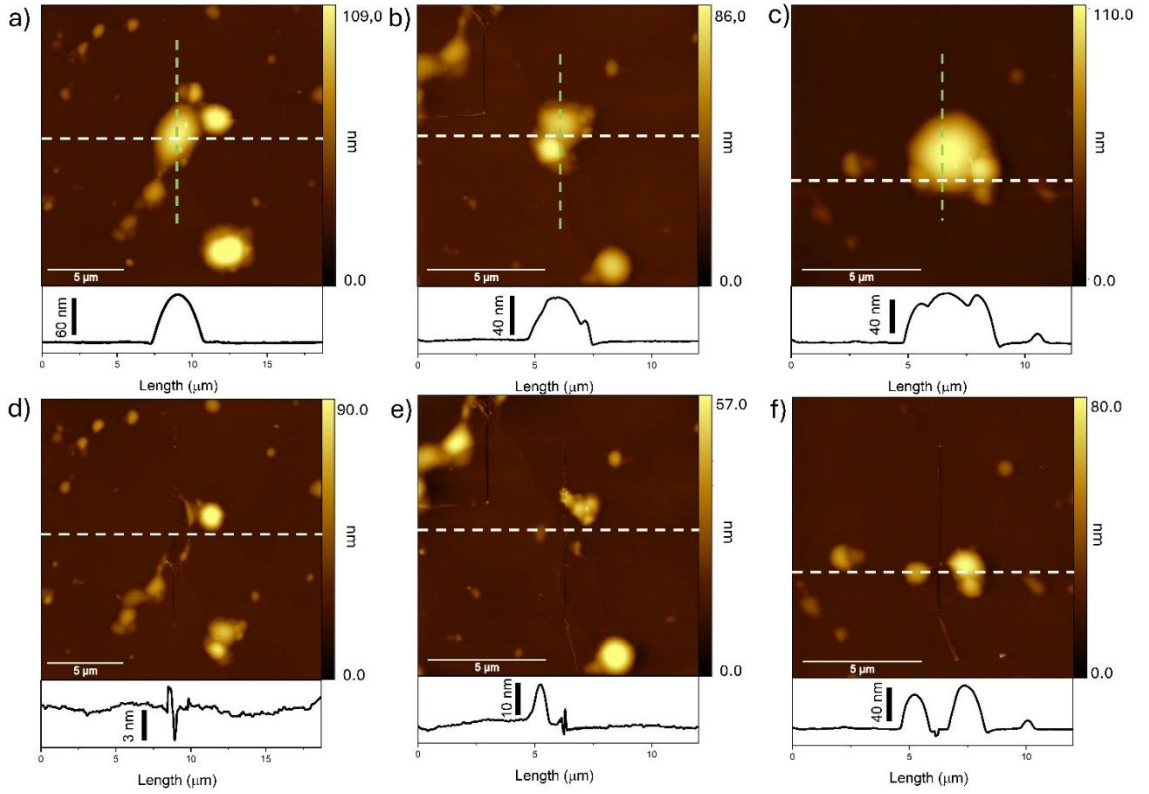


Figure 4.5: Topography images, acquired in situ and in non-contact mode, of three different blisters; respectively before (a, b, c) and after (d, e, f) their cut. The green vertical dash line evidences the path of the tip during the cut. The white dash line is in correspondence of the cross-section, reported at the bottom of each panel.

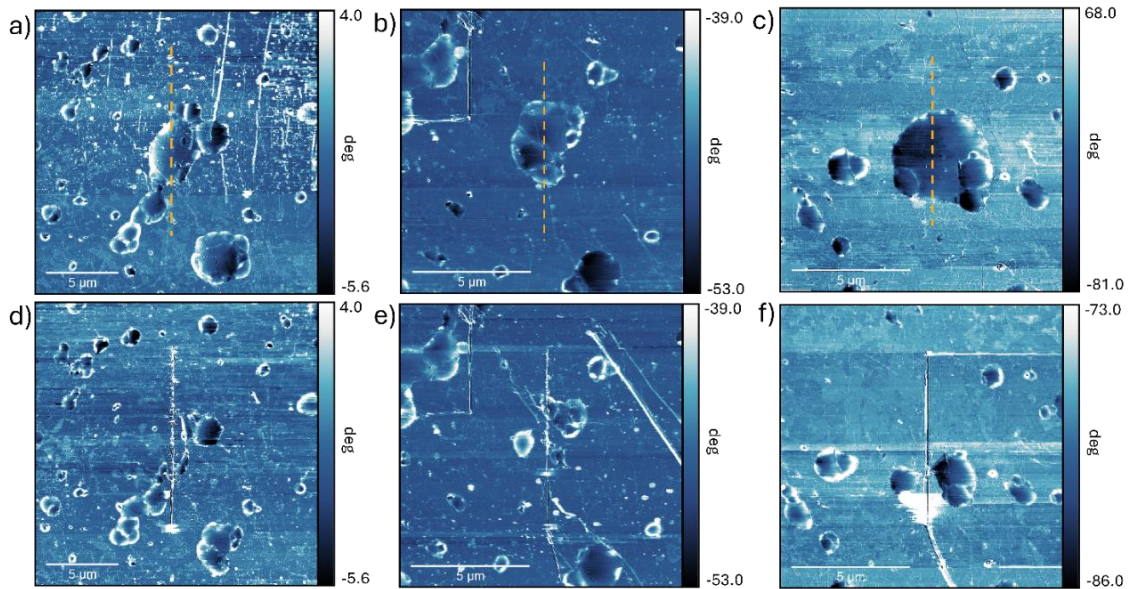


Figure 4.6: Phase-contrast images of three different blisters before (a, b, c) and after the cut (d, e, f). The vertical green dash line highlights the position of the cut.

Following the cutting operation, the swollen region collapses completely. The blister vanishes, leaving behind only small, irregular fragments that correspond to

remnants of the former graphitic shell. The incision created by the tip, which typically ranges between 5 and 10 nm in depth, demonstrates that the cutting process is intrusive yet remains confined to a shallow region of the surface. The remaining fragments can be interpreted as the collapsed edges of the blister, which lose their inflated configuration once the internal gas is released. Part of this material is displaced toward the end of the cutting path, as particularly evident in panels (e) and (f).

From these observations, it becomes evident that the blistered region undergoes complete mechanical relaxation once the shell is ruptured. The area that was previously swollen reattaches seamlessly to the basal plane, recovering its initial flat morphology without displaying measurable discontinuities or height mismatches. This behaviour demonstrates that the graphite layers forming the blister are highly flexible and capable of returning to their equilibrium configuration once the internal pressure is removed. The collapse mechanism closely resembles that of a gas-filled bubble: once the thin shell is punctured, the trapped gas escapes and the structure collapses, deflating in a manner analogous to a soap bubble being pierced.

This interpretation is further supported by the analysis of the phase-contrast images shown in Figure 4.6, where it is reported the phase signal of the same AFM morphologies reported in Figure 4.5. Before rupture, the blister exhibits a distinct phase signal relative to the surrounding basal plane, reflecting a local variation in stiffness due to the internal pressure. After cutting, this contrast disappears entirely, and the deflated region becomes indistinguishable from the unperturbed graphite surface. Since the AFM phase signal is highly sensitive to local variations in elastic response, the disappearance of the contrast indicates that the material fully regains the stiffness characteristic of the pristine basal plane.

A more detailed analysis of the basal plane before and after cutting, as shown in the saturated images of Figure 4.7, provides additional insight. First, the monoatomic steps visible in panels (a) and (b), which maintain their orientation and continuity after the cut. This demonstrates that the gases trapped below the steps do not escape laterally but through the step, and that the mechanical perturbation remains confined to the blistered region. Second, neighbouring blisters often belonging to the same cluster—retain their shape, position, and apparent volume, confirming that the cutting process is highly localized and does not influence adjacent structures. Finally, small monoatomic domains and surface defects created during the electrochemical intercalation remain continuous across the area where the blister previously existed, indicating that the surface reattaches

smoothly without forming voids or structural discontinuities. This is really evident in Figure 4.7 panel c, if is observed the upper part of the old blister skin, thanks to the saturate colour bar, is it possible to appreciate the domain continuity, without showing any trace of the previous swelling deformation.

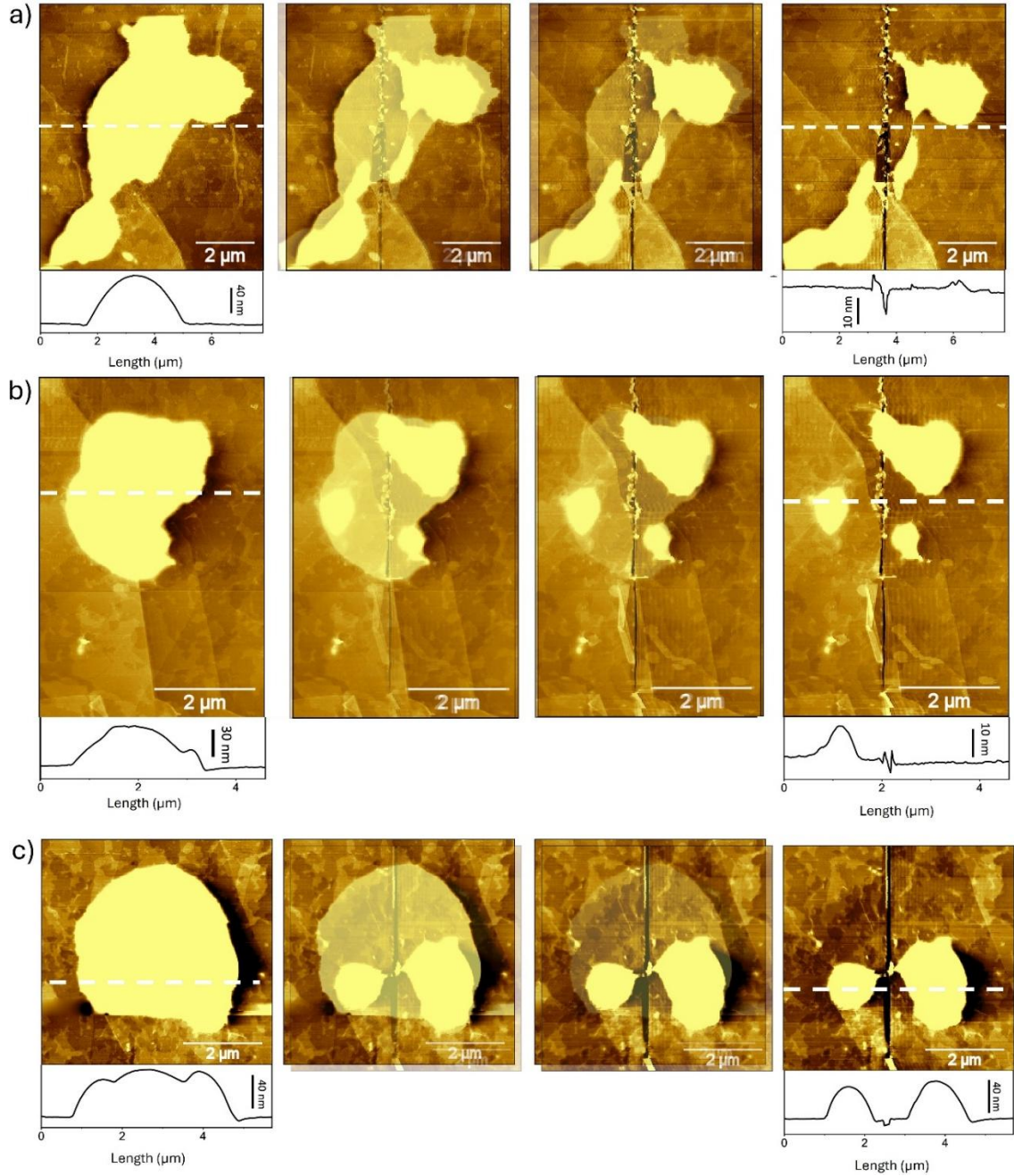


Figure 4.7: Superposition of AFM topography images highlighting the consequences of blister cutting. In panels (a–c), magnified details of the blisters previously shown in Figure 4.5 are overlaid before and after incision; the shaded regions represent the original swollen morphology. The images show that, once the blister is removed, the basal plane does not retain any detectable trace of the prior deformation. The dashed white lines indicate the positions of the cross-sections reported below each panel.

Taken together, the results presented in section 4.1 and 4.2, provide strong experimental evidence that blister deformation in HOPG is predominantly elastic in nature. The internal pressure generated during intercalation causes a reversible bending of the upper graphitic layers rather than permanent delamination or structural damage. Once the internal gas is released through mechanical rupture, the layers collapse and adhere again to the underlying basal plane, restoring their initial morphology and mechanical characteristics. Overall, the process can be interpreted as the elastic response of a layered material to a confined internal pressure, with the AFM tip acting as a precise nanoscale tool capable of probing, modifying, and ultimately relieving this stress in a controlled and reproducible manner.

4.3 Raman analysis of mechanically collapsed blister regions

As a final consideration regarding the mechanical manipulation experiments, it is relevant to verify whether the structural modifications induced by blister cutting can be detected from a spectroscopic point of view. While AFM provides direct information on surface morphology and mechanical response, Raman spectroscopy allows one to probe possible modifications of the vibrational properties of the graphite lattice following the collapse of the blister.

Figure 4.8 shows a large-area AFM image acquired in air after the mechanical cutting of a blister. Compared to the corresponding image acquired in liquid, residual products of the intercalation process can be clearly observed on the surface. The region corresponding to the former blister is highlighted by a continuous contour in all panels. As indicated by the height profile, the incision produced by the AFM tip has a depth of approximately 6–7 nm and a lateral extension of about 500 nm.

In order to assess whether the cutting operation induces detectable structural or vibrational modifications, Raman mapping was performed on the same area investigated by AFM. Three quantities were analysed: the intensity of the G band (Figure 4.8b), the spatial variation of its peak position (Figure 4.8c), and the intensity of the D band (Figure 4.8d).

The G-band intensity map reveals that the signal is not uniform across the entire analysed area. The origin of these fluctuations will be discussed in detail in the following chapter. At this stage, however, the relevant observation is that, when

the region corresponding to the former blister is compared with neighbouring areas of the basal plane, no significant difference in G-band intensity is observed.

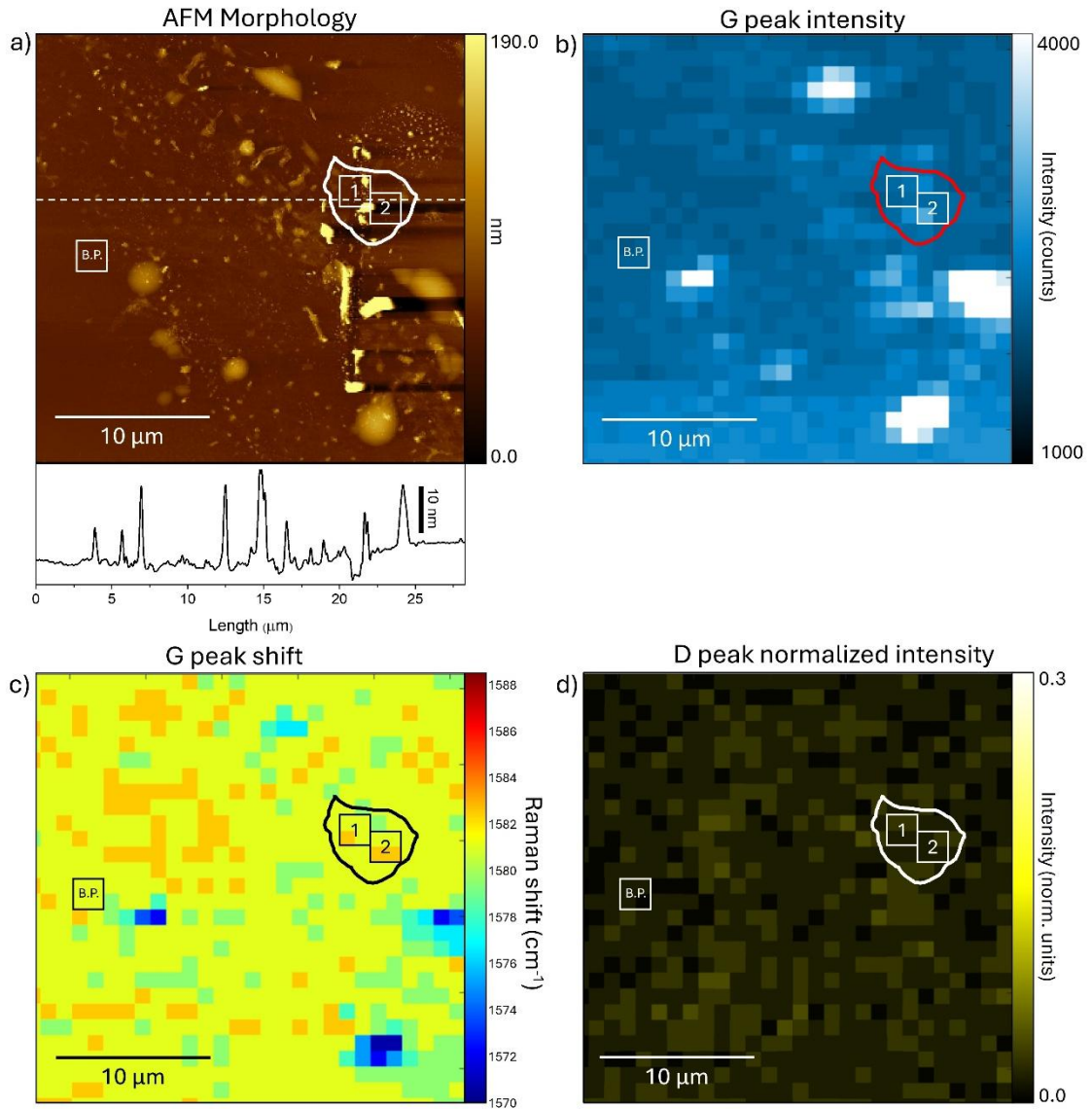


Figure 4.8: AFM and Raman analysis of a mechanically cut blister region (air). **a)** Large-area AFM topography showing the incision produced by the AFM tip; the contour of the former blister is indicated. **b)** Raman G-band intensity map. **c)** Spatial map of the G-band peak position. **d)** Raman D-band intensity map.

Although local variations are present throughout the map, the former blister region does not display any distinct contrast relative to the surrounding graphite surface.

A similar conclusion can be drawn from the analysis of the G-band peak position. Small spatial variations are observed across the mapped area, indicating that the surface is not perfectly homogeneous. Nevertheless, when the region of the former blister is directly compared with representative areas of the basal plane, the

peak position remains unchanged within experimental uncertainty. No measurable spectroscopic signature associated with the previous blister swelling is therefore detected after mechanical collapse.

Particular attention was devoted to the D band, which is commonly associated with lattice defects. The D-band intensity is negligible across the entire analysed area and remains indistinguishable from the noise level, not only on the basal plane but also within the region of the former blister and along the incision line itself. This observation is especially noteworthy, as the mechanical cutting operation constitutes an artificial perturbation of the surface. The absence of a detectable D-band enhancement indicates that the procedure does not introduce a measurable increase in defect density at the atomic scale, thereby confirming the highly localized and controlled nature of the mechanical incision. Moreover, the elevated topographic features attributed to intercalation residues do not exhibit any detectable Raman signature, further supporting their interpretation as adsorbed by-products rather than structural defects of the graphite lattice.

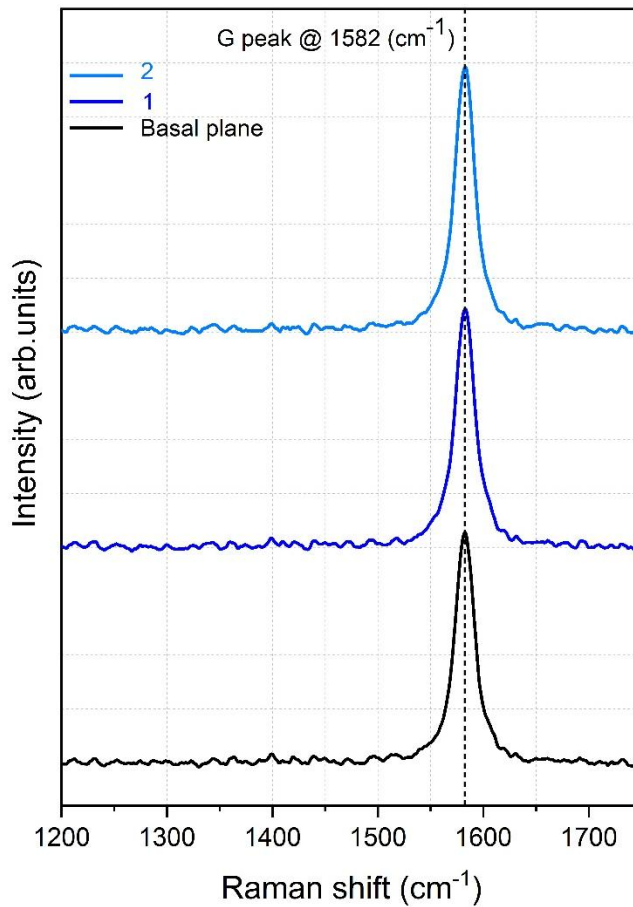


Figure 4.9: Representative Raman spectra acquired at positions 1 and 2 within the former blister region and on the basal plane (see Figure 4.8 for location).

To further substantiate these observations, representative Raman spectra acquired at the positions marked as 1 and 2 within the former blister region are compared with a spectrum obtained from the basal plane, (e.g. b.p), as shown in Figure 4.9. The spectra confirm the conclusions drawn from the maps: the G-band intensity and peak position are essentially identical within experimental resolution, and no D-band contribution is observed in any of the analysed points. The vibrational response of the region previously occupied by the blister is therefore indistinguishable from that of the surrounding basal plane.

Taken together, these results demonstrate that the graphene layers forming the blister skin collapse onto the underlying graphite without leaving a spectroscopically detectable trace. Neither defect-related features nor persistent modifications of the vibrational response are observed after the mechanical manipulation, in full agreement with the AFM analysis presented above. The collapsed region does not retain any apparent “memory” of the previous swelling state.

It is worth noting that this behaviour contrasts with the spectroscopic response observed in regions where intact blisters are present. As will be discussed in detail in Chapter 5, significant and systematic variations of the Raman response are detected in swollen regions, in particular affecting both the intensity of the G band and its peak position. The absence of such variations in the mechanically collapsed area further supports the conclusion that the blister skin re-adheres to the underlying graphite layers in a reversible manner, restoring a vibrational response equivalent to that of the pristine basal plane.

The physical origin of the Raman variations observed in swollen blisters, and their quantitative interpretation in terms of local mechanical modifications, will be addressed in the following chapter.

The mechanical measurements presented in this chapter demonstrate that HOPG blisters behave as mechanically compliant and confined systems, whose response is governed by elastic deformation and internal pressure. The AFM manipulation experiments further indicate that the deformation of the blister skin is largely reversible and does not introduce detectable structural damage upon collapse. Such mechanical deformation is expected to generate significant local modifications of the lattice configuration within the graphitic layers. An essential question therefore arises: how is the mechanical state of swollen blisters reflected in their vibrational response? In order to establish a direct and quantitative connection

between morphology, mechanical behaviour, and Raman features, the following chapter introduces a correlative AFM–Raman spectroscopy approach.

5

Correlative AFM–Raman Characterization of Intercalated HOPG

In this chapter, it will be presented and discussed the results obtained through the combined use of Atomic Force Microscopy (AFM) and Raman spectroscopy. As already explained in Chapter 2, this integrated system allows the simultaneous acquisition of both topographical and spectroscopic information over the same scanned area, thus providing a direct correlation between the morphological and vibrational characteristics of the analysed surface.

Although some preliminary data acquired with this setup in acidic electrolyte environments have already been reported in the literature, the results presented here provide a deeper understanding of the intercalation process in HOPG and, in particular, of the vibrational and mechanical behaviour of the blisters formed during electrochemical cycling.

For clarity and completeness, the results discussed in this chapter are organized into **two distinct sub-chapters**, each focusing on a specific aspect of the analysis.

The **first sub-chapter** concerns the large-scale investigations carried out over areas of $50 \times 50 \mu\text{m}^2$. These measurements were essential to obtain an overall understanding of the intercalation phenomenon. This section presents the most extended correlation achievable between AFM signals, Raman spectral features, and optical microscopy images, allowing a comprehensive overview of how morphological modifications are linked to spectroscopic variations and vice versa. All the data in this part were acquired in a 1 M H_2SO_4 solution, under electrochemical conditions conducive to the formation and evolution of blisters.

The **second sub-chapter** is dedicated to the statistical analysis of individual blisters performed using the AFM–Raman spectroscopy system. In this case,

although the setup allows operation in acidic environments, the data were intentionally collected in air to ensure higher imaging stability and improved signal quality. This methodological choice is justified by the fact that, HOPG blisters remain stable once removed from the electrolyte [49]. Their structural and mechanical properties, particularly their elasticity and stiffness are preserved under ambient conditions. This approach therefore guarantees both experimental flexibility and reliable characterization of the blisters' intrinsic mechanical properties.

5.1 AFM Raman correlation on intercalate HOPG in 1M H_2SO_4

In the following section, I present the results obtained using the NT-MDT system. The sample was immersed in a 1 M H_2SO_4 solution, and the characterization was carried out after three cyclic voltammetry (CV) cycles.

The analysed area with the combined AFM–Raman setup measured $48 \times 48 \mu\text{m}^2$. The AFM images were acquired in contact mode, and for each figure, both the topographical morphology and the AFM phase-contrast signals are reported. These two signals are crucial for establishing a reliable correlation with the Raman data, and for this reason, they are consistently presented to facilitate the interpretation of the results.

The Raman map was acquired using a laser wavelength of 532 nm, impinging power on the sample of 5 mW for a total of 0.5 seconds for each spectra point. Raman mapping is a technique which consist in the acquisition of many individual spectra on a grid of points. For all the mapping the point-spacing was fixed at $0.85 \mu\text{m}$.

For this kind of measurements the alignment is crucial, as explained in chapter 2, the system permits you to know the exact tip-laser beam distance with a precision in the limits given by the Raman spectroscopy resolution. In all the following panels I'll show just the common areas between AFM image and Raman spectroscopy map.

Except for the G peak, all other intensity map of the other characteristic band of intercalated HOPG, i.e. D, 2D and G_i , the intensity values have been normalized with respect to the G peak, in order to enable direct comparison between different features. Furthermore, for the G peak, I analysed the spectral variation of its position (Raman shift) across the entire scanned region, in order to visualize how

intercalation phenomena locally modify the vibrational response of the HOPG surface.

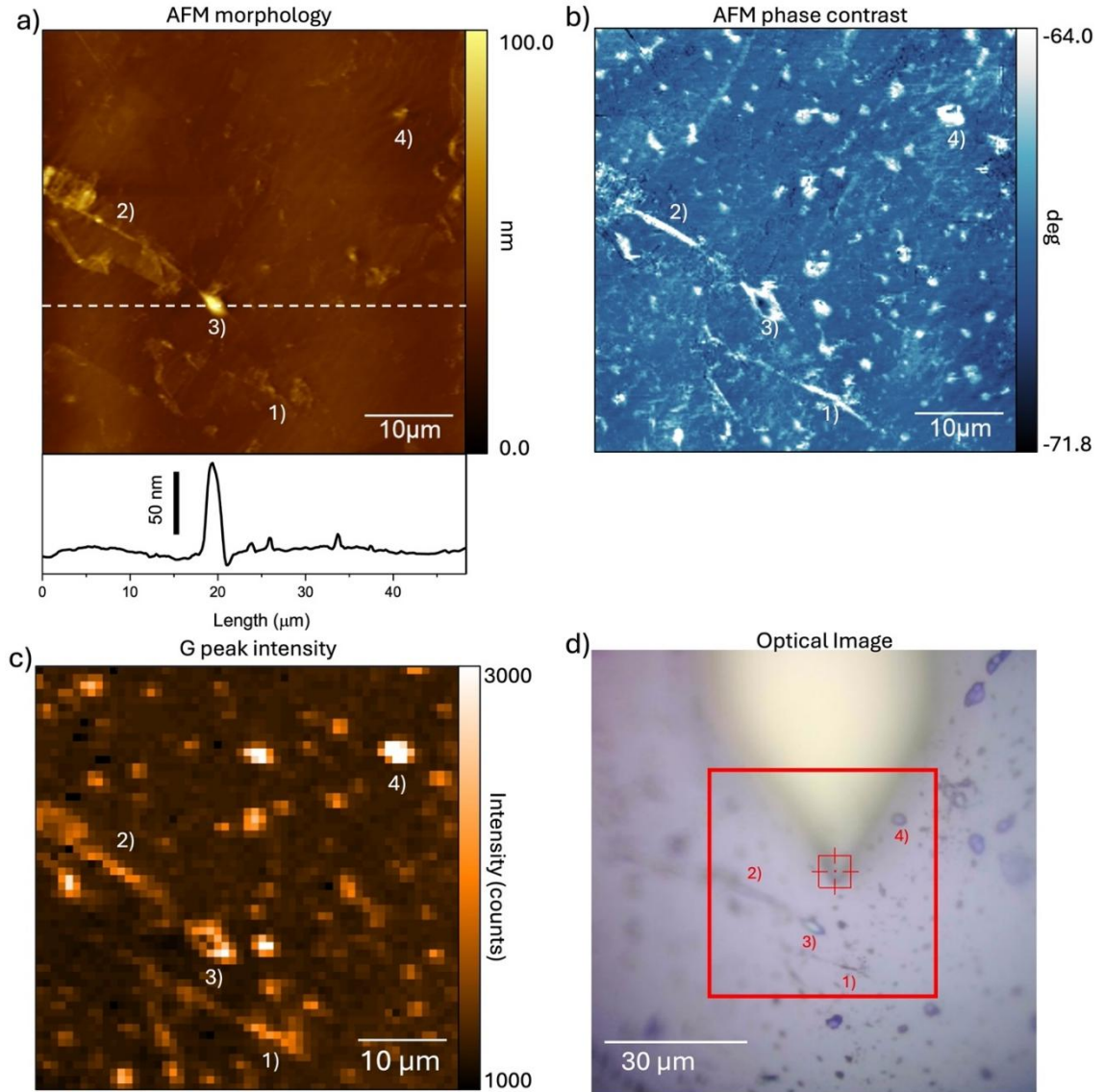


Figure 5.1: All the images were acquired in 1M solution of H₂SO₄. **a)** $48 \times 48 \mu\text{m}^2$ AFM morphology **b)** AFM phase contrast image **c)** G peak Raman Map intensity same region of the AFM image **d)** Optical Image, the red square indicates the area analysed with AFM and Raman spectroscopy. The numbered points highlight some points of interest, reported in all the panels.

The first relevant result is shown in Figure 5.1. Panel (a) displays the topographic image of the analysed area, acquired with the full height range. In this image, several regions affected by layer corrosion are visible, particularly in the lower central part (region 1) and the middle-left part (region 2). Although the chosen colour scale does not emphasize them clearly, the presence of multiple blisters can be inferred, the most prominent being the large one in the central part of the image (region 3), also visible in the profile section, and another in the upper

right corner (region 4), partially distorted due to a tip–sample jump during scanning. These four characteristic points are highlighted consistently across all panels.

It has been demonstrated that the phase of an oscillating AFM tip is sensitive to variations in the local stiffness of the sample for instance [137], it changes when the tip moves from a hard crystalline surface, such as HOPG, to a softer region containing adsorbed or organic species. In the phase-contrast image shown in **panel (b)**, several bright spots are visible. Some of these correspond to topographic features, such as those surrounding the blisters in regions 3 and 4, while others, like those near regions 1 and 2, appear associated with areas of partial surface modification. However, a large number of bright points appear independently of any morphological variation, indicating that the phase signal is also sensitive to other local properties of the surface. In any case, according to the literature, the swelling that create the blisters, reduce the hardness of the surface. As reported in chapter 4 The young modulus of blisters is reduce from ~ 100 GPa to ~ 1 -10 MPa.

The **G peak intensity map**, corrected for background, is shown in panel (c). By comparing this map with the phase-contrast image, it becomes evident that all the bright regions in panel (b) correspond to higher Raman intensities. Analysis of the individual spectra confirms that the total Raman intensity, including the G band contribution, is systematically higher in the regions corresponding to increased Raman map intensity. The G peak was selected as reference because, unlike the D, G_i , or 2D bands, it is not strongly affected by defects or intercalation effects and thus provides a more reliable intensity correlation.

This correlation becomes even more evident when comparing panels (b) and (c) with the **optical image** shown in **panel (d)**, where a red square outlines the area analysed by AFM and Raman mapping. In the optical micrograph, the four previously described regions are clearly visible as darker zones, particularly region 3, where a dark ring surrounds a brighter centre. Moreover, several other dark optical spots correspond precisely to the bright regions observed in the phase-contrast and G peak intensity maps.

To summarize, the results reported in panels a and d are consistent with what is already known in the literature. In particular, panel a shows a typical intercalated HOPG surface, characterized by the coexistence of blisters, structural defects, and locally degraded regions. The optical micrograph allows clear identification of large fractured areas (1 and 2) as well as extended swollen regions. It has been previously

demonstrated that optical techniques can distinguish not only multilayer graphene domains but also localized swelling phenomena [132,138].

The phase-contrast image in panel b also reflects observations commonly reported for intercalated HOPG [51]. Although these bright regions have frequently been described, the underlying physic-chemical origin of their contrast is still not fully clarified. With regard to blisters, the phase variation can be reasonably attributed to a local change in their mechanical response, namely a modification of the Young's modulus [108]. A similar interpretation may be tentatively extended to defected or degraded zones of the basal plane.

The most relevant new result is shown in panel c. In this case never reported before a pronounced enhancement of the G-band Raman intensity is observed in the very same regions that exhibit both an increased phase-contrast signal and optical visibility. It is known that, in few layer graphene system, the intensity of the G peak is higher respect the HOPG ones for a number of layers between 2 and 50 [139,140], this will be explained in detail in section 5.2., during the blister Raman spectra analysis. However this behaviour may reasonably apply to blisters, which according to Murray's model are finite number of graphite/graphene layer swelled and detached from the crystal below by pressurized gas [141]. However, this explanation accounts only for the Raman-enhanced regions that spatially coincide with blistered areas. A significant fraction of the intensity-enhanced domains does not correspond to morphologically identifiable blisters, indicating that additional mechanisms must contribute to the observed Raman contrast.

Unfortunately, within the explored spectroscopic window, no additional Raman features provide a clear chemical interpretation for the enhanced intensity observed in the remaining regions. A possible explanation although still speculative could involve local modifications of the surface mechanical response (as inferred from phase contrast), which might induce interference or confinement effects leading to a localized enhancement of the electromagnetic field. At the present stage, this must be considered only a hypothesis.

In any case, the strong correlation observed between the AFM phase-contrast map and the spatial distribution of the G-peak intensity has allowed an accurate and reliable registration between the AFM dataset and the Raman maps, enabling a direct comparison of morphological and spectroscopic information on the same microscopic region.

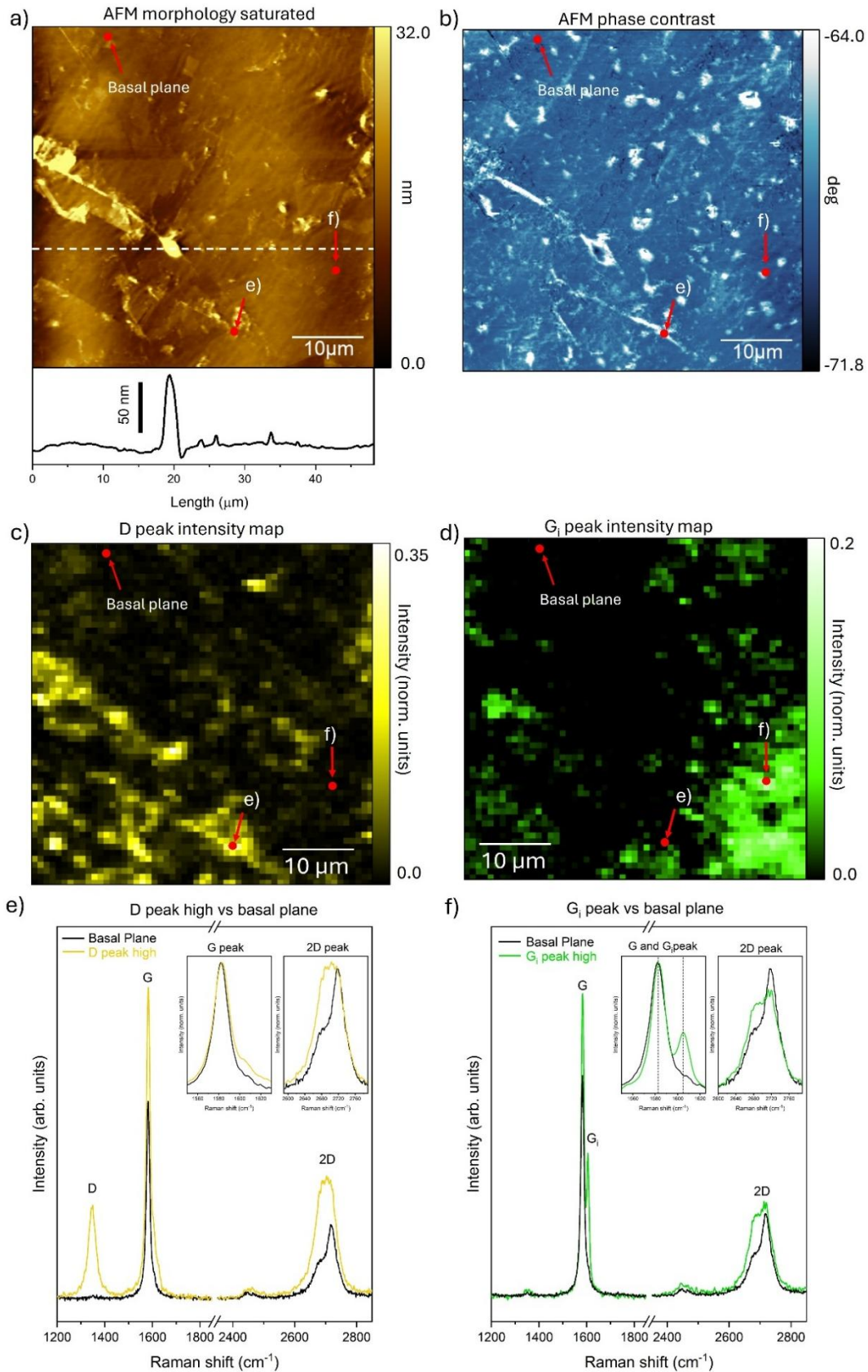


Figure 5.2: Same region of the AFM and Raman maps presented in Figure 5.1. **a)** AFM morphology **b)** AFM phase contrast image **c)** D peak normalised intensity Raman map. **d)** G_1 peak normalised intensity Raman map. **e** and **f)** Raman spectra of the point indicated as “e” and “f” respectively, compared with a region with an almost pristine basal plane.

Figure 5.2 presents the multichannel correlation between AFM measurements namely topography and phase-contrast signals (panels a and b) and D and G_i band intensity map (panels c and d). In particular, panel a displays the topographic map, whose dynamic range has been intentionally saturated to enhance subtle corrugations of the basal plane and thus improve visual readability. Complementarily, panels c and d report the spatial distribution of the D and G_i Raman bands, while panels e and f show representative spectra collected in regions where these features are present, compared to a reference spectrum acquired on an almost pristine portion of the basal plane. These datasets provide a coherent framework that links morphological, mechanical, and spectroscopic properties.

When directly comparing AFM morphology with the D-band intensity map, a clear correlation emerges. Regions exhibiting structural degradation such as local etching or terrace disruption—show a pronounced increase in the D-band signal [128]. Interestingly, the D band is also detected in areas that appear apparently undisturbed in the topographic image. This behaviour can be rationalized considering that the cyclic-voltammetry process introduces chemical etching over the entire basal plane; however, the resulting structural disorder may be too subtle to be resolved via tip-based height sensing. In these cases, Raman spectroscopy acts as a more sensitive probe of in-plane lattice perturbations, revealing defective regions that would otherwise remain undetected by AFM alone [51]. Consequently, this combined analysis demonstrates that Raman mapping provides essential complementary information, expanding the interpretative power of AFM imaging.

The spatial distribution of the G_i-band intensity reveals a strongly heterogeneous intercalation pattern across the analysed area. These results could be in contrast with the literature but actually is full consistent. Previous results give average situation, analysing a large portion of the intercalated sample. Here instead we reach a precision never obtain before [45,52]. This observation is fully consistent with the quasi-reversible character of the intercalation–deintercalation process during cyclic voltammetry. Indeed, not all anions inserted between graphite layers are removed when the potential sweep is reversed. The absence of a detectable G_i contribution does not necessarily imply complete deintercalation; rather, it may indicate that the local intercalant concentration falls below the sensitivity limit of the present Raman measurements. Moreover, no significant spatial correlation is found between the G_i-band intensity and either the AFM topography or the phase-contrast signal. This confirms that intercalation may occur locally without

generating morphological signatures or appreciable variations in mechanical response at the nanoscale [51].

The behaviour of the 2D band reflects the local perturbations of interlayer coupling and charge distribution induced by electrochemical intercalation. Although the central frequency of the 2D feature remains essentially unchanged, both the relative intensity and the apparent maximum of its two components ($2D_1$ and $2D_2$) exhibit noticeable variations. This behaviour aligns with extensive literature reporting that the 2D band being a double-resonant, second-order process is highly sensitive to multiple concurrent factors, such as lattice disorder, modulation of interlayer coupling, and charge-transfer effects. All these mechanisms are expected to be simultaneously active in electrochemically intercalated HOPG, therefore compounding their influence on the 2D-band profile.

In regions characterized by a strong D-band response, one must recall that both D and 2D modes arise from phonons near the K point of the Brillouin zone. In pristine graphite, first-order Raman selection rules suppress the D band, while the 2D transition becomes intense, with a characteristic $2D_1/2D_2$ amplitude ratio close to 0.25 (see section 3.2). When structural defects are introduced, however, these selection rules relax, enabling D-band activation and modifying the double-resonance pathways involved in the 2D process [53,128]. As a result, a redistribution of oscillator strength between the two 2D components takes place, consistent with what is observed experimentally.

A similar spectral reshaping occurs upon ion intercalation. Interlayer spacing modulation perturbs the electronic band structure and modifies the resonance conditions governing the 2D process. Therefore, both structural disorder and interlayer-distance variation contribute synergistically to the observed changes in the 2D-band envelope [133].

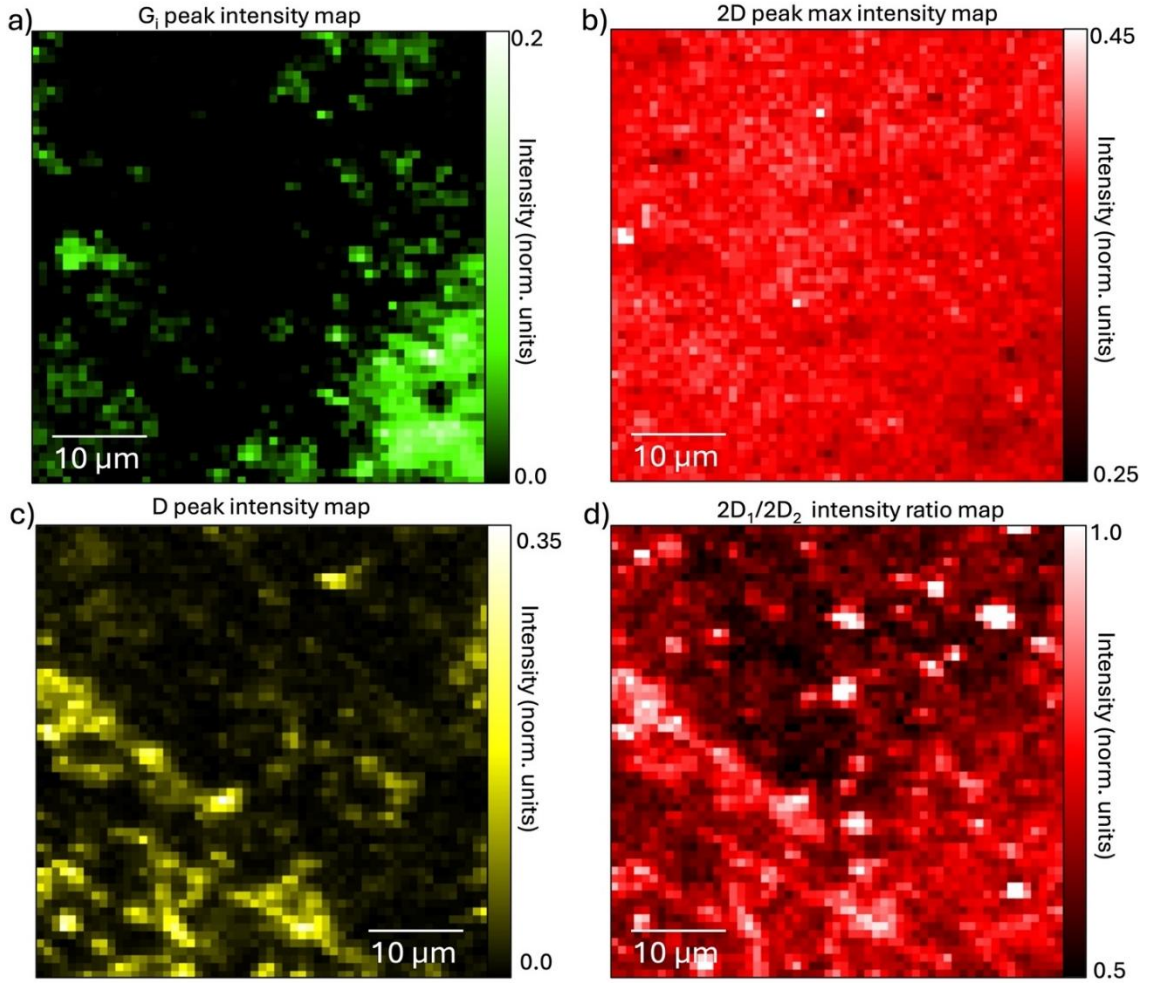


Figure 5.3: Same region of the AFM and Raman maps presented in Figure 5.1. **a)** G_i peak normalised intensity Raman map. **b)** 2D peak normalized intensity Raman map **c)** D peak normalised intensity Raman map. **d)** 2D₁/2D₂ Raman intensity ratio map. For the intensity of the 2D₁ component I selected the spectrum intensity at 2684 cm⁻¹, for the 2D₂ I selected the maximum intensity of the whole band, in order to follow the redshift of the band.

To clarify these aspects, the spatial distribution of the D and G_i signals (panels a and c of Figure 5.3) was compared to two distinct representations of the 2D feature: panel b reports the normalized maximum intensity of the 2D band, whereas panel d maps the 2D₁/2D₂ intensity ratio. Due to the complexity of the 2D peak shape in the intercalated HOPG, I have to clarify how the map in panel D was constructed. For the intensity of the 2D₁ component I selected the spectrum intensity at 2680 cm⁻¹, for the 2D₂ I selected the maximum intensity of the whole band in order to follow the redshift of the band in some areas. Although this criterion does not fully capture the redshift of the 2D₁ component in some regions, it is sufficient to generate a map that faithfully reflects the complexity of the 2D-band profile and enables a meaningful correlation with the other Raman features and the AFM signals.

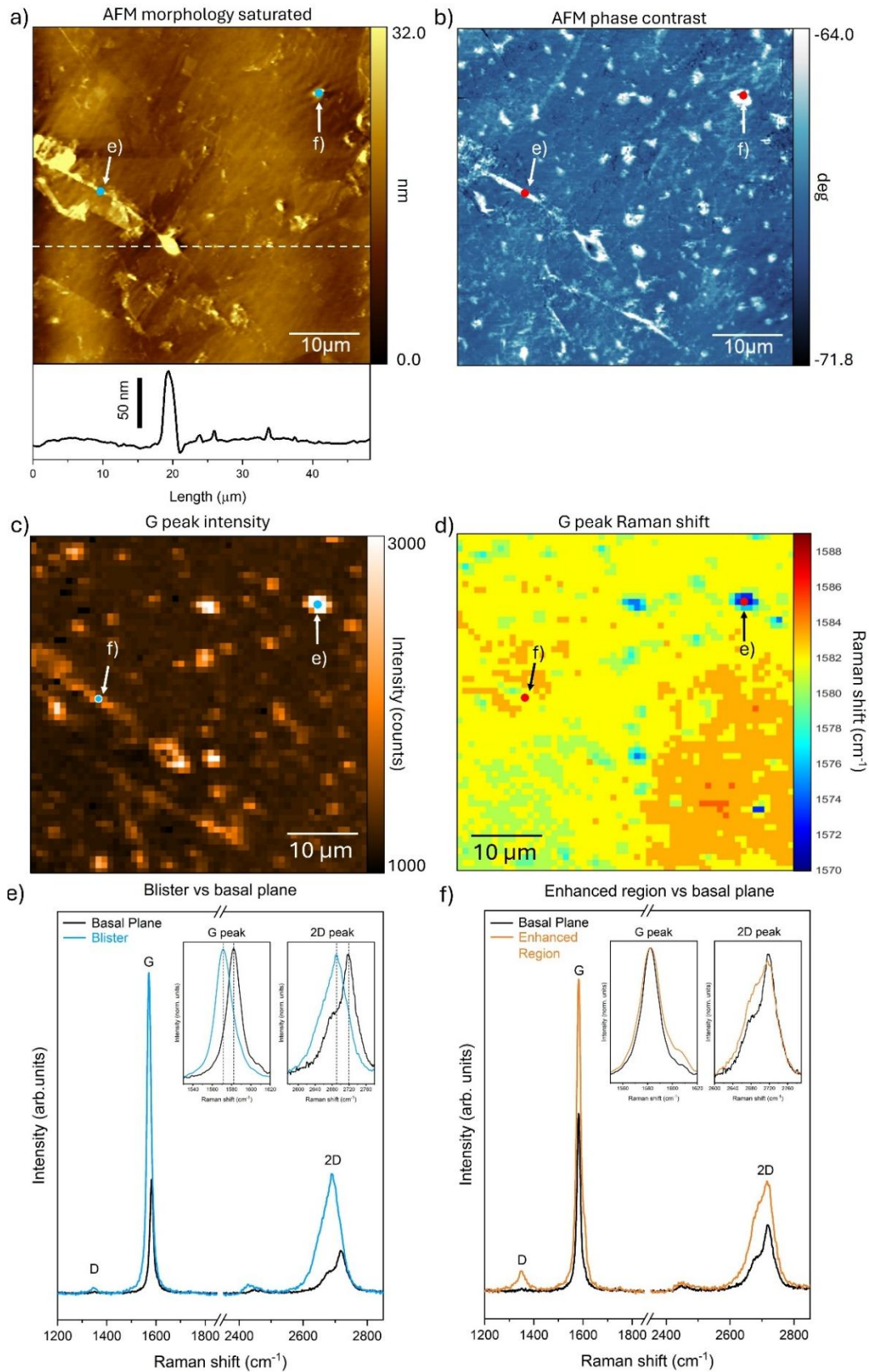


Figure 5.4: Same region of the AFM and Raman maps presented in Figure 5.1. **a)** AFM morphology **b)** AFM phase contrast image **c)** G peak intensity Raman map, the colour depends on the Raman shift of the G peak in that position. **e** and **f**) Raman spectra of the point indicated as “e” and “f” respectively, compared with a region with an almost pristine basal plane.

A comparison between panels a and b reveals that the normalized 2D signal tends to decrease in regions where the G_i band is strongly expressed, suggesting that intercalation plays a dominant role in modulating the overall 2D response. Furthermore, when panel d is compared with panels a and c, it becomes evident that where the G_i or D band intensity is high, the 2D₁/2D₂ intensity ratio is high, the latter instead is at its minimum where both the G_i and D peaks have a null intensity. However there are some areas where the latter conclusion is not true, I will go more in detail later.

The final part of the AFM–Raman analysis in acidic environment is devoted to a more detailed examination of selected Raman-enhanced regions and their correlation with the local morphology, with particular emphasis on the behaviour of the G band. Specifically, we analyse the Raman shift of the G peak in areas exhibiting spectral enhancement.

Figure 5.4 reports the AFM topography (panel a) and phase-contrast (panel b) images, together with the G-peak intensity map (panel c) and the corresponding G-peak shift map (panel d). Unlike the intensity map, the latter displays the Raman shift associated with the peak maximum. The pristine graphite G-peak position lies at 1582 cm⁻¹; across most of the investigated area, the measured values remain within (1582.0 ± 1.5) cm⁻¹. Only a few isolated sites exhibit a pronounced redshift, reaching values up to $\Delta \approx 12$ cm⁻¹, as exemplified by the red spot marked “e”. By comparing panels a and d, it becomes clear that these redshifted regions spatially coincide with blister-like features. Such redshifts have previously been reported in the literature for several graphene bubble system [9,142], the physical origin for the blister of this response will be discussed in the next section.

What can be seen from this correlative analysis between morphology and spectroscopy is that blisters shown an enhancement of the G-peak intensity, which can be rationalised considering their structure: few-layer of graphite locally lifted by pressurised, trapped species. As previously discussed, a finite number of graphene layers leads to an enhancement of the Raman signal. A representative spectrum from a blister (panel e) displays both the enhancement and a redshift of the G and 2D bands, relative to spectra recorded on pristine-like areas. In addition, the 2D band shows a systematic modification of its line shape. Since the 2D-band profile is known to be highly sensitive to the number of graphene layers, the local swelling associated with blister formation likely changes the balance between the 2D₁ and 2D₂ contributions.

However, it is important to note that not all Raman-enhanced areas exhibit a concurrent G-peak redshift. As shown in the representative spectrum in panel f, regions with strong G-peak enhancement may nonetheless display neither G nor 2D redshift. It is possible to conclude that blister formation lead to a spectral enhancement, it cannot be said that spectral enhancement indicates blister formation, suggesting a more complex scenario, as already seen in image 4.1.

To assess whether blistering also impacts the intensity distribution within the 2D band Figure 5.5, we compare the AFM morphology and the G-peak shift map with the 2D-band intensity and $2D_1/2D_2$ contribution maps (the latter already shown in Figure 5.3). I find that the energy shift does not globally affect the integrated 2D-band intensity. Instead, panel b highlights local variations in the $2D_1/2D_2$ ratio, which mirrors the behaviour seen in Figure 5.3: the highest ratio values (white regions) correspond to blister-like areas.

Taken together with the data presented in Figure 5.3, these results highlight the intrinsic complexity of analysing the 2D band in intercalated graphite. Its spectral evolution is governed not by a single dominant mechanism but by the convolution of several simultaneous perturbations including lattice disorder, variable stacking order, and intercalation-induced electronic modification each contributing to the final spectral profile. This reinforces the importance of integrating morphological, mechanical, and spectroscopic datasets to achieve a unified and coherent interpretation of the intercalation process.

In conclusion, this analysis provides an overall picture of the initial HOPG intercalation processes that can be accessed via AFM–Raman correlation in acidic environments. The principal findings are summarised below:

- There is a clear correlation among the optical image, G-peak Raman enhancement, and AFM phase-contrast response.
- The G_i band is not uniformly distributed and does not correlate with AFM topography or phase-contrast images.
- Defects and intercalated ions strongly influence the 2D-band shape, making its interpretation more complex.
- In blistered regions, the Raman spectrum exhibits both an intensity enhancement and a redshift; the **detailed** and **statistics** spectroscopic behaviour of blisters will be the focus of the following section.

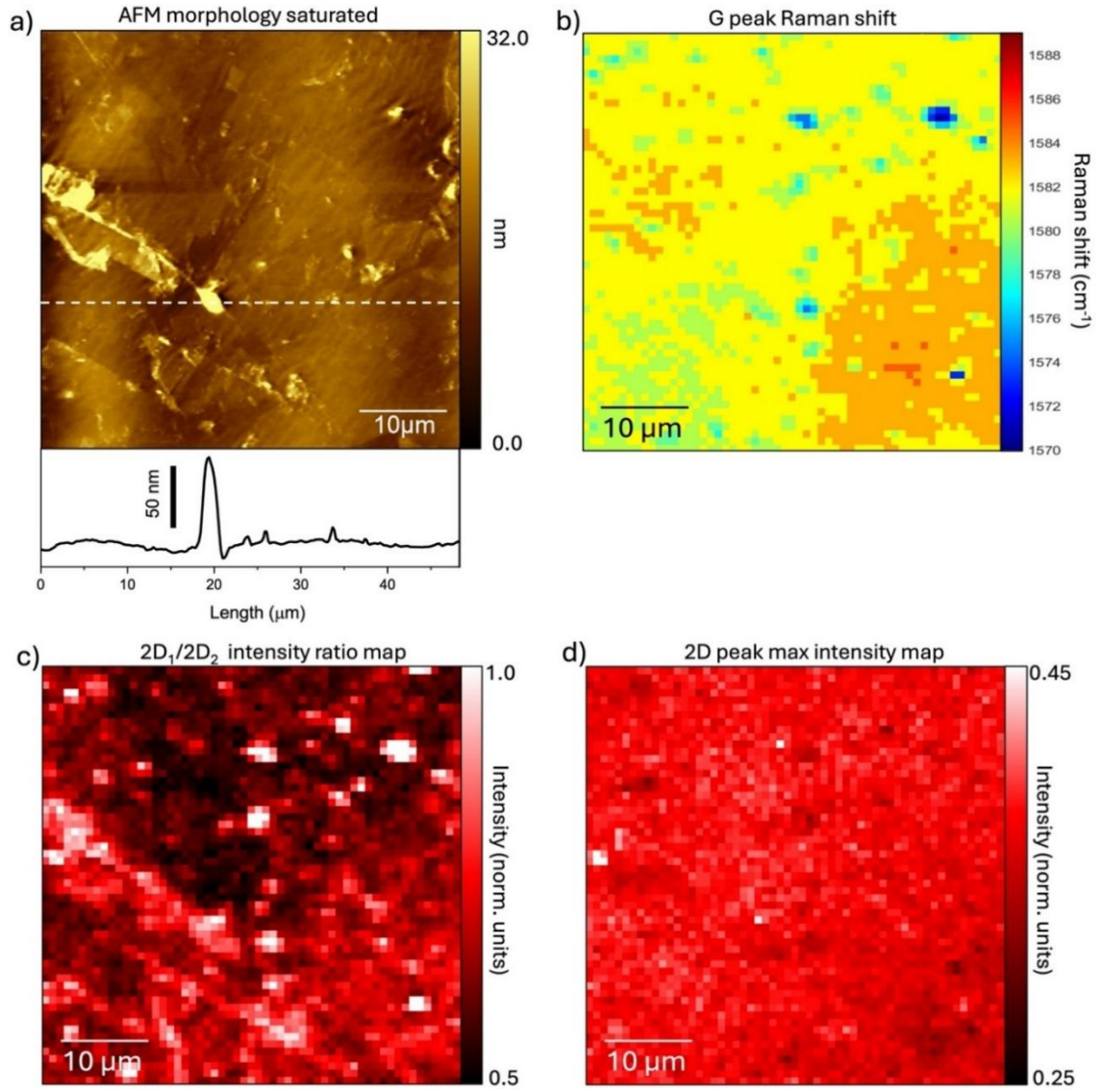


Figure 5.5: Same region of the AFM and Raman maps presented in Figure 5.1. **a)** AFM morphology **b)** G peak Raman shift map **c)** 2D₁/2D₂ Raman intensity ratio map **d)** 2D peak normalized intensity Raman map.

5.2 Statistics AFM Raman spectroscopy correlation on blisters

In the second part of this chapter, I present the results obtained through the combined AFM–Raman approach, focusing specifically on the characterization of individual blisters. To the best of my knowledge, this represents the first systematic investigation in which these structures are analysed individually and their structural and spectroscopic properties directly compared. A total of 23 distinct blisters were examined under identical experimental conditions, enabling a statistically grounded analysis aimed at identifying recurring trends as well as deviations among different structures.

For greater experimental flexibility, all measurements presented in this section were carried out under ambient conditions. Although the blisters were initially formed in acidic environments, previous studies have demonstrated that, once created, these structures remain stable upon transfer to air, preserving both their morphology and spectroscopic signatures. Thus, measurements performed in air can be considered fully representative of their behaviour in liquid.

The choice of perform the characterization in ambient condition was due to the long-time Raman Mapping that was needed (30-60 min) to get a good signal to noise ratio, and so a high quality and stability in the AFM was needed.

The findings presented here are, in several respects, unprecedented. Prior work has largely focused on blister formation as a secondary by-product of intercalation, without directly addressing their distinctive Raman response. In contrast, the present study reports, for the first time, the Raman fingerprints characteristic of the blister state, thereby offering a new interpretative perspective. Rather than viewing blisters merely as passive topographical irregularities, I consider each structure as an autonomous nanoscale system: a confined pocket of few-layer graphene, mechanically uplifted by internal pressure, electronically decoupled but still physically connected to the underlying graphite lattice. Such a framework not only refines the conceptual understanding of blister formation, but also motivates a revised experimental strategy in which blisters are treated as individual nanoscale systems with intrinsic mechanical and spectroscopic properties. This perspective provides a more rigorous basis for analysing the local effects of intercalation at the nanoscale.

5.2.1 AFM and Raman correlation

In order to obtain a comprehensive overview of the spatial distribution and morphological variability of the blistered regions, a large-area AFM scan ($40 \times 40 \mu\text{m}^2$) was systematically acquired for each sample. This approach allowed the reliable identification of blisters suitable for subsequent, more detailed structural and spectroscopic analyses. A representative AFM topography image of the intercalated HOPG surface is reported in Figure 5.6. In this image, blister-like features are clearly visible, together with minor surface residues that can be reasonably attributed to residual traces of the electrolyte. Importantly, the step edges characteristic of pristine HOPG remain well defined, indicating that the long-range crystalline order of the graphite lattice is largely preserved despite the electrochemical I⁻ intercalation process.

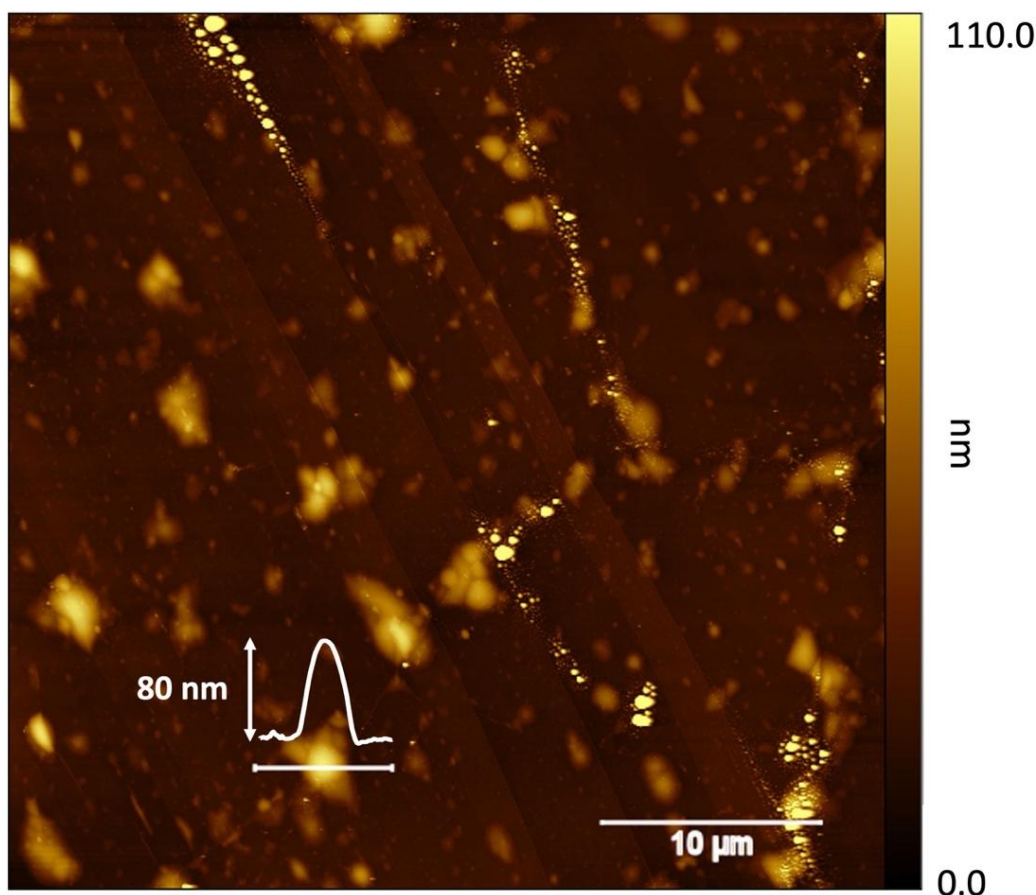


Figure 5.6: $40 \times 40 \mu\text{m}^2$ AFM image of 2CVs intercalated HOPG.

Figure 5.7 schematically illustrates the experimental procedure adopted for the systematic analysis of individual blisters. A crucial preliminary step in this workflow is the definition of appropriate selection criteria for blisters suitable for Raman spectroscopic investigation. Given that the lateral spatial resolution of conventional

Raman microscopy is approximately $1\ \mu\text{m}$, this value was adopted as the selection criterion for blister inclusion in the dataset. Specifically, only blisters with a lateral diameter larger than $1\ \mu\text{m}$ were considered for analysis, in order to ensure that the acquired Raman spectra were spatially representative of the blistered region and not significantly affected by signal averaging with the surrounding basal plane.

Following the initial large-area AFM survey, a higher-resolution AFM image was acquired, centred on the selected blister, as indicated by the red square in panels (a) and (b). Subsequently, a Raman map of the same region was recorded in order to establish a direct correlation between the local topographic features and the corresponding spectroscopic response revealed by AFM.

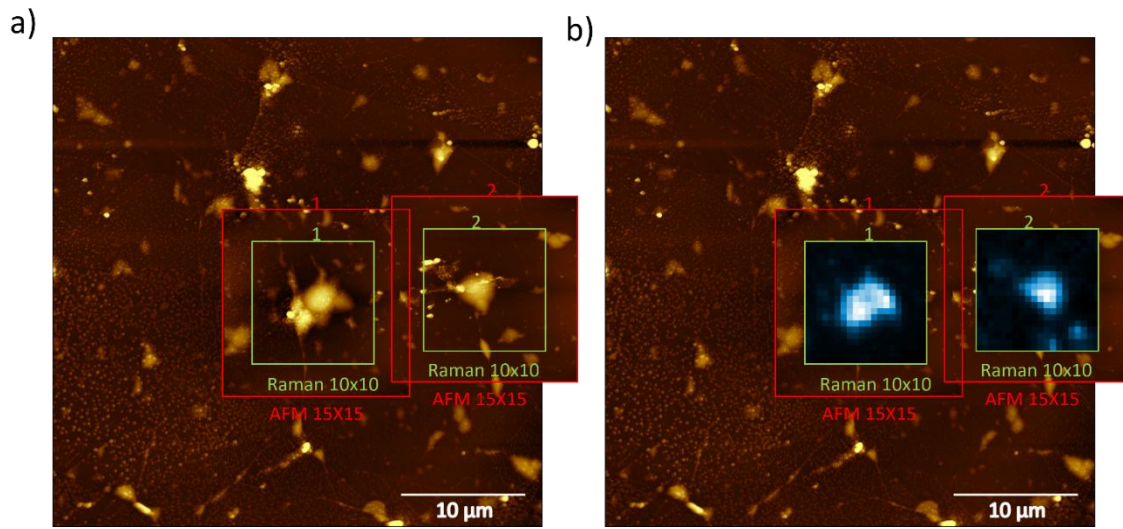


Figure 5.7: **a)** $40 \times 40\ \mu\text{m}^2$ in air AFM scan of intercalated HOPG with highlighted the regions chosen for detail scans and Raman maps **b)** Detail $15 \times 15\ \mu\text{m}^2$ AMF scan and $10 \times 10\ \mu\text{m}^2$ G peak intensity Raman maps of selected blisters.

All AFM measurements were performed in semi contact (tapping) mode to minimise tip–sample interaction forces while preserving high spatial resolution. Raman maps were acquired using a laser excitation wavelength of 532 nm, with an incident power of 1 mW on the sample and an acquisition time of 30 s for each spectral point. The experimental sequence illustrated in Figure 5.7 was repeated for each of the 23 blisters analysed in this study, with Raman mapping performed after careful laser alignment. For all Raman maps, the point-to-point spacing was fixed at $0.53\ \mu\text{m}$, while the overall map size and consequently the number of acquired spectra was adjusted according to the dimensions of the selected blister. As introduced at the beginning of the chapter, a complete Raman spectrum is collected at each point; specific spectral features of interest can then be selected to

generate false-colour maps, in which the colour intensity reflects the local intensity of the chosen Raman band.

Accurate AFM–Raman spatial alignment is a critical requirement for the analysis of individual blisters, as even small misalignments can introduce significant errors in the interpretation of the local spectroscopic response. Accurate spatial correlation between topographic and Raman maps is therefore a critical prerequisite to ensure that the acquired spectra are truly representative of the blistered regions.

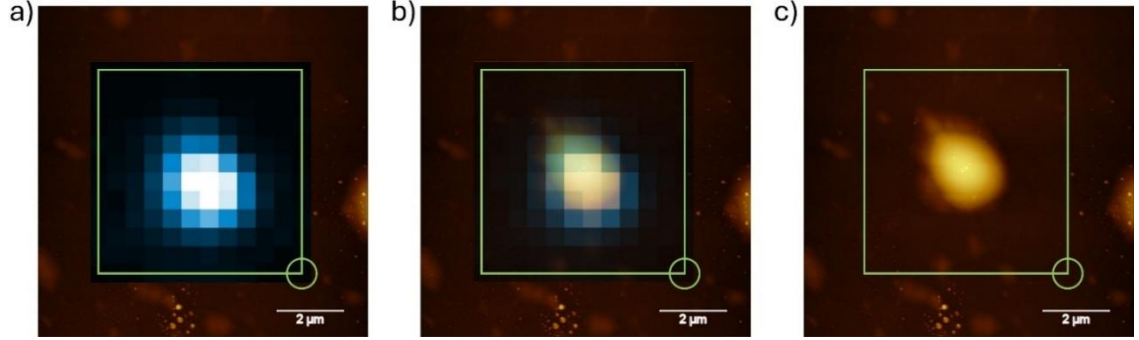


Figure 5.8: a) $6 \times 6 \mu\text{m}^2$ Raman map of a selected blister. b) Same Raman map superimposed with partial transparency onto the corresponding $10 \times 10 \mu\text{m}^2$ AFM topography, allowing direct spatial comparison between spectroscopic and morphological features. c) AFM morphology of the blister shown in full. The yellow circle indicates the Rayleigh diffraction limit.

Figure 5.8 illustrates a representative example of the alignment between a $10 \times 10 \mu\text{m}^2$ AFM image and a $6 \times 6 \mu\text{m}^2$ Raman map acquired on the same blister. In this context, careful alignment is particularly important to guarantee that the characteristic Raman response of the blister is correctly sampled. The green square indicates the area covered by the piezo-tube scanner during the Raman acquisition; due to the finite size of the laser spot and its higher spatial extent compared to the piezo movement, the effective Raman-mapped area appears larger than the nominal scanner displacement.

The experimental setup allows precise determination of the relative position between the AFM tip and the laser focus, within the limits imposed by the spatial resolution of Raman spectroscopy. This resolution is schematically represented by the green circle in Figure 5.8.

As discussed in the previous section, blister regions are associated with a systematic enhancement of the overall Raman intensity. Given that the G band is intense, well-defined, and present across the entire mapped area, it was adopted as the primary spectral marker to assess the spatial alignment between AFM and Raman datasets. Additional validation was obtained by analysing defect-rich

regions, where the correspondence between AFM features and the normalised intensity of the D-band Raman map provided an independent check. In all measurements, the residual misalignment between AFM and Raman maps was found to be within the Rayleigh diffraction limit (i.e., less than 1 μm), as shown in Figure 5.8, confirming the reliability of the spatial correlation employed throughout this study.

Once the alignment procedure has been accurately validated, it is possible to proceed with the detailed spectral analysis of the Raman response characteristic of individual blisters. Figure 5.9 summarises the outcome of this analysis. Panels (a) and (b) report the AFM topography and the corresponding G-band intensity map, respectively. Based on these datasets, representative spectral points were selected both on the blister apex (black square) and on the surrounding basal plane (red square), as shown in panel (c). In the upper-right inset of panel (c), a magnified view of the normalised G-band spectra acquired on the blister and on the basal plane is presented for direct comparison.

Both spectra display the characteristic Raman features of intercalated graphite, namely the first-order G band and the second-order 2D band also present in pristine HOPG together with the G_i peak associated with intercalation phenomena and the D band related to structural disorder (see Figure 2.4). The most striking result emerging from this correlation is the pronounced enhancement of the Raman signal measured on the blisters with respect to the surrounding basal plane. When considering the G band which is largely insensitive to both defects and intercalations the intensity ratio between blister and basal plane ranges from approximately 1.8 to 5, depending on the specific blister analysed (see Table 5.1 in section 5.2.3).

As discussed in the previous section, Raman intensity enhancement in intercalated HOPG is not limited to morphologically identifiable blisters, indicating that multiple factors may contribute to the observed signal increase. Although the microscopic origin of the enhancement cannot be conclusively determined from the present measurements alone, established results on few-layer graphene systems provide a physically grounded framework for its interpretation. It is important to recall that the blister “skin” consists of a finite number of swollen graphene layers locally detached from the underlying HOPG substrate. Previous studies have shown that Raman intensity depends strongly on the number of graphene layers [139]. In particular, while single-layer graphene and bulk HOPG exhibit comparable G-band intensities, Wang et al. demonstrated that when the number of layers lies between approximately 2 and 50, the G-band intensity can increase by up to a factor of six.

Although the present system differs from graphene exfoliated or grown on silicon oxide substrates, the finite layering within the blistered regions likely plays a key role in the observed Raman enhancement.

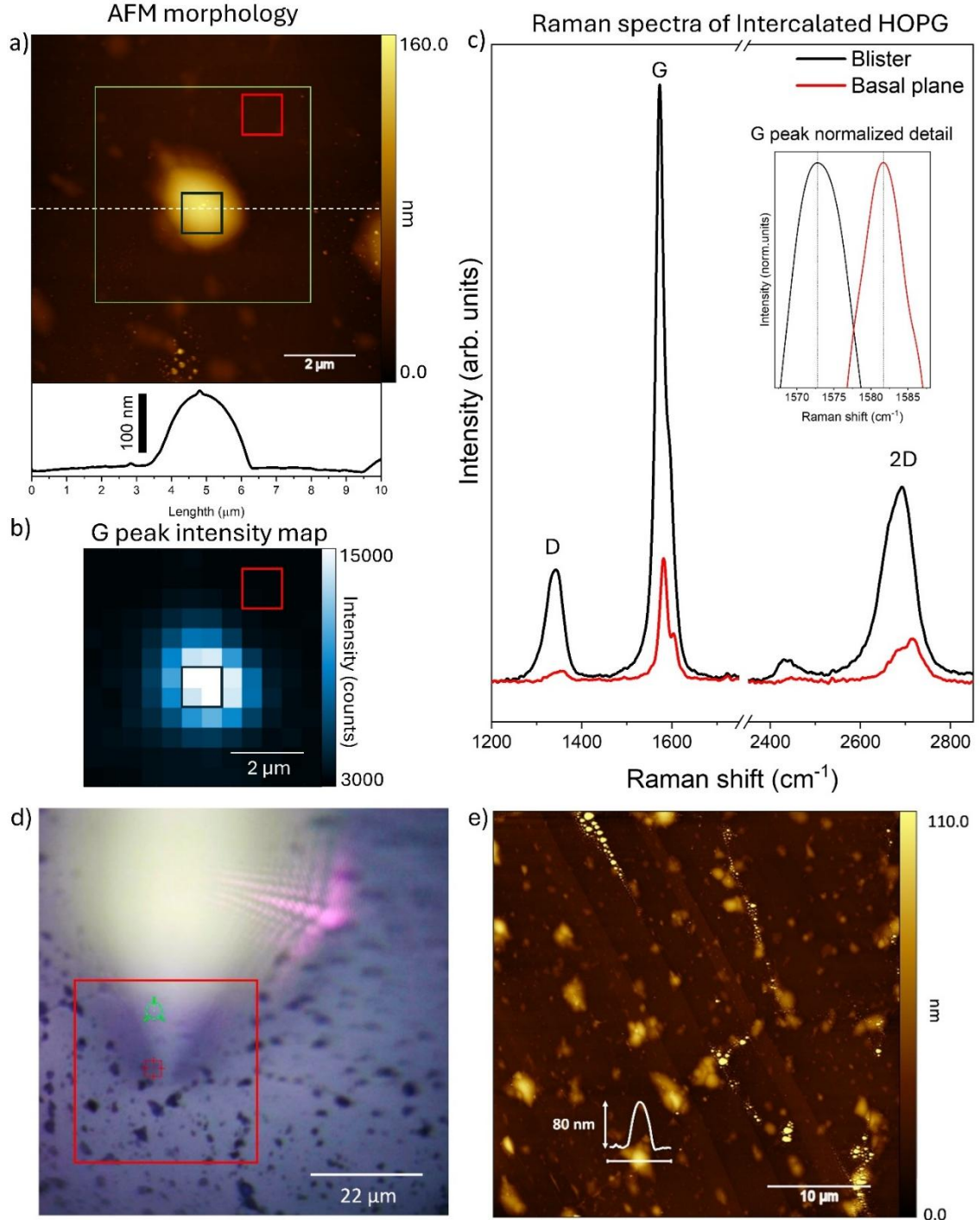


Figure 5.9: a) 10 x 10 μm^2 AFM scan of a blister with highlighted the region chosen for the Raman map b) 6 x 6 μm^2 G peak intensity Raman map of the same area c) Raman spectrum of the selected regions: the black spectrum is representative of the blister while the red of intercalated HOPG basal plane. d) Optical image of intercalated HOPG, the red square indicate the area of the AFM image in panel e. e) AFM image acquired in the red square of panel d.

Further support for this interpretation is provided by the correlation between AFM morphology and optical microscopy. In Figure 5.9, panels (d) and (e) show the optical image corresponding to the AFM topography reported in Figure 5.6, with the AFM-scanned area highlighted by the red square. A direct comparison reveals that regions identified as blisters by AFM correspond to darker spots in the optical images of intercalated HOPG surfaces. Similar observations were reported by Hoffmann et al. and Bayle et al. [131,132], who attributed such dark regions to graphene flakes or locally modified graphite regions. In particular, Ref. [132] combined optical and Raman analyses to provide a more comprehensive interpretation of this behaviour.

The darker appearance of blisters in optical microscopy can reasonably be attributed to local modifications of optical reflectivity, potentially arising from thin-film interference effects at the interface between the delaminated graphene layers and the trapped gas. While the exact optical mechanism is not directly resolved here, such variations in local optical response are consistent with the enhanced Raman signal observed on blistered regions. Regardless of the exact microscopic origin, the Raman intensity enhancement constitutes a powerful experimental advantage: it enables a clear and unambiguous correlation between morphology and spectroscopy, allowing the Raman response of the blister apex to be reliably distinguished from that of the basal plane and directly compared with the spectrum of pristine HOPG. Importantly, these observations are fully consistent with the results presented earlier in this chapter (Figures 5.1 and 5.4), and demonstrate that the spectral enhancement persists both when the sample is immersed in solution and after it is extracted and dried.

Having established the presence of a systematic Raman intensity enhancement on blisters, the analysis can be extended to the behaviour of individual Raman bands. Both the blister and basal-plane spectra exhibit a noticeable D-band intensity; however, as discussed in section 5.1 and further elaborated later in this chapter, the Raman peak does not show a clear correlation with blister formation and should therefore be regarded as incidental in this specific case. Similarly, while the G_i band is observed on the basal plane, it is absent on the blister; once again, no direct correlation between the G_i -band intensity and the presence of blisters can be established.

Focusing on the G band, a clear redshift is observed for the blister spectrum relative to the basal plane. This behaviour was already identified in section 5.1, but here it emerges systematically across all analysed blisters. According to the

interpretative framework introduced in Ref. [13], blister formation is driven by pressurised gases trapped beneath the surface. A useful analogue for understanding this behaviour is provided by graphene blisters or bubbles, for which controlled pressure experiments have demonstrated a linear correlation between internal pressure and G-band redshift. Studies by Zabel and Wang, in particular, showed that increasing pressure inside graphene blisters induces a progressive downshift of the G band. Although HOPG blisters represent a more complex, multilayered system, it is reasonable to hypothesise that a similar mechanism is at play, whereby internal gas pressure induces local strain and consequently shifts the G-band position to lower energies.

Finally, the 2D band also exhibits significant modifications compared to pristine graphite. In particular, its line shape differs markedly, in agreement with the observations reported in the first part of this chapter. A closer inspection of the 2D₂ component reveals a redshift analogous to that observed for the G band. This behaviour is again consistent with previous studies on graphene blisters and bubbles [4,9,138,142] further supporting the interpretation of blister-induced strain as a key factor governing the Raman response of intercalated HOPG.

While the AFM–Raman correlation provides direct access to the spatial relationship between morphology and vibrational response, the correct interpretation of the Raman spectra acquired on blistered regions requires a precise understanding of how the Raman features of graphitic systems evolve as the number of layers is reduced from bulk graphite to few-layer graphene. In particular, both the absolute intensity and the line shape of the G and 2D bands in graphitic systems are highly sensitive to the strength of interlayer coupling, which evolves as the number of graphene layers decreases from bulk graphite to few-layer graphene. For this reason, a reference framework based on the Raman signatures of few-layer graphene is introduced in the following section.

5.2.2 Raman signatures of few-layer graphene: reference framework for blister analysis

The interpretation of Raman spectra acquired on blistered regions of intercalated HOPG requires a clear reference framework describing how the vibrational response of graphitic systems evolves when the number of layers is reduced from bulk graphite to few-layer and single-layer graphene. As discussed in the previous sections, HOPG blisters can be regarded as structures involving a finite number of graphene layers that are locally lifted and mechanically decoupled from

the underlying bulk crystal by a pressurized confined phase. As a consequence, their Raman response is expected to deviate from that of bulk graphite and to share key similarities with few-layer graphene systems.

Raman spectroscopy provides a particularly sensitive probe of such differences, owing to the strong dependence of both the absolute intensity and the line shape of the characteristic bands on the number of layers and on the interlayer coupling. Among these features, the G band and, most importantly, the 2D band play a central role in discriminating between bulk-like and few-layer behaviour, see Figure 5.10.

In bulk graphite, the 2D band exhibits a complex line shape arising from the electronic band structure and from the strong interlayer coupling associated with Bernal stacking [125]. When the number of layers is reduced, this coupling progressively weakens, leading to a marked evolution of the 2D-band profile. In single-layer graphene, the 2D band collapses into a single, sharp Lorentzian peak, whereas in bilayer and few-layer graphene it can be decomposed into multiple components whose relative intensity and spectral weight depend on the stacking order and on the number of layers (panel c) [129].

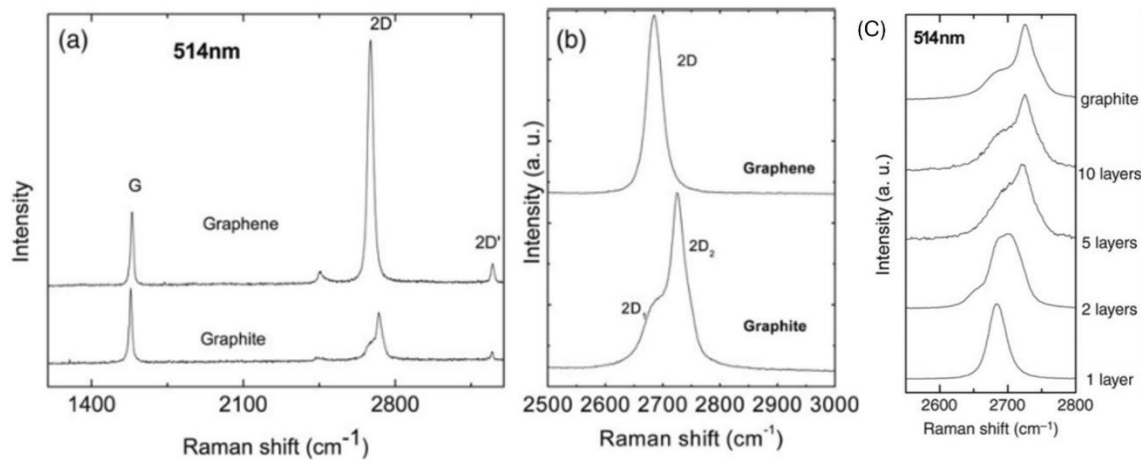


Figure 5.10: **a)** Raman spectrum of graphene and HOPG for 514 nm excitation **b)** detail of the 2D peak showing a single 2D component for graphene and the two 2D₁ and 2D₂ components for HOPG, **c)** Number of layers 2D peak dependence, laser wavelength 514 nm [129].

However, it is important to emphasize that both the absolute intensity of Raman bands and the detailed 2D-band line shape are not solely determined by the number of layers. Substrate optical properties, excitation wavelength, stacking order, strain, and interfacial coupling can all significantly modify the Raman response. As a result, thickness estimation based on a single spectroscopic parameter may lead to ambiguous or even misleading conclusions if applied without caution.

For this reason, the combined analysis of absolute G-band intensity and 2D-band line shape has been proposed as a practical and more robust method for estimating the thickness of graphene flakes.

On this basis, the combined analysis of absolute G-band intensity and 2D-band line shape has been proposed as a practical method for estimating the thickness of graphene flakes [139]. Further investigations are required to clarify the extent to which such effects apply to other substrates and interfacial environments, including the blistered regions of intercalated HOPG.

In the following section, these concepts are applied to the analysis of AFM–Raman data acquired on intercalated HOPG, with the aim of assessing whether similar spectroscopic markers can be reliably used to characterize individual blisters and to gain insight into their local structural and mechanical state.

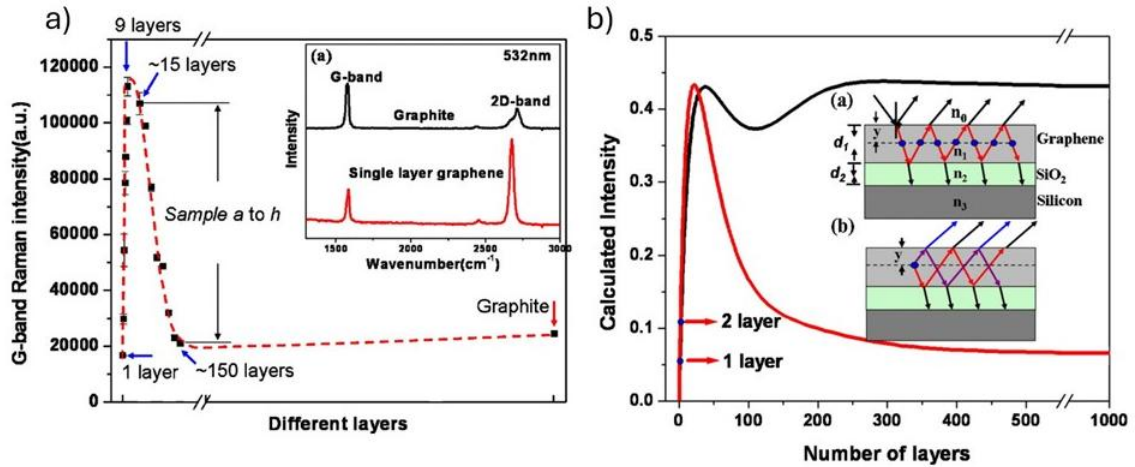


Figure 5.11: a) experimental enhancement of the G band in few-layers graphene flakes of different thicknesses deposited on SiO₂ b) theoretical curve calculated based on an interference mechanism model, which happens at the interface with SiO₂ [139].

5.2.3 Basal plane subtraction and peak analysis

Building on the reference framework introduced above, the Raman spectra acquired on intercalated HOPG were analysed by separating the contribution of blistered regions from that of the surrounding basal plane. Here, the term *basal plane* refers to the flat, non-blistered (i.e., non-delaminated) HOPG surface used as the local reference. This subtraction step is essential to isolate the intrinsic spectroscopic signatures of the blisters from the dominant response of bulk graphite.

It should be noted that this procedure represents an approximation, based on the assumption that the Raman contribution arising from the graphite layers underlying the blister is nearly identical to that of the surrounding basal plane. The

physical justification and limitations of this assumption are discussed in detail in the following section.

In the following, the basal-plane subtraction procedure and the quantitative analysis of the G and 2D bands are described in detail.

Some blisters showed a G peak with two components (1582 and $\sim$ 1560–1570 cm^{-1}), Figure 5.12. Panels (a) and (b) report, respectively, the AFM topography and the corresponding Raman G-band intensity map. The green square in panel (a) indicates the region over which the Raman map was acquired. The black and red squares, visible in both panels, mark the representative locations selected for extracting Raman spectra characteristic of the blister apex and of the surrounding basal plane, respectively.

The full Raman spectra acquired at these positions are shown in panel (c), with the blister spectrum displayed in black and the basal-plane spectrum in red. The reported intensities correspond to raw, unprocessed data. Consistent with previous observations (see Figure 5.9), a clear enhancement of the overall Raman signal is evident in the blistered region. The inset in the upper-right corner of panel (c) presents a magnified view of the normalised G-band region for both spectra. Notably, the blister spectrum exhibits a clear splitting of the G peak: one component coincides with the G-band position of the basal plane, while a second component is distinctly redshifted.

Panel (d) shows the fitting of the G band in the blister spectrum using a two-component model. The redshifted contribution is centred at 1567 cm^{-1} , whereas the second component is located at 1583 cm^{-1} , matching the G-band position of pristine HOPG. Interpreting this behaviour is not straightforward. An initial hypothesis could involve optical convolution with the underlying basal plane; however, this possibility can be excluded, since the G-band splitting is observed exclusively at the blister centre and not at positions closer to the blister edges.

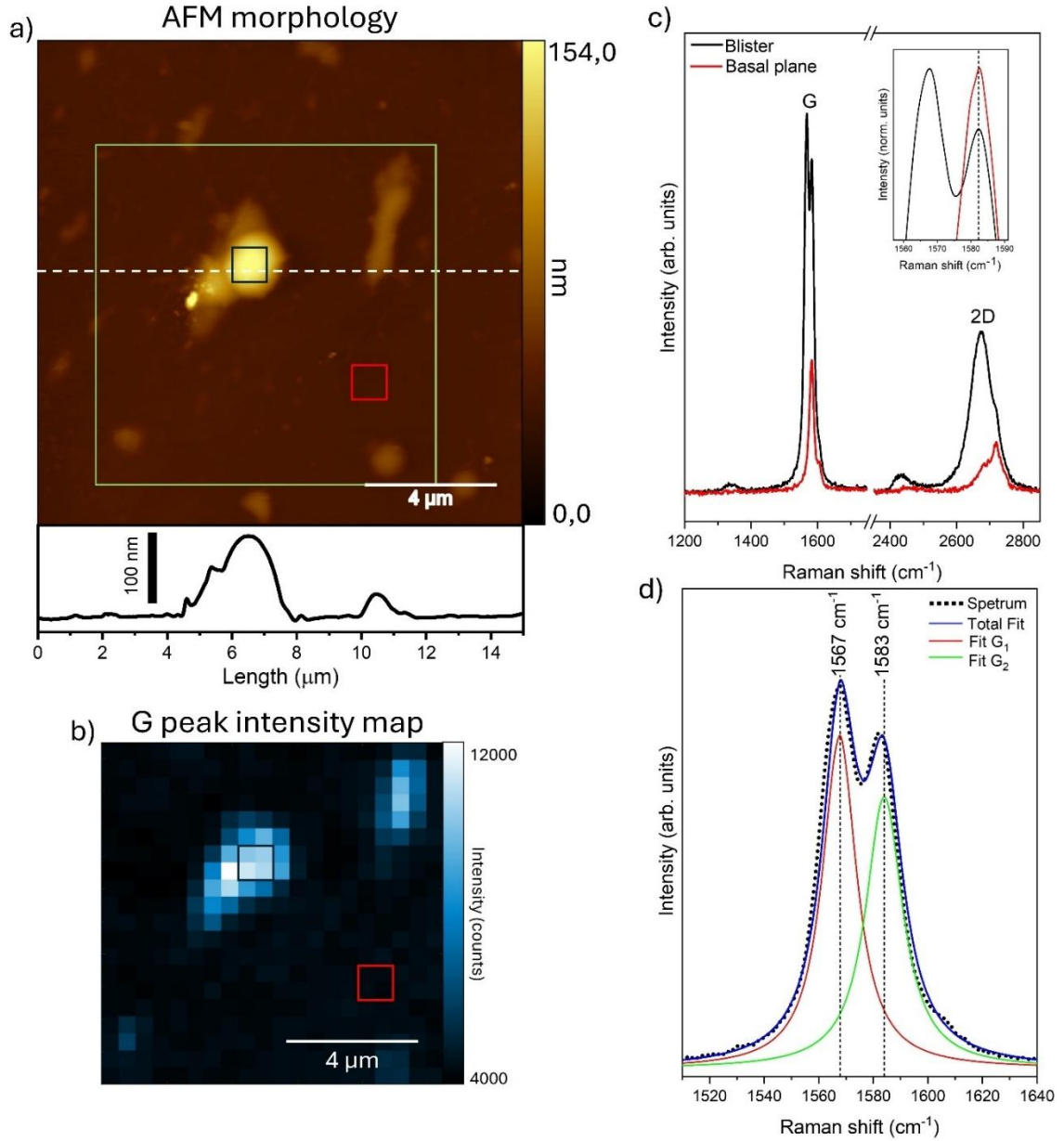


Figure 5.12: **a)** 15 x 15 μm^2 AFM scan of a blister with highlighted the region chosen for the Raman map **b)** 10 x 10 μm^2 G peak intensity Raman map of the same area **c)** Raman spectrum of the selected regions: the black spectrum is representative of the blister while the red of intercalated HOPG basal plane. **d)** Detail of the G peak acquired in presence of the blister, two component fitting was performed.

In the literature, G-band splitting has been reported in graphene subjected to uniaxial strain. However, this scenario can be excluded in the present case. The blister geometry implies an approximately isotropic strain distribution, as expected for a membrane inflated by internal pressure, rather than a uniaxial deformation. In closely related systems, such as self-suspended graphene blisters under controlled pressure, G-band splitting is typically not observed.

Furthermore, under genuine uniaxial strain both G-band components are expected to redshift relative to pristine graphite. In the present case, however, only one component exhibits a measurable redshift, which is inconsistent with the characteristic spectroscopic signature of uniaxial strain [9,142–146].

A more plausible interpretation is that the Raman signal collected from the blister region contains contributions from both the blister “skin” and the intercalated basal plane beneath it. Since the blister skin is only a few graphene layers thick and largely transparent to visible light, the Raman response from the underlying graphite cannot be neglected. Because the spectra from these two regions are intrinsically similar, disentangling their respective contributions is nontrivial. On this basis, the G-band component at 1583 cm^{-1} is attributed to the basal plane beneath the blister, while the redshifted component reflects the strained blister shell. It is worth noting that the maximum blister height is approximately 100 nm, which is well below the vertical resolution of conventional Raman spectroscopy (see section 2.3), making it impossible to selectively probe the blister skin by optical focusing alone.

To isolate the spectral contribution of the blister shell, each spectrum acquired on top of a blister was corrected by subtracting a reference spectrum collected from a nearby basal-plane region, as illustrated in Figure 5.13. In this figure, the grey curve represents the original blister spectrum, showing both G-band components and a shoulder at approximately 2720 cm^{-1} in the 2D band. After subtraction of the basal-plane contribution (red curve), the G-band component at 1582 cm^{-1} and the 2D-band shoulder are effectively removed, yielding the corrected spectrum shown in black. These features are therefore attributed to the underlying HOPG rather than to the blister skin itself. Within this first-order approximation, the subtraction procedure provides a reasonable estimate of the Raman spectrum intrinsic to the blister shell. This approach was systematically applied to all blister spectra included in the statistical analysis.

Using this subtraction procedure, a corrected Raman spectrum was obtained for each individual blister and used as the basis for the subsequent statistical analysis. A statistical analysis of the peak intensities was then performed. After fitting the relevant Raman bands, all peak intensities were normalised to that of the G band in order to facilitate comparison across different blisters. The resulting statistics are summarised in Table 5.1.

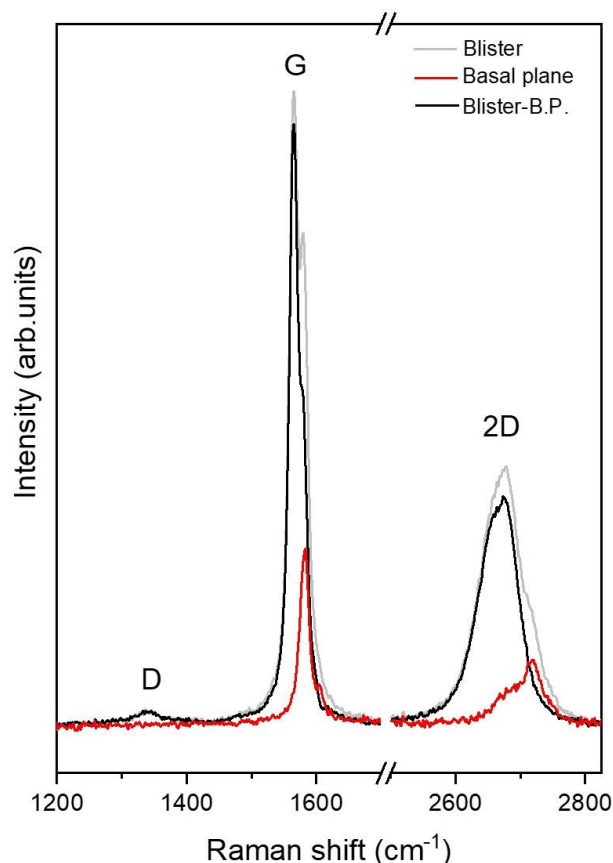


Figure 5.13: example of basal plane subtraction. The blister spectrum (grey line shape) presents two G contributions at 1582 cm^{-1} and 1566 cm^{-1} , and a 2D peak with a shoulder at 2720 cm^{-1} .

The statistical results confirm that the blister swelling process does not correlate with the intensity of the D band, although it should be emphasised that defect formation remains an important phenomenon influencing the overall properties of the blistered regions. The analysis of the G and G_i bands is more straightforward: each spectrum was fitted using two components, corresponding to the G and G_i peaks, as shown in Figure 5.14 (a). Consistent with previous observations and as discussed in detail later (see Table 2), a systematic redshift of the G band is observed for all analysed blisters.

The analysis of the 2D band in the presence of blisters is intrinsically more complex. As discussed previously, in pristine HOPG the 2D band is commonly described by a two-component deconvolution: a lower-energy, lower-intensity contribution ($2D_1$) and a higher-energy, more intense component ($2D_2$). In the blistered regions, the situation is further complicated by the fact that the structure consists of several graphitic layers that are locally swollen due to the presence of pressurised gas trapped during the cathodic phase of the cyclic voltammetry process.

In the case of graphene, the fitting strategy adopted for the 2D band strongly depends on the number of layers, as extensively reported in the literature. For bilayer graphene, the 2D band is typically decomposed into four components, two of which exhibit significantly higher intensity than the remaining two. When a laser excitation wavelength of 532 nm is employed and the number of graphene layers is equal to or greater than three, previous studies by Ni *et al.* and Graf *et al.* have shown that a two-component fitting model provides an adequate description of the 2D band [106,147].

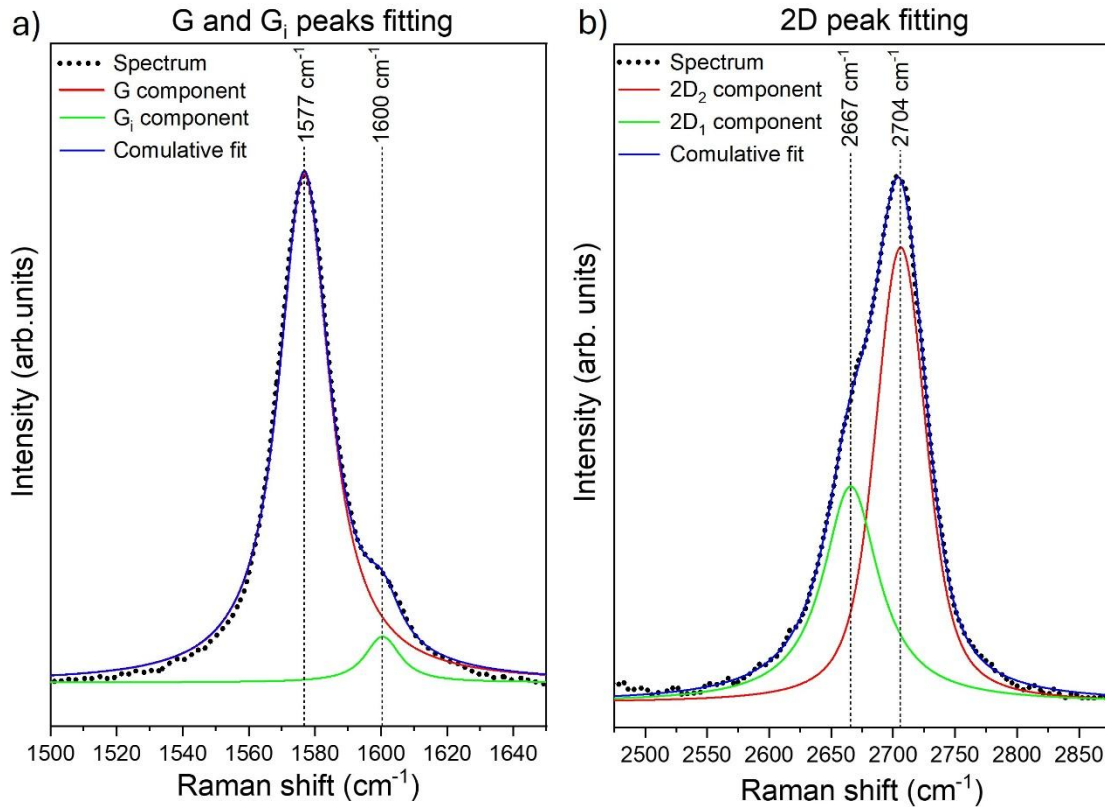


Figure 5.14: a) Fitting of the G and G_1 peak, with Voigt functions, red curve G peak, green curve G_1 peak, blu line cumulative fit, dot line spectrum. b) Fitting of the 2D peak, with Voigt functions, green curve $2D_1$ component, red curve $2D_2$ component, blu line cumulative fit, dot line spectrum.

Building upon established results on few-layer graphene, a two-component fitting approach was adopted for the analysis of the 2D band in blistered regions. This choice represents a reasonable compromise between physical relevance and fitting robustness for a system composed of a finite number of swollen graphitic layers. A representative example of this fitting procedure is shown in Figure 5.14 (b), where the $2D_1$ and $2D_2$ components are indicated by the red and green peaks, respectively. The blue curve corresponds to the overall fit, while the experimental spectrum is represented by the dotted line.

One of the primary objectives of this detailed analysis of the blisters was to obtain an estimate of the number of graphitic layers involved in the swelling process for each individual structure. This estimation was pursued by analysing the intensity ratio between the two components of the 2D band, following an approach conceptually analogous to that commonly adopted for few-layer graphene deposited on SiO₂ substrates. However, in the present system, the coexistence of multiple physical and chemical effects significantly complicates the interpretation.

Within the framework of the Murray model, the blister shell is expected to consist of only a limited number of monoatomic layers, typically ranging between two and fifteen. Such a finite thickness should modify the electronic band structure of the system and, consequently, affect the shape and relative intensity of the 2D band components. In principle, this behaviour could be compared to that observed in few-layer graphene flakes, where systematic variations in the 2D-band line shape as a function of layer number have been reported by Ferrari *et al.* [125,129].

In practice, however, the present system cannot be directly assimilated to isolated few-layer graphene, since the blistered regions are embedded in an intercalated graphite environment where defect formation and charge-transfer effects simultaneously modify the electronic structure. This situation departs from the simplified configuration introduced in section 3.4, where the blister skin was treated, as a first approximation, as a chemically pristine multilayer membrane mechanically decoupled from the bulk. The experimental evidence discussed here indicates that additional spectroscopic contributions must be considered when analysing the 2D-band response.

As discussed in the first part of this chapter, the 2D band of intercalated graphite already differs substantially from that of pristine HOPG. The presence of structural defects and intercalated ions, both well known to alter the electronic properties of graphite, strongly influences the Raman response and, in particular, the 2D band. As a consequence, as shown in Figure 5.4, the simultaneous presence of D and G_i bands is associated with a modified 2D-band profile and with 2D₁/2D₂ intensity ratios that differ markedly from those observed in pristine HOPG.

When examining the Raman spectra acquired on individual blisters, it is evident that both the D and G_i peaks exhibit non-negligible intensities. This observation further complicates the quantitative analysis, as it becomes challenging to disentangle the effects arising purely from the finite number of swollen layers from those induced by defect formation and ion intercalation during the electrochemical process. The overlap of these contributions prevents a straightforward application

of models developed for ideal few-layer graphene systems and highlights the intrinsically complex nature of blistered, intercalated graphite.

Despite the complexity discussed above, it is still possible to obtain a qualitative estimate of the number of layers forming the blister shell. To this end, the analysis is based on two complementary spectroscopic indicators: the $2D_1/2D_2$ intensity ratio, which is sensitive to changes in interlayer coupling, and the relative enhancement of the G-band intensity on the blister with respect to the basal plane. All these quantities are summarised in Table 1.

Blister	$I_G \text{ blister} / I_G \text{ HOPG}$	$I G_i \text{ (n. u.)}$	$I D \times 10^{-3} \text{ (n. u.)}$	$I 2D_1 \text{ (n. u.)}$	$I 2D_2 \text{ (n. u.)}$	$2D_1 / 2D_2$
1	$3.03 \pm 0,01$	Not resolved	$21,1 \pm 0,2$	$0,281 \pm 0,006$	$0,250 \pm 0,006$	$1,12 \pm 0,05$
2	$3.85 \pm 0,01$	$0,261 \pm 0,003$	$20,3 \pm 0,2$	$0,231 \pm 0,005$	$0,282 \pm 0,005$	$0,82 \pm 0,03$
3	$3,46 \pm 0,01$	$0,298 \pm 0,003$	$10,5 \pm 0,1$	$0,258 \pm 0,006$	$0,290 \pm 0,006$	$0,89 \pm 0,04$
4	$2.77 \pm 0,01$	$0,227 \pm 0,002$	$23,1 \pm 0,2$	$0,233 \pm 0,005$	$0,252 \pm 0,005$	$0,92 \pm 0,04$
5	$2.77 \pm 0,01$	$0,401 \pm 0,004$	Not resolved	$0,190 \pm 0,004$	$0,229 \pm 0,004$	$0,83 \pm 0,03$
6	$1.90 \pm 0,01$	$0,412 \pm 0,004$	Not resolved	$0,192 \pm 0,004$	$0,234 \pm 0,004$	$0,82 \pm 0,03$
7	$3.53 \pm 0,01$	Not resolved	$19,8 \pm 0,2$	$0,287 \pm 0,006$	$0,256 \pm 0,006$	$1,12 \pm 0,05$
8	$1.84 \pm 0,01$	$0,316 \pm 0,003$	120 ± 1	$0,182 \pm 0,005$	$0,281 \pm 0,003$	$0,64 \pm 0,03$
9	$2.41 \pm 0,01$	$0,304 \pm 0,03$	$50,4 \pm 0,5$	$0,183 \pm 0,005$	$0,252 \pm 0,003$	$0,72 \pm 0,03$
10	$4.29 \pm 0,01$	$0,152 \pm 0,008$	$94,2 \pm 0,9$	$0,190 \pm 0,006$	$0,258 \pm 0,003$	$0,74 \pm 0,03$
11	$2.29 \pm 0,01$	$0,080 \pm 0,04$	$68,4 \pm 0,7$	$0,151 \pm 0,005$	$0,280 \pm 0,003$	$0,54 \pm 0,02$
12	$3.50 \pm 0,01$	Not resolved	$47,6 \pm 0,5$	$0,163 \pm 0,005$	$0,314 \pm 0,003$	$0,52 \pm 0,02$
13	$2.17 \pm 0,01$	$0,531 \pm 0,05$	129 ± 1	$0,391 \pm 0,008$	$0,269 \pm 0,006$	$1,45 \pm 0,06$
14	$4.27 \pm 0,01$	$0,346 \pm 0,004$	$92,3 \pm 0,9$	$0,199 \pm 0,005$	$0,260 \pm 0,004$	$0,77 \pm 0,03$
15	$3.64 \pm 0,01$	$0,061 \pm 0,003$	$37,5 \pm 0,4$	$0,143 \pm 0,004$	$0,291 \pm 0,003$	$0,49 \pm 0,02$
16	$3.76 \pm 0,01$	$0,228 \pm 0,002$	$91,5 \pm 0,9$	$0,146 \pm 0,005$	$0,257 \pm 0,003$	$0,57 \pm 0,02$
17	$3.94 \pm 0,01$	$0,224 \pm 0,002$	$56,3 \pm 0,5$	$0,170 \pm 0,005$	$0,263 \pm 0,003$	$0,65 \pm 0,03$
18	$3.73 \pm 0,01$	$0,129 \pm 0,007$	$48,3 \pm 0,5$	$0,201 \pm 0,005$	$0,249 \pm 0,003$	$0,80 \pm 0,03$
19	$3.28 \pm 0,01$	$0,104 \pm 0,005$	$82,1 \pm 0,8$	$0,182 \pm 0,005$	$0,270 \pm 0,003$	$0,67 \pm 0,03$
20	$4.37 \pm 0,01$	Not resolved	$49,2 \pm 0,5$	$0,162 \pm 0,005$	$0,298 \pm 0,003$	$0,54 \pm 0,02$
21	$3.34 \pm 0,01$	$0,084 \pm 0,004$	$59,4 \pm 0,6$	$0,153 \pm 0,005$	$0,281 \pm 0,003$	$0,54 \pm 0,02$
22	$4.89 \pm 0,01$	$0,202 \pm 0,002$	190 ± 2	$0,169 \pm 0,004$	$0,201 \pm 0,004$	$0,85 \pm 0,04$
23	$2.67 \pm 0,01$	$0,212 \pm 0,002$	$73,8 \pm 0,7$	$0,178 \pm 0,005$	$0,282 \pm 0,003$	$0,63 \pm 0,03$

Table 5.1: I numbered each blister, for everyone I calculated or extrapolated the following values: ($I G_{\text{blister}} / I G_{\text{basal plane}}$) ratio, Intensity of G_i and D peaks, Intensity of $2D_1$ and $2D_2$ component, ($I 2D_1 / I 2D_2$) ratio.

According to the literature, the intensity of the $2D_2$ component becomes larger than that of $2D_1$ when the number of graphene layers is equal to or greater than

five. Furthermore, when the number of layers exceeds approximately 15–20, the overall shape of the 2D band approaches that of pristine HOPG, with a characteristic $2D_1/2D_2$ ratio of about 0.25 (see Figure 3.2). Concerning the G band, Ni et al. [106] reported a nearly linear increase in intensity with the number of layers up to 9–10 layers, reaching values up to six times higher than bulk HOPG.

For larger thicknesses, the G-band intensity progressively decreases until it converges to the bulk graphite response.

Based on the present statistical analysis, the majority of the investigated blisters excluding blisters #1, #7, and #13 exhibit a $2D_2$ component that is more intense than $2D_1$. This observation suggests that these blisters are composed of at least five graphitic layers. However, as discussed in the first part of this chapter, both the D and G_i bands contribute to an increase in the $2D_1/2D_2$ ratio in intercalated HOPG. This effect complicates the determination of an upper bound for the number of layers. For instance, a blister composed of approximately 15 layers would already display a $2D_1/2D_2$ ratio similar to that of pristine HOPG (see Figure A24 in the appendix section), but the additional presence of defects or intercalated ions can artificially increase this ratio, leading to an underestimation of the actual thickness.

In some specific cases where both the D and G_i band intensities are relatively low such as blisters #12 and #20, the $2D_1/2D_2$ ratio falls in the range 0.50–0.55. The corresponding 2D-band line shape (see appendix section), closely resembles that reported for pristine graphene systems consisting of 9–10 layers supporting a comparable thickness for these blisters.

An attempt was also made to correlate the $2D_1/2D_2$ intensity ratio with the G-band intensity ratio between blister and basal plane, with the aim of improving the accuracy of the layer-number estimation.

Based on the combined analysis of the 2D-band behaviour and the G-band intensity trends reported in the literature, the maximum number of layers is expected to be on the order of 20. However, no clear correlation between the $2D_1/2D_2$ and $G_{\text{blister}}/G_{\text{basal-plane}}$ ratios could be identified. As a result, this approach does not allow a precise and unambiguous determination of the number of layers for each individual blister.

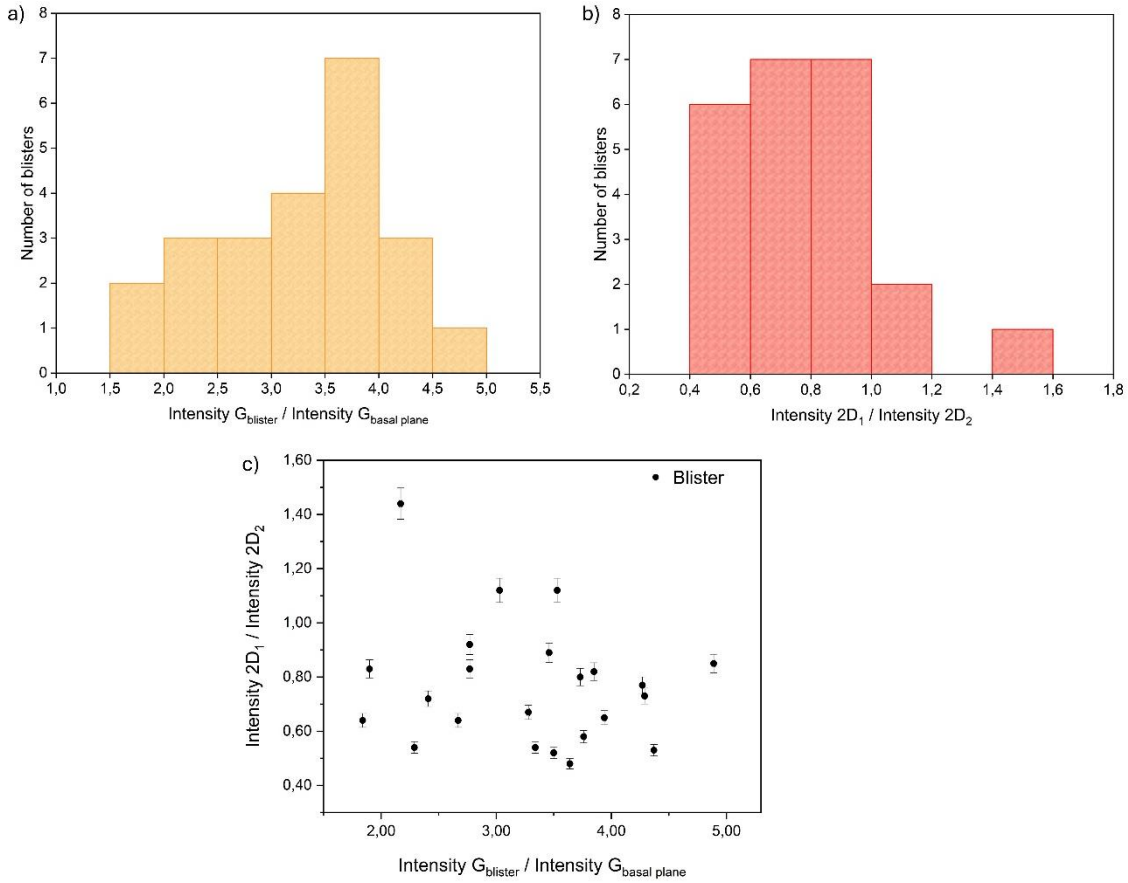


Figure 5.15: a) Distribution of the G-band intensity ratio $I_G^{blister} / I_G^{basal}$. b) Distribution of the 2D-band intensity ratio I_{2D_1} / I_{2D_2} . c) Correlation between the two ratios for individual blisters; error bars reflect fitting uncertainties.

In contrast, blisters #1, #7, exhibit $2D_1/2D_2$ ratios greater than unity. Since, for these cases, the contributions of the D and G_i bands are negligible or weak, it is reasonable to infer that these blisters consist of a smaller number of layers, likely in the range of 2 to 4. Also blister #13 exhibit $2D_1/2D_2$ ratios greater than one, but the higher intensity of D and G_i peaks they make a qualitative analysis of the 2D peak difficult for this blister.

These conclusions are in good agreement with previous work by Goss et al., who employed Auger electron spectroscopy to estimate blister thicknesses between 5 and 12 layers [13]. The present analysis extends these findings by indicating that some blisters may also consist of as few as 2–4 layers. This interpretation is consistent with STM observations, which systematically reveal a graphitic lattice within the blisters, implying the presence of at least two graphene layers.

Overall, it can be concluded that the number of layers forming the blister shell typically ranges between 2 and 15, with the majority of blisters consisting of 5 to 15 layers.

Finally, neither defect formation nor ion intercalation alone induces a redshift of the 2D-band components. Such a redshift is observed exclusively in the presence of blisters and can therefore be attributed to the swelling-induced strain of the graphitic layers. This observation indicates that the 2D-band redshift can be decoupled from other phenomena affecting the 2D-band shape and intensity. A more detailed analysis of this aspect will be presented in the next section.

5.2.4 AFM-Based Strain Estimation and Raman Correlation in Blisters

As discussed previously, the presence of blisters induces a systematic redshift of both the G and 2D Raman bands. This behaviour has also been reported in systems closely related to HOPG blisters, such as graphene bubbles and suspended bilayer or multilayer graphene structures inflated by a controlled internal gas pressure. In all these studies, the redshift of the Raman peaks is generally attributed to an isotropic stretching of the C–C bonds within the graphene layers, generated by the internal pressure.

Numerous studies have demonstrated a direct linear relationship between externally applied strain and the redshift of the G and 2D Raman bands. However, in the case of graphene bubbles, a direct and quantitative measurement of the applied strain remains challenging, and no experimental configuration has yet enabled the application of a truly isotropic, directly measurable, and continuously tuneable strain. The closest experimentally accessible analogue to isotropic strain is biaxial strain, which is commonly used as a reasonable approximation for describing the mechanical state of pressurised graphene membranes.

For single-layer graphene, multiple studies have reported a linear relationship between applied biaxial strain and the redshift of the G and 2D bands. In particular, the average reported shift rates are approximately -60 cm^{-1} per percent of strain for the G band, while the corresponding value for the 2D band is nearly twice as large, around -140 cm^{-1} per percent [148–151].

The situation becomes more complex as the number of layers increases. In multilayer systems, strain can induce a progressive loss of the crystalline ABAB stacking order described in the introduction. For bilayer graphene, the maximum strain that can be sustained without disrupting the stacking is approximately 1.2 % [9]. Below this threshold, the strain sensitivities remain comparable to those observed in single-layer graphene, with reported values of about $-58\text{ (cm}^{-1}\text{/\%)}$ for the G band and $-135\text{ (cm}^{-1}\text{/\%)}$ for the 2D band.

Androulidakis *et al.* [152] extended this analysis to multilayer graphene systems. For tri layer graphene, they reported strain sensitivities of approximately $-48 \text{ cm}^{-1}/\%$ for the G band and $-120 \text{ cm}^{-1}/\%$ for the 2D band, while for few-layer graphene the values further decrease to roughly $-39 \text{ (cm}^{-1}/\%)$ and $-90 \text{ (cm}^{-1}/\%)$ for the G and 2D bands, respectively. A key outcome of this work is that the strain sensitivity systematically decreases with increasing layer number. This behaviour was attributed to an incomplete transmission of strain from the bottom layer to the upper layers. Moreover, the authors observed that the linear correlation between strain and Raman shift is lost beyond approximately 0.25% strain, suggesting that, for systems thicker than bilayers, strain application rapidly leads to a partial decoupling or rearrangement of the layer stacking.

The central idea pursued here is to investigate whether a similar strain–Raman-shift relationship holds for HOPG blisters and whether their behaviour resembles that reported for few-layer graphene by Androulidakis *et al* [152]. In the case of blisters, however, the strain cannot be measured directly. Therefore, an indirect estimation approach is required.

To this end, the strain is estimated from AFM topographic data using a simple geometrical model. Specifically, each blister is approximated as a spherical cap, allowing the strain to be inferred by comparing the arc length of the swollen surface with the corresponding projected basal-plane distance. Although simplified, this model provides a first-order estimate of the tensile strain induced by the internal gas pressure and enables a quantitative correlation between blister morphology and Raman peak redshift.

The average blisters radius R_m was extracted from the AFM images as mean of the maximum and minimum radius (Figure 5.16b). From the AFM scans also the maximum height h of the blisters from the HOPG basal plane was extracted. A statistical analysis of the blister morphological parameters is reported in appendix. No simple correlation between radius and height was observed.

From this data the mean estimation of the % variation on the C–C bond of each the blisters was calculated with a spherical cap approximation of the blister morphology. Panel b) shows the spherical cap model used and a depiction of biaxial strain on the hexagonal structure of a monoatomic layer. R and h are the known parameters, the % strain is connected the ratio between the unstrained length l and the strained arc l' [7].

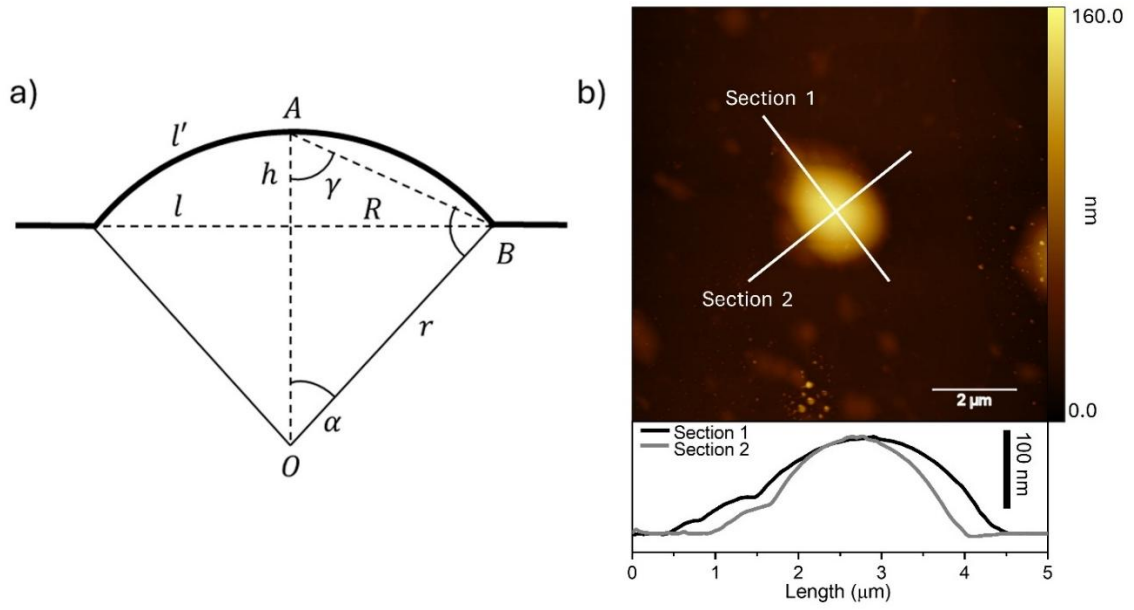


Figure 5.16: a) Scheme to calculate the blister, b) AFM image with two profiles, used to calculate the average radius.

From the morphological parameters R and h , extracted from the AFM scan, the % strain is calculated by the following equations. The angle γ can be obtained as:

$$\gamma = \arctan\left(\frac{R}{h}\right) \quad (5.1)$$

Since the AOB triangle is isosceles the angle α is:

$$\alpha = \pi - 2\gamma \quad (5.2)$$

Therefore the length l' of the circle arc is:

$$l' = \frac{4\pi r \alpha}{2\pi} = \frac{2\alpha R}{\sin(\alpha)} \quad (5.3)$$

The % strain is obtained as the normalized difference between the unstrained length $l = 2R$ and the strained arc l' , which is directly linked to the C-C bond elongation:

$$\% \text{ strain} = \frac{l' - l}{l} * 100 \quad (4)$$

Table 5.2 shows the extracted blister average radius R (μm) and height h (nm), the calculated % strain together with G and 2D positions (cm^{-1}). The data reported in Table 5.2 suggest a tendency for blisters with higher strain percentages to exhibit larger redshifts of the G and 2D peaks.

Blister/HOPG	h (nm)	R (μm)	% Strain	G shift (cm^{-1})	2D ₁ shift (cm^{-1})	2D ₂ shift (cm^{-1})
HOPG pristine	0	0	0	1582,0 $\pm$ 1,5	2677,0 $\pm$ 3,3	2720,0 $\pm$ 1,3
HOPG b.p. intercalated	0	0	0	1582,0 $\pm$ 1,5	2684,0 $\pm$ 3,3	2719,5 $\pm$ 1,3
1	118,5 $\pm$ 1,5	1,43 $\pm$ 0,02	0,461 $\pm$ 0,014	1568,3 $\pm$ 1,5	2656,2 $\pm$ 3,3	2684,2 $\pm$ 1,3
2	40,0 $\pm$ 0,5	1,03 $\pm$ 0,02	0,101 $\pm$ 0,003	1577,8 $\pm$ 1,5	2675,4 $\pm$ 3,3	2705,7 $\pm$ 1,3
3	90,2 $\pm$ 1,2	1,85 $\pm$ 0,03	0,165 $\pm$ 0,005	1579,0 $\pm$ 1,5	2678,7 $\pm$ 3,3	2708,3 $\pm$ 1,3
4	76,0 $\pm$ 1,0	1,95 $\pm$ 0,3	0,102 $\pm$ 0,003	1580,1 $\pm$ 1,5	2684,1 $\pm$ 3,3	2715,6 $\pm$ 1,3
5	44,8 $\pm$ 0,6	2,23 $\pm$ 0,04	0,030 $\pm$ 0,001	1578,3 $\pm$ 1,5	2679,8 $\pm$ 3,3	2712,8 $\pm$ 1,3
6	42,1 $\pm$ 0,5	1,35 $\pm$ 0,02	0,058 $\pm$ 0,002	1578,7 $\pm$ 1,5	2680,6 $\pm$ 3,3	2715,1 $\pm$ 1,3
7	114,5 $\pm$ 1,5	1,63 $\pm$ 0,03	0,334 $\pm$ 0,010	1566,8 $\pm$ 1,5	2652,4 $\pm$ 3,3	2680,0 $\pm$ 1,3
8	68,1 $\pm$ 0,9	1,93 $\pm$ 0,03	0,082 $\pm$ 0,002	1575,7 $\pm$ 1,5	2677,1 $\pm$ 3,3	2710,4 $\pm$ 1,3
9	70,0 $\pm$ 0,9	1,60 $\pm$ 0,03	0,129 $\pm$ 0,004	1577,2 $\pm$ 1,5	2670,8 $\pm$ 3,3	2703,6 $\pm$ 1,3
10	101,9 $\pm$ 1,3	1,70 $\pm$ 0,03	0,243 $\pm$ 0,007	1575,0 $\pm$ 1,5	2664,5 $\pm$ 3,3	2698,9 $\pm$ 1,3
11	67,3 $\pm$ 0,9	1,3 $\pm$ 0,02	0,177 $\pm$ 0,005	1578,4 $\pm$ 1,5	2670,6 $\pm$ 3,3	2707,2 $\pm$ 1,3
12	115,0 $\pm$ 1,5	2,00 $\pm$ 0,03	0,216 $\pm$ 0,007	1574,9 $\pm$ 1,5	2669,3 $\pm$ 3,3	2703,1 $\pm$ 1,3
13	97,7 $\pm$ 1,3	1,50 $\pm$ 0,03	0,284 $\pm$ 0,008	1570,0 $\pm$ 1,5	2667,1 $\pm$ 3,3	2696,4 $\pm$ 1,3
14	95,0 $\pm$ 1,2	1,38 $\pm$ 0,02	0,320 $\pm$ 0,010	1573,4 $\pm$ 1,5	2664,0 $\pm$ 3,3	2696,8 $\pm$ 1,3
15	85,4 $\pm$ 1,1	1,13 $\pm$ 0,02	0,379 $\pm$ 0,011	1572,8 $\pm$ 1,5	2667,4 $\pm$ 3,3	2704,0 $\pm$ 1,3
16	64,6 $\pm$ 0,8	1,45 $\pm$ 0,02	0,131 $\pm$ 0,004	1577,1 $\pm$ 1,5	2670,2 $\pm$ 3,3	2704,5 $\pm$ 1,3
17	65,2 $\pm$ 0,8	1,50 $\pm$ 0,03	0,127 $\pm$ 0,004	1574,0 $\pm$ 1,5	2667,0 $\pm$ 3,3	2700,3 $\pm$ 1,3
18	100,1 $\pm$ 1,3	2,00 $\pm$ 0,03	0,169 $\pm$ 0,005	1573,2 $\pm$ 1,5	2665,8 $\pm$ 3,3	2696,9 $\pm$ 1,3
19	84,9 $\pm$ 1,1	1,60 $\pm$ 0,03	0,194 $\pm$ 0,006	1576,4 $\pm$ 1,5	2669,9 $\pm$ 3,3	2706,0 $\pm$ 1,3
20	80,5 $\pm$ 1,0	1,25 $\pm$ 0,02	0,275 $\pm$ 0,008	1570,7 $\pm$ 1,5	2655,6 $\pm$ 3,3	2691,4 $\pm$ 1,3
21	105,4 $\pm$ 1,4	2,20 $\pm$ 0,04	0,148 $\pm$ 0,005	1576,2 $\pm$ 1,5	2668,0 $\pm$ 3,3	2704,2 $\pm$ 1,3
22	119,8 $\pm$ 1,6	1,65 $\pm$ 0,03	0,346 $\pm$ 0,011	1572,7 $\pm$ 1,5	2662,3	2695,7 $\pm$ 1,3
23	70,0 $\pm$ 0,9	1,45 $\pm$ 0,02	0,160 $\pm$ 0,005	1576,8 $\pm$ 1,5	2673,0 $\pm$ 3,3	2704,8 $\pm$ 1,3

Table 5.2: Morphological parameters extracted from the AFM analysis and correlated % strain estimation; position of the G and 2D band extracted from the fitting procedure.

A clear trend is observed between the G-band downshift and the strain estimated from AFM morphology: blisters exhibiting larger Raman shifts correspond to higher strain levels. Figure 5.17 illustrates the dependence of the G-band position on the strain percentage derived from the AFM-based geometrical analysis. In this representation, increasing strain is associated with progressively larger G-band redshifts.

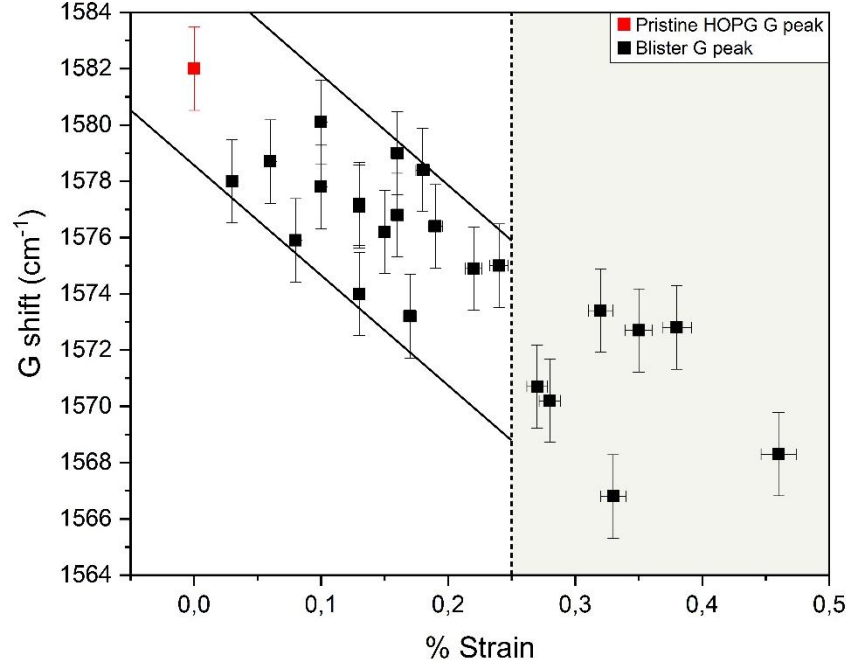


Figure 5.17: G peak Raman shift of 23 blisters spectrums as function of the calculated % strain, the downshift of the G peak in few layer graphite is consistent with a linear behaviour on the blister strain for a value less than 0.25%. For the confidence interval we considered the blister that has a strain less than 0.25 %. G peak confidence interval slope $m = (-39.3 \pm 0.2) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+1582 \pm 4) \text{ cm}^{-1}$.

In order to determine a reliable confidence interval for this correlation, only blisters experiencing strain levels below 0.25% were considered, since beyond this threshold the Raman response is expected to depart from the simple linear regime characteristic of coherently strained few-layer systems. This threshold was chosen based on the considerations discussed in section 5.2.3, according to which blisters subjected to higher strain are more likely to undergo sliding between the layers, that causes the losing of the crystal stacking ABAB (Figure 1.1). The resulting statistical analysis yields a confidence interval for the G-band shift characterised by a slope $m = (-39.3 \pm 0.2) \text{ cm}^{-1}/\%$ and an intercept $q = (1582 \pm 4) \text{ cm}^{-1}$.

As discussed in section 5.2.3, the majority of the analysed blisters are expected to consist of between 5 and 15 graphitic layers. Inspection of Table 5.2 further reveals that blisters presumed to have a smaller number of layers such as blisters #1, #7, and #13 exhibit strain values exceeding 0.25% and were therefore excluded from the correlation analysis. Under these assumptions, the present results can be directly compared with those reported by Androulidakis et al. [152] for few-layer graphene, who obtained a slope coefficient of $-38.9 \text{ cm}^{-1}/\%$ for the G band.

The excellent agreement between the two values strongly supports the validity of the AFM-based strain estimation and the applicability of the few-layer graphene

framework to the blister system within the regime of moderate deformation. At the same time, the exclusion of blisters with strain values above approximately 0.25% should not be regarded merely as a statistical choice, but as a physically meaningful indication that the linear strain–Raman-shift correlation progressively loses validity at larger deformation. In this regime, the mechanical response can no longer be described as that of an ideal uniformly strained multilayer membrane, since interlayer sliding, partial decoupling, or local rearrangement of the graphite stacking are likely to occur. More generally, these results establish a direct experimental link between the mechanical description of pressurised multilayer membranes and their Raman spectroscopic response. This demonstrates that, within the appropriate deformation regime, Raman spectroscopy can be used as a quantitative probe of strain in confined graphitic systems, bridging morphological, mechanical, and vibrational information.

A similar strain-dependent behaviour is observed for the 2D band, as shown in Figure 5.18. Separate correlations were performed for the two 2D components, yielding the following confidence intervals: for the 2D₂ component, a slope $m = (-98.8 \pm 0.3) \text{ cm}^{-1}/\%$ and an intercept $q = (2719 \pm 8) \text{ cm}^{-1}$; for the 2D₁ component, a slope $m = (-83.2 \pm 0.4) \text{ cm}^{-1}/\%$ and an intercept $q = (2686 \pm 8) \text{ cm}^{-1}$.

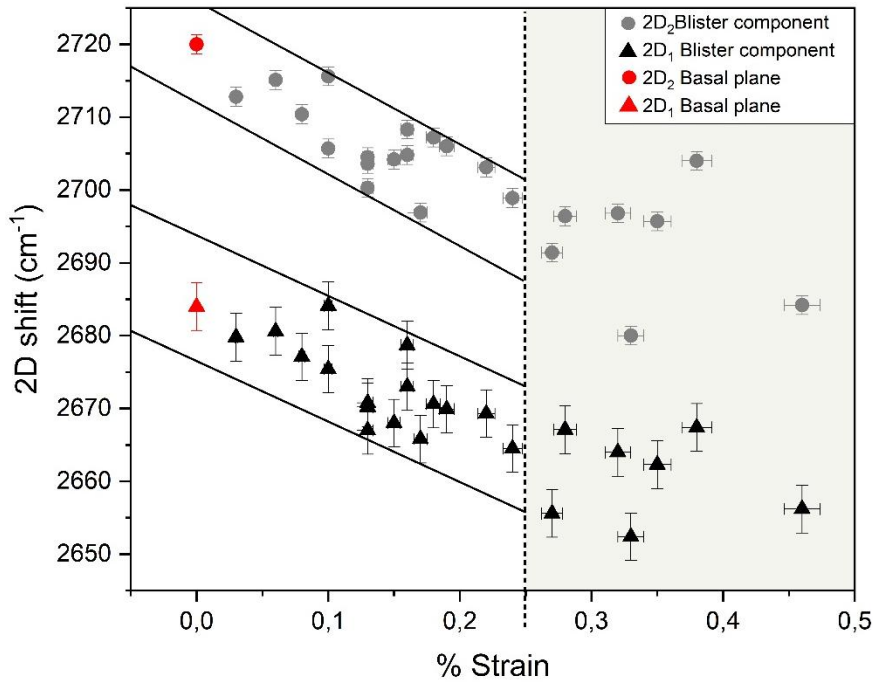


Figure 5.18: 2D peak components position as a function of the % strain, 2D₂ confidence interval slope $m = (-98.8 \pm 0.3) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+2719 \pm 8) \text{ cm}^{-1}$, 2D₁ confidence interval slope $m = (-83.2 \pm 0.4) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+2686 \pm 8) \text{ cm}^{-1}$.

These values are again consistent with the findings of Androulidakis et al. [152], who described the strain dependence of the 2D band in few-layer graphene using a single-component fit and reported a slope of $-90.9 \text{ cm}^{-1}/\%$. The agreement confirms that, despite the increased complexity of the blistered HOPG system, the strain-induced Raman response closely resembles that of few-layer graphene subjected to biaxial strain.

Also the dependence of the 2D-band components on the G-band position was evaluated, following the standard approach used in graphene-based systems to discriminate strain effects. [143]. The resulting correlations are shown in Figure 5.18.

For the present dataset, the confidence intervals obtained from the linear fits are as follows: for the $2D_2$ component, a slope $m = 2.18 \pm 0.01$ with an intercept $q = -728 \pm 4 \text{ cm}^{-1}$; for the $2D_1$ component, a slope $m = 2.05 \pm 0.01$ with an intercept $q = -553 \pm 7 \text{ cm}^{-1}$. These values are in excellent agreement with previous studies on graphene, which report slope coefficients for the correlation between the 2D band and the G band typically ranging between 2 and 2.5. This agreement further supports the interpretation of the Raman shifts observed in the blistered regions as being primarily driven by strain effects.

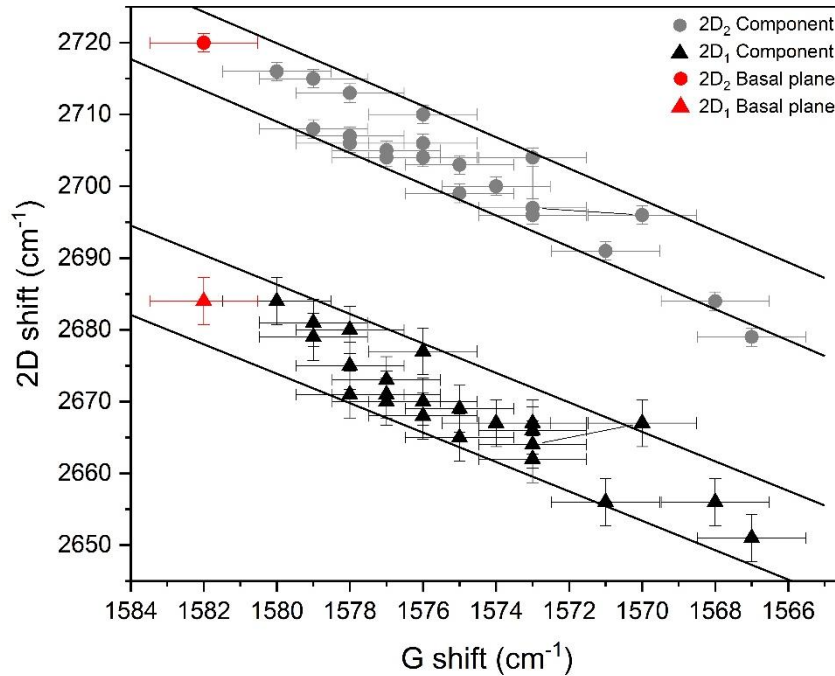


Figure 5.19: 2D peak components position as a function of the G position on blisters and basal plane, the downshifts are correlated, the points less bright are represents the blister under a strain $> 0.25\%$. The two lines represents the confidence interval: $2D_2$ confidence interval slope $m = 2,18 \pm 0,01$, confidence interval intercept $q = -728 \pm 4 \text{ cm}^{-1}$; $2D_1$ confidence interval slope $m = 2,05 \pm 0,01$, confidence interval intercept $q = -553 \pm 7 \text{ cm}^{-1}$.

In order to assess whether the Raman intensity enhancement is related to the blister morphology, the ratio $I_G^{\text{blister}}/I_G^{\text{basal plane}}$ was plotted as a function of the strain estimated from AFM morphology (Figure 5.20). As discussed in appendix, neither the blister radius nor the height alone exhibits a direct correlation with the spectroscopic response. However, their combined geometrical contribution allows the strain to be estimated.

Since strain represents the morphological parameter that shows a clear correlation with the Raman peak shifts, it is therefore natural to examine whether a similar dependence exists for the G-band intensity ratio.

The data, however, do not reveal a well-defined linear or monotonic trend. This indicates that, unlike the Raman peak shifts, the G-band intensity enhancement is not directly governed by the mechanical strain of the blister shell. Rather, it likely arises from a combination of optical interference effects, finite-layer contributions, and local chemical inhomogeneities

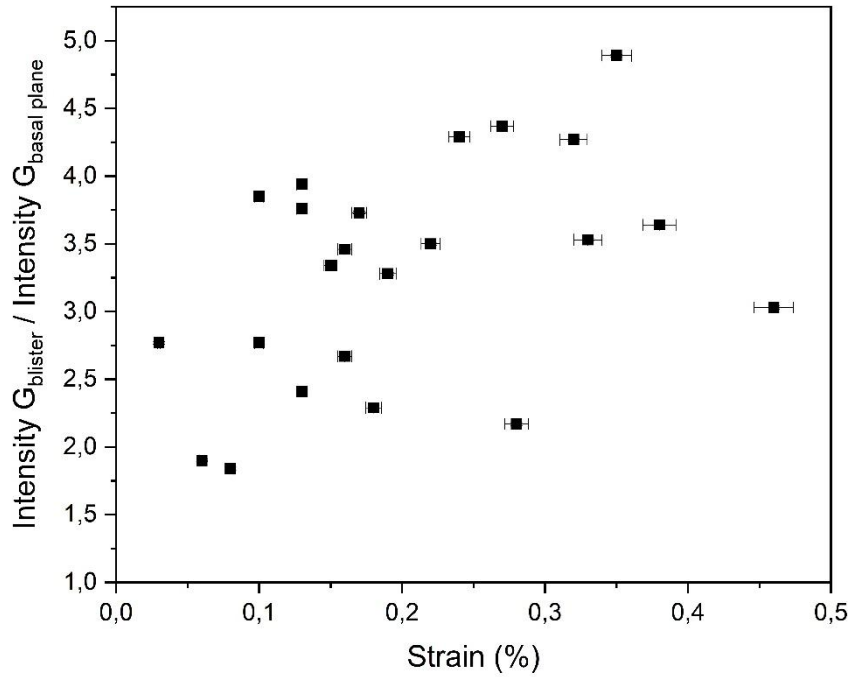


Figure 5.20: $I_G^{\text{blister}}/I_G^{\text{basal plane}}$ as a function of the strain percentage estimated from AFM morphology.

In conclusion, the results obtained from the combined analysis of G and 2D band shifts as a function of strain are particularly significant, especially when considering the intrinsic complexity of the 2D band in intercalated HOPG, where factors other than strain such as defects and ion intercalation—also influence its spectral properties. The robustness of the observed correlations confirms that the

subtraction procedure employed to remove the spectral contribution of the underlying basal plane provides a simple yet effective method for isolating the Raman response of the blister shell. Furthermore, despite the geometric and structural complexity of the blistered regions, the spherical-cap approximation used to estimate strain from AFM morphology proves to be remarkably effective in capturing the essential physics governing the Raman response.

This statement, however, must be understood within the validity range identified above. The spherical-cap approximation and the associated linear strain–Raman correlation provide a reliable effective description only as long as the blister shell preserves a sufficiently coherent multilayer stacking. At higher strain, the observed deviations suggest that the graphite layers no longer respond as an ideal few-layer membrane, and that stacking perturbation or local interlayer rearrangement must be taken into account.

In order to contextualize the Raman response observed in the present multilayer HOPG blisters, it is instructive to compare the geometric parameters and Raman observables reported in the literature for graphene membranes subjected to biaxial or isotropic strain conditions. Table 5.3 summarizes representative studies focusing exclusively on graphene systems, including controlled equibiaxial mechanical loading experiments and spontaneous or pressure-induced blister configurations.

Reference	Thickness class	Strain / blister generation	Bubble radius (μm)	Bubble height (nm)	Max. applied strain (%)	max G shift (cm^{-1})	$(d\omega_2/D / d\omega_{-G})$
Androulidakis et al., 2015	Monolayer	Controlled equibiaxial loading	–	–	~ 0.42	~ -25	$\sim 2.2 - 2.4$
	Bilayer	Same	–	–	~ 0.35	~ -22	$\sim 2.1 - 2.3$
	Few-layer	Same	–	–	~ 0.25	~ -18	$\sim 2,34$
Zabel et al., 2012	Monolayer and bilayer bubbles	Spontaneous gas-trapped bubbles	$\sim 2.5 - 5.0$	$\sim 50 - 200$	~ 1	~ -60	~ 2.4
Metten et al., 2014	suspended monolayer	Optical blister test	~ 2	pressure dependent	~ 1	~ -55	~ 2.3
Pandey et al., 2022	Multilayer graphene	Spontaneous blistering (AFM model)	$\sim 1-3$	~ 100	0,05	1,9	–
This work	Multilayer ($\sim 5 - 10$ L)	Electrochemical intercalation	1–2.5	50–120	~ 0.25	~ 12	2.02 ($2D_1/G$), 2.18 ($2D_2/G$)

Table 5.3: Literature comparison of graphene membranes under biaxial or isotropic strain conditions.

As previously discussed Androulidakis et al. provide a benchmark case of controlled equi biaxial strain applied to exfoliated graphene flakes. In that study, the maximum strain reached $\sim 0.42\%$ for monolayer graphene, progressively decreasing for bilayer and few-layer systems. The corresponding G-band redshift scales accordingly, confirming the reduced Raman sensitivity with increasing thickness under identical strain conditions. In the biaxial regime, the ratio between the 2D and G shifts, typically $d\omega_{2D}/d\omega_G \approx 2.2\text{--}2.4$ for monolayer graphene, establishes a well-known reference for isotropic tensile strain [152].

Spontaneously formed mono- and bilayer graphene bubbles, as reported by Zabel et al., exhibit larger strain values, reaching approximately 1% for monolayer graphene and up to $\sim 1.2\%$ in bilayer configurations before significant stacking modifications occur. In these systems, strain originates from gas trapping beneath the graphene membrane. The spherical geometry of the bubbles results in an approximately biaxial tensile state at the apex, with G-peak redshifts approaching -60 cm^{-1} and 2D/G correlation slopes again close to $\sim 2.3\text{--}2.4$, consistent with isotropic strain [9].

Similarly, the optical blister test developed by Metten et al. enables controlled pressurization of suspended monolayer graphene membranes. Under increasing pressure, the central region experiences biaxial stretching, with maximum strains approaching $\sim 1\%$, accompanied by significant G-band softening. These experiments provide a direct link between membrane bulging and Raman response under well-defined boundary conditions, with 2D/G correlation values comparable to those observed in other biaxial configurations [138].

More recently, Pandey et al. investigated multilayer graphene blisters formed on substrates, where strain values were derived from AFM-based geometric modelling, in full analogy with the approach adopted in the present work. In their study, the average strain at the blister apex was found to be on the order of $\sim 0.05\%$, associated with an average G-band redshift of approximately $-1,9\text{ cm}^{-1}$. Although their work primarily focused on mechanical instabilities rather than on a systematic Raman–strain calibration, the simultaneous reporting of geometrically derived strain and Raman shifts makes their results directly comparable to those presented here [4].

In comparison with these studies, the blisters investigated in the present work exhibit radii in the range $1.0\text{--}2.5\text{ }\mu\text{m}$ and heights between 50 and 120 nm, fully comparable to those reported for graphene bubbles in the literature. The maximum strain extracted from AFM geometry reaches $\sim 0.25\%$ in the linear correlation

regime, with corresponding G-band redshifts of -12 cm^{-1} , while larger shifts are observed outside the strict linear regime.

In order to quantitatively assess the strain state, the correlation between the 2D and G bands was analysed by taking the linear trend of ω_{2D_i} as a function of ω_G . The resulting slopes, defined as the coefficients $m = d\omega_{2D_i}/d\omega_G$, represent the ratio between the 2D and G softening rates under strain. In the present case, the extracted values $d\omega_{2D_1}/d\omega_G = 2.02$ and $d\omega_{2D_2}/d\omega_G = 2.18$ fall within the range reported for biaxially strained graphene. This agreement confirms that, despite the multilayer nature of the blister shell and the presence of interlayer shear effects, the strain state at the blister apex remains predominantly isotropic.

5.2.5 Evaluating the shift spatial variation on the blister

As a final step of the analysis, the correlation between the G-band shift and the blister morphology was investigated in order to evaluate how the strain-induced Raman response evolves spatially across the analysed area. Figure 5.17 shows the AFM topography of the same blister previously discussed in Figure 5.9.

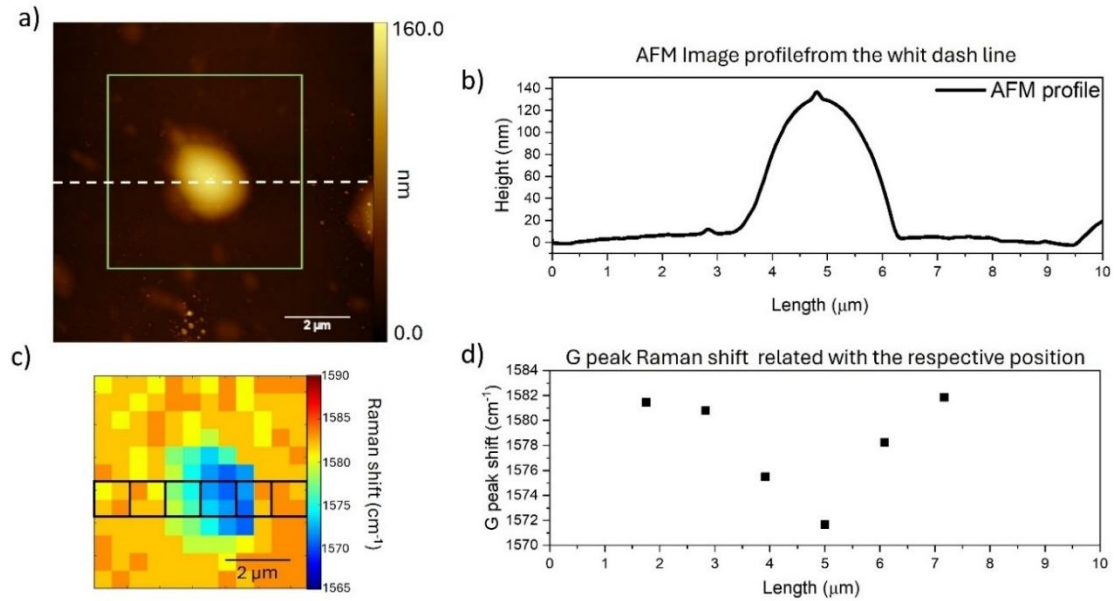


Figure 5.21: a) 15x15 μm AFM scan b) blister profile along the white section of panel a. c) 6x6 Raman map of the selected area showing the position of the G peak d) G peak position along the profile obtained with a Lorentzian fit, the data is correct with the subtraction of the basal plane data.

The height profile extracted along the white dashed line is reported in panel (b). Panel (c) presents a Raman map in which, instead of the peak intensity, the

colour scale represents the spatial variation of the G-band peak position, with each pixel indicating the locally measured Raman shift.

In panel (c), the pixels selected for extracting the G-band shift along the topographic profile are explicitly highlighted. For each square region, the Raman spectra corresponding to the four enclosed pixels were extracted and averaged in order to improve the signal-to-noise ratio. The resulting averaged spectrum was then fitted to determine the precise position of the G band at that location.

By correlating panels (b) and (c), it becomes evident that the maximum G-band downshift occurs at the apex of the blister, where the Raman shift reaches approximately 1572 cm^{-1} , corresponding to a redshift of about 10 cm^{-1} with respect to the G-band position of pristine HOPG. Moving away from the blister centre, the G-band position progressively recovers, following an approximately symmetric trend, until it reaches the basal-plane value of 1582 cm^{-1} .

A closer inspection of panel (c) reveals that, when moving radially outward from the blister centre, the G-band downshift gradually decreases. A small but detectable residual variation persists slightly beyond the apparent blister boundary into the surrounding HOPG regions. This behaviour is fully consistent with previous reports on biaxially strained graphene bubbles, which exhibit a very similar spatial distribution of the Raman G-band shift [9,142].

The AFM–Raman correlation discussed in this chapter provides clear evidence that blister formation is accompanied by measurable modifications of the Raman response, which can be interpreted in terms of strain and local structural changes.

However, due to the diffraction-limited spatial resolution of conventional Raman spectroscopy, these measurements necessarily yield spatially averaged information over regions much larger than the characteristic size of individual blisters. As a result, local strain heterogeneities particularly between the blister centre and its perimeter cannot be fully resolved. To overcome this limitation and access vibrational information at the nanometric scale, the following chapter explores the application of tip-enhanced Raman spectroscopy (TERS).

6

Tips Enhanced Raman Spectroscopy on HOPG

The previous chapter demonstrated how the combined use of AFM imaging, nanoindentation, and mechanical manipulation provides a powerful experimental framework to investigate the mechanical response of graphite blisters formed during electrochemical intercalation. Through these approaches, it was possible to describe how internal gas pressure, strain distribution, and elastic relaxation govern the deformation of the upper graphitic layers. While these results offer an essential understanding of the blister morphology and mechanics at the micrometre scale, they also raise a number of questions regarding the structural and spectroscopic signatures associated with such highly deformed regions of the graphite surface. In particular, the extent to which nanoscale defects, strain fields, and local variations in lattice symmetry emerge during the swelling process cannot be fully resolved through conventional Raman spectroscopy, whose spatial resolution is ultimately limited by optical diffraction.

For this reason, in the present chapter, the investigation of intercalated graphite systems is extended through the use of Tip-Enhanced Raman Spectroscopy (TERS), well explained in the experimental set-up section . By exploiting the electromagnetic field enhancement generated at the apex of a metallic AFM or STM tip, TERS makes it possible to acquire Raman spectra with nanometric spatial resolution several orders of magnitude smaller than the diffraction limit. This capability is particularly valuable when studying systems such as steps and blisters in intercalated HOPG, where the structural modifications induced by intercalation occur over length scales that are far smaller than those accessible with conventional far-field Raman measurements.

In recent years, Tip-Enhanced Raman Spectroscopy has emerged as a powerful tool for investigating graphene and other layered materials at the nanometric scale. Early demonstrations of TERS on graphene established the capability of the

technique to spatially resolve edge-related defect signatures and local variations of the D and G bands beyond the diffraction limit [153]. These studies showed that step edges, grain boundaries, and structural discontinuities exhibit enhanced defect-related Raman features when probed with near-field excitation, highlighting the intrinsic sensitivity of TERS to local lattice symmetry breaking.

Subsequent works extended the application of TERS to more complex graphene-based systems, including epitaxial graphene on SiC and multilayer configurations, demonstrating that local variations in stacking order, strain, and electronic structure can be directly correlated with modifications of the Raman line shape and peak positions [154]. In particular, the capability of TERS to detect nanoscale strain distributions has been reported in systems such as graphene wrinkles and suspended nanostructures, where local redshifts of the G and 2D bands were mapped with spatial resolutions below 50 nm [155].

More recently, nano-Raman and TERS approaches have been successfully applied to twisted bilayer graphene and reconstructed superlattices, revealing spatial localization of vibrational modes and strain solitons that are inaccessible to conventional far-field spectroscopy [156]. These results demonstrate that near-field Raman techniques provide direct access to localized phonon behaviour and electronic-vibrational coupling in structurally modulated graphene systems.

Despite these advances, the application of TERS to electrochemically intercalated graphite and, in particular, to pressurized blister structures remains largely unexplored. The present work therefore extends the use of TERS to a system in which curvature, intercalation, and strain coexist, enabling the investigation of lattice vibrations at the nanometre scale in a mechanically deformed bulk layered material.

TERS is especially suited to address some of the outstanding questions left open by the previous analyses. First, the technique allows one to probe the presence and distribution of defects with unprecedented precision, clarifying whether morphological irregularities such as step edges or blister borders act as preferential sites for lattice disorder or not. Second, it enables the direct measurement of Raman peak shifts in highly localized regions, allowing one to evaluate how strain evolves when transitioning from the basal plane to the swollen areas. This level of detail is essential for understanding the interplay between mechanical deformation, lattice symmetry breaking, and the associated vibrational response of graphite. Finally, TERS provides a means to assess the consistency between nanoscale Raman measurements and the more global spectroscopic signatures previously obtained

through AFM-Raman correlation, offering an important validation of the methodologies used throughout this work.

The chapter is therefore structured to gradually build a coherent picture of the spectroscopic and morphological properties of HOPG under intercalation conditions. It begins with TERS measurements performed on pristine graphite. The analysis is then extended to intercalated HOPG, where the modifications induced by the electrochemical process become evident at the nanometre scale. Finally, TERS is applied to the blisters themselves, allowing a direct comparison with the AFM-based observations presented in Chapter 5 and providing new insights into the strain distribution across these unique nanostructures.

In this way, the combination of TERS and STM described in this chapter offers a complementary perspective to the mechanical investigations performed earlier, enabling a deeper and more complete understanding of how intercalation affects the graphite lattice from both structural and spectroscopic viewpoints.

6.1 TERS on pristine HOPG

In Figure 6.1 are reported the experimental results obtained on pristine HOPG. The 3D STM image shows a portion of the surface where it is possible to clearly distinguish a multiatomic step, approximately 10 nm in height, indicated as region (i). It is important to underline that the STM image dimension is considerably smaller than the typical spatial resolution achievable by spontaneous Raman spectroscopy. In fact, considering that the diameter of the Airy disk produced by the 633 nm laser beam is around 800 nm, it would not be possible, without the TERS contribution, to separate the Raman spectrum collected on the step from the one corresponding to the adjacent basal plane. Under these conditions, TERS generates a strongly confined electromagnetic field at the tip-sample junction, enabling the acquisition of Raman information with nanometric spatial resolution beyond the diffraction limit.

In Figure 6.1a, two TERS spectra are reported: the first acquired directly on the step region, and the second on the basal plane near it. It can be observed that in the spectrum recorded on the step, both the D and D' peaks are present. These peaks are typically associated with the presence of structural defects within the carbon lattice, as previously discussed in the theoretical background [53,128]. Their appearance therefore confirms that the step region acts as a defect site within the otherwise ordered graphitic structure of HOPG. The result obtained through TERS

is particularly significant because it provides a direct spectroscopic proof of this phenomenon on a nanometric scale.

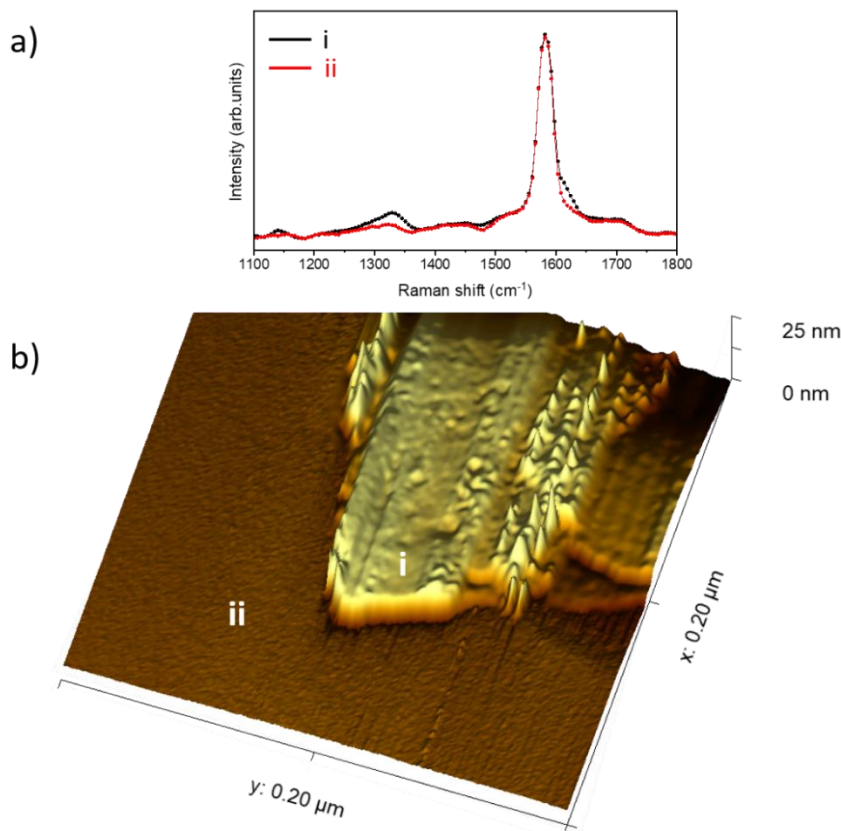


Figure 6.1: **a)** TERS spectra, i) taken on the edge of a pristine HOPG step, ii) TERS spectra taken on the HOPG basal plane. **b)** 3D STM image, $200 \times 200 \text{ nm}^2$, $V_{\text{bias}} = 0.1 \text{ V}$, $I_T = 1 \text{ nA}$.

This observation is in agreement with what has been already reported in literature [131,157]; however, it acquires particular relevance in this work since, to the best of current knowledge, no Raman spectrum has ever been directly collected on a step of an HOPG crystal. The experiment, in this case, was intentionally limited to the acquisition of two single spectra instead of performing a complete mapping. This choice was mainly dictated by the need to achieve a sufficiently high signal-to-noise ratio to clearly distinguish the D and D' peaks. To reach this level of accuracy, it was necessary to set an integration time of approximately 20 seconds for each acquisition point.

A complete map under these conditions would have required a much longer acquisition time, making the experiment particularly sensitive to possible mechanical drifts between the TERS signal and the STM topographic image. Such drifts could have compromised the precise correspondence between the structural information obtained from the STM image and the spectroscopic data collected through TERS. Therefore, a point-by-point acquisition was preferred, ensuring a

better control of both the signal quality and the spatial alignment of the measurements.

6.2 TERS on steps of intercalated HOPG

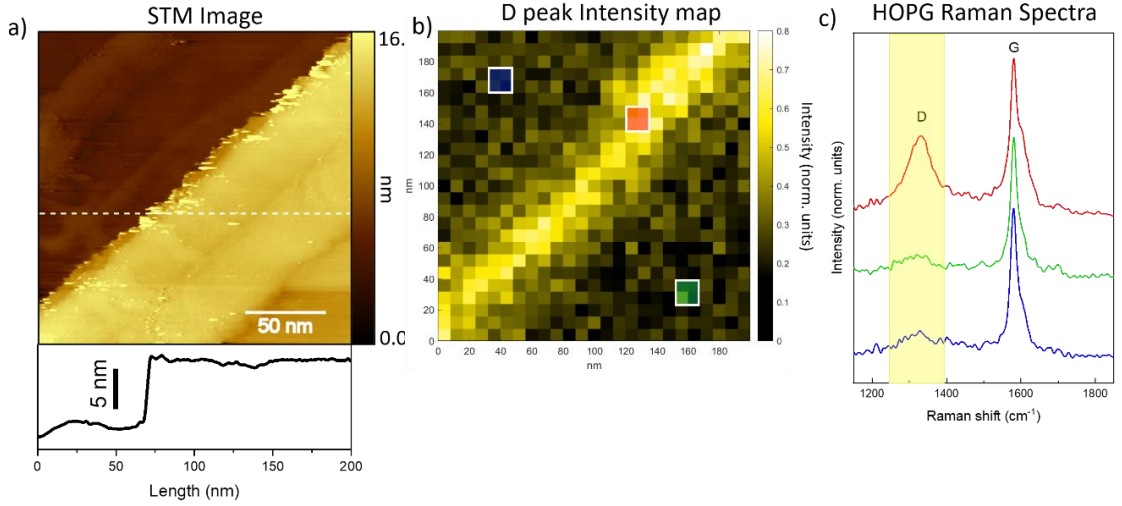


Figure 6.2: a) STM image of the a step of the Intercalated HOPG $200 \times 200 \text{ nm}^2$ $V_{\text{bias}} = 0.1 \text{ V}$, $I_T = 950 \text{ pA}$. b) TERS D peak map, each squares indicate the position of the 3 spectra in panel c, with the respective colour, c) 3 TERS spectra extracted from the TERS map.

The results obtained from the analysis of the intercalated HOPG surface are presented in Figure 6.2. Although the observed step height is comparable to that previously discussed in Figure 6.1, the intensity of the D peak is remarkably higher. This pronounced enhancement of the Raman signal allows the acquisition of a complete TERS map (Figure 6.2b), which makes it possible to establish a direct correlation between the spectroscopic response and the morphological characteristics of the investigated region. It is important to emphasize that both the lateral dimensions of the TERS map and the corresponding STM image are significantly smaller than the spatial resolution attainable through conventional spontaneous Raman spectroscopy. This highlights the capability of TERS to probe localized regions at the nanometric scale, providing spectroscopic information that would otherwise be inaccessible.

As shown in panel c), the intensity of the D peak does not vanish across the entire analysed area, but it is clearly more pronounced along the step edge. This behaviour can be interpreted considering the partial dissolution of HOPG occurring during the intercalation process [47,51]. Indeed, step edges act as preferential sites for the insertion of anions and consequently undergo a higher dissolution rate, which

leads to the formation of localized regions characterized by an increased defect density [47].

Furthermore, during the cyclic voltammetry (CV) process, the step regions are progressively damaged due to the corrosive action of the acidic medium. As a consequence, an enhancement of the D peak is observed at step edges, particularly for low-height steps (e.g., ~ 1.5 nm) compared to higher steps (e.g., ~ 8 nm, Figure 6.2), as shown in Figure 6.3.

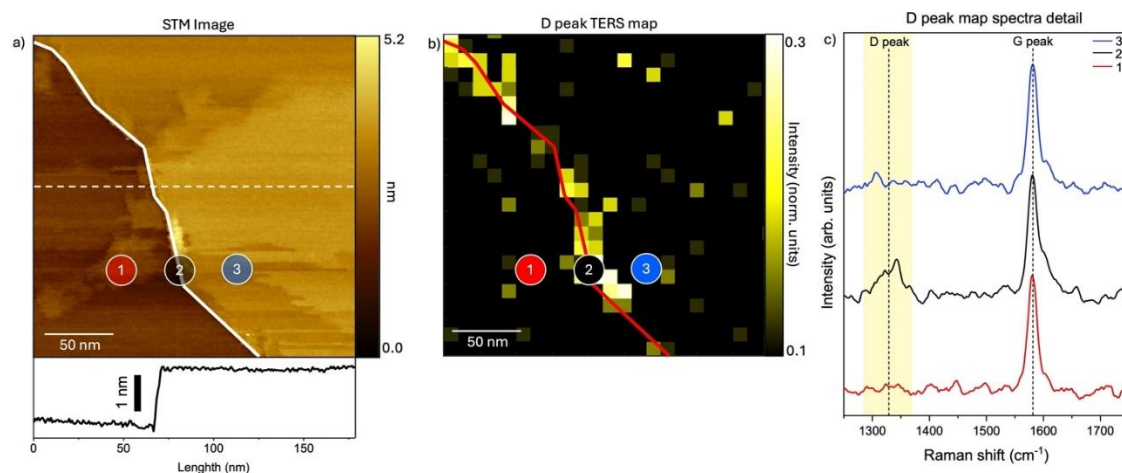


Figure 6.3: a) STM image of the a step of the Intercalated HOPG 200×200 nm², the white line highlight the step position, the numerate circles indicates the position of the three spectra in panel c, $V_{\text{bias}} = 0.1$ V, $I_T = 900$ pA. b) TERS D peak map, the red line highlight the step position, the numerate circles indicates the position of the three spectra in panel c. c) 3 TERS spectra extracted from the TERS map.

In Figure 6.3, panel a) displays an STM image of an intercalated HOPG surface, where a step feature outlined by a white dashed line is clearly visible. The measured step height, approximately 1.6 nm, is nearly one order of magnitude smaller than those previously reported in Figure 6.2, indicating the presence of a finer structural irregularity. For clarity, the same step is also highlighted in panel b), allowing a direct visual correlation between the topographic and spectroscopic data.

From the TERS map shown in panel b), it can be observed that the D peak remains distinctly visible in correspondence with the step region, whereas its intensity gradually decreases in the surrounding areas. This behaviour provides further confirmation that even at smaller step heights, structural defects persist as a consequence of the intercalation and partial dissolution processes affecting the graphite lattice.

The combined interpretation of the STM and TERS data thus enables a deeper understanding of the mechanisms through which electrochemical intercalation alters

the HOPG surface morphology at the nanoscale. As emphasized in panel c), the D peak signal is not homogeneously distributed across the analysed area but rather localized near specific defect sites, demonstrating the capability of TERS to resolve variations in defect density with nanometric precision. In this way, it becomes possible to achieve an unprecedented level of detail in the study of the intercalation process, directly linking morphological modifications to the corresponding spectroscopic signatures.

6.3 TERS investigation of intercalated HOPG blisters

Before discussing the experimental results, it is important to consider the vertical spatial resolution of TERS, as already introduced in Chapter 2. The effective vertical resolution in TERS is governed by the decay length of the near-field electromagnetic enhancement at the tip apex (section 2.5). This field distribution is, in turn, strongly influenced by the exact geometry of the metallic tip. Because the tips are fabricated by electrochemical etching, their shape can vary significantly from one tip to another, leading to variations in the effective probing depth.

Despite these uncertainties, Experimental and theoretical studies typically report a vertical resolution in the range of approximately 1–2 nm [158]. Considering the interlayer spacing in HOPG (0.335 nm), this corresponds to a probing depth of roughly 3 to 6 graphitic layers from the surface.

This aspect becomes particularly critical when correlating the observed Raman shifts with the strain within the blister. If the blister shell consists of more than six layers, TERS predominantly probes only the uppermost layers rather than the entire swollen structure. Previous studies have shown that the outermost layers experience a lower degree of strain compared to the deeper ones, which are more directly affected by the internal pressure. Consequently, the Raman shifts measured by TERS may reflect primarily the strain of the uppermost layers of the blister shell, rather than the average strain of the entire swollen structure. This aspect should be considered when interpreting the quantitative strain–shift relationship.

As discussed at the end of this chapter, a detailed analysis of the 2D band and therefore a precise determination of the number of layers forming the blister shell was not feasible due to the presence of etalon effects. This limitation further constrains the interpretation of the TERS results and highlights the need to

carefully account for the finite probing depth of the technique when drawing quantitative conclusions.

At first, a comparison was carried out between the TERS results obtained on the top of the blister and those previously acquired through Raman-AFM correlation measurements. The selected blister, shown in Figure 6.4, presents morphological characteristics comparable to those discussed and analysed in Chapter 5, with an average diameter of approximately 1 μm and a height of around 40 nm. These dimensions make it representative of the typical structures formed during the intercalation process, allowing a consistent comparison with the data previously acquired through other experimental techniques.

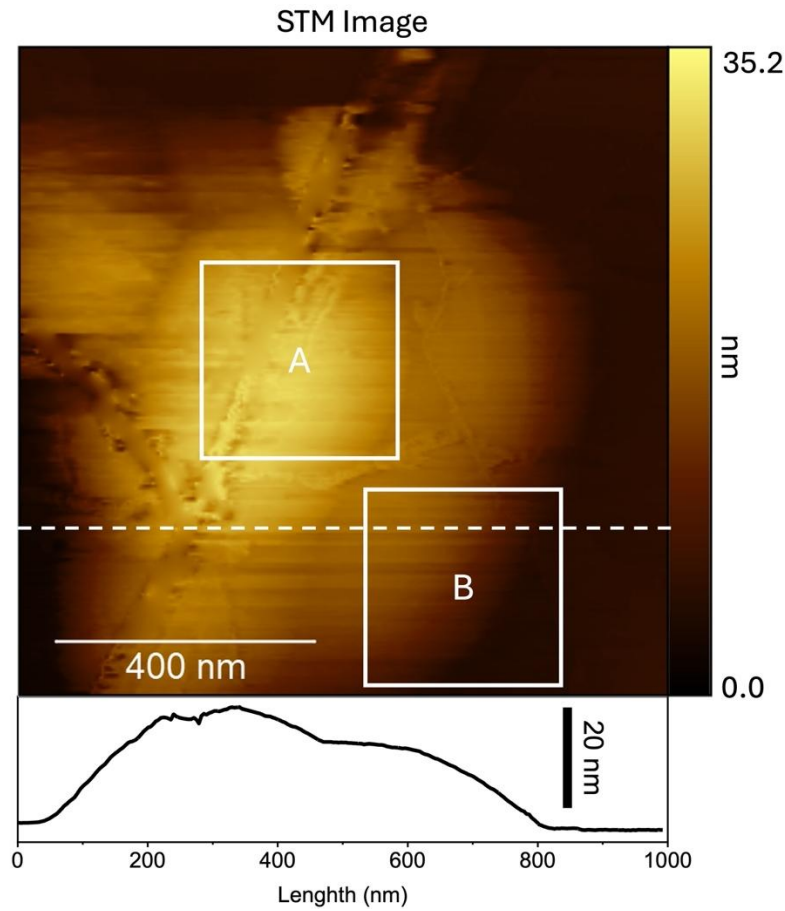


Figure 6.4: STM image of the wall blister $1000 \times 1000 \text{ nm}^2$, the square indicates the two areas analysed with STM and the TERS $V_{\text{bias}} = 0.1 \text{ V}$, $I_{\text{T}} = 900 \text{ pA}$.

In the following analysis, two distinct regions of investigation were considered. The first, referred to as *region A*, corresponds to the uppermost part of the blister. This specific area was selected in order to directly compare the TERS spectra with those obtained from the AFM-Raman spectroscopy correlation, thereby verifying the consistency of the spectroscopic response between the two methodologies. Such

a comparison is essential to confirm the reliability of TERS measurements when applied to highly curved and strained regions of the graphite surface.

The second investigated region was instead selected along the blister edge. In this case, the purpose was to monitor the evolution of the TERS spectra as a function of position, moving progressively from the edge toward the central part of the swollen region. This approach was conceived in order to understand how the Raman spectral features, particularly the frequency shifts of the characteristic vibrational modes, evolve in relation to the blister morphology and the local mechanical strain.

In this way, it was possible to address one of the most relevant questions arising from the previous AFM-Raman correlation study namely, the relationship between the strain induced by the pressurized gas and the corresponding Raman shift. The TERS measurements allowed to directly observe whether the downshift of the Raman bands gradually increases from the edge toward the centre of the blister, or whether it is already present as soon as the upper graphite layers detach from the underlying ones due to the internal gas pressure. This analysis, therefore, provides new insights into the interplay between strain distribution, morphological deformation, and the resulting spectroscopic response at the nanoscale.

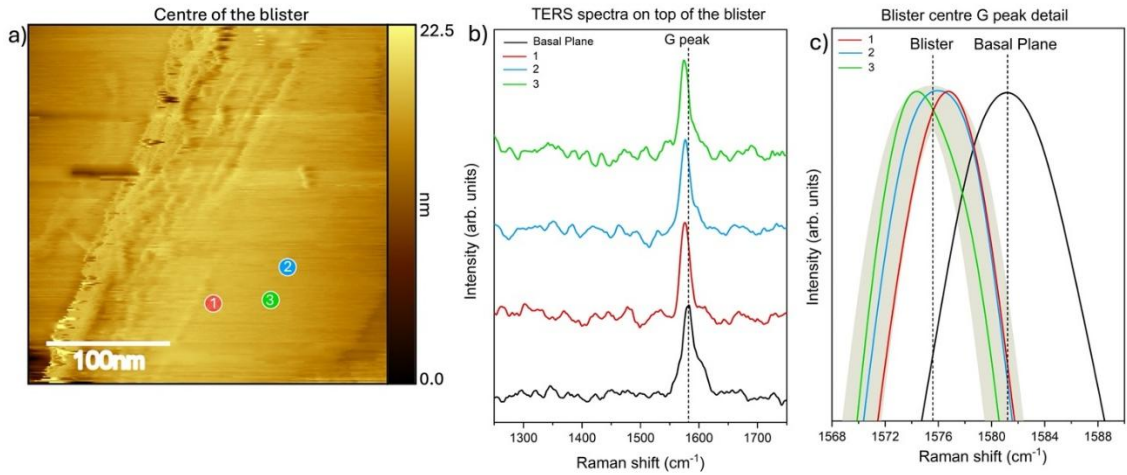


Figure 6.5: **a)** $300 \times 300 \text{ nm}^2$ STM image, the number in the circle indicates the position of the TERS spectra, in panel b. Each circle has the dimension of the TERS spatial resolution, $V_{\text{bias}} = 0.1 \text{ V}$, $I_{\text{T}} = 900 \text{ pA}$. **b)** full TERS spectrum of the three point indicate in panel a, the black spectrum is the on of the basal plane. **c)** Detail of the G peak presented in panel b, the grey region indicates the variance of the different spectra acquire in the analysed region.

In Figure 6.5 the results obtained on region A of the selected blister are presented. In panel (a), the STM image is shown, together with the exact positions where the individual TERS spectra were acquired, which are reported in panels (b)

and (c). The diameter of the circles marking each acquisition point corresponds to the estimated lateral spatial resolution of the TERS measurement. It is worth noting that, on the left side of panel (a), a step crossing the blister surface is visible, running above the swollen region.

It is important to emphasise that the three spectra were collected in extremely close proximity to one another, with an average lateral separation smaller than 80 nm. This short distance highlights the high spatial resolution achieved through TERS, allowing the detection of variations in the Raman response at the nanoscale.

As explained in Chapter 2, single-point TERS spectra were acquired in this case for two main reasons. The first was the need to record two spectra for each position: one with the tip in contact with the sample and another with the tip retracted by 50 nm. This procedure enables the acquisition of both spectra with and without the TERS enhancement, while still remaining within the vertical resolution of spontaneous Raman spectroscopy. In this way, by subtracting the retracted-tip spectrum from the one acquired in contact, it is possible to isolate the pure TERS contribution. Moreover, this subtraction efficiently removes the Raman contribution arising from the basal plane underneath the blister a similar post-processing strategy to the one applied in the AFM-Raman correlation experiments described earlier. Since the vertical resolution of the near-field interaction is smaller than the blister height, the TERS signal can be confidently attributed to photons scattered exclusively from the blister surface.

Figure 6.5 b shows the complete TERS spectra acquired at the selected points, together with a reference TERS spectrum collected on the basal plane, in an almost pristine region of the graphite surface. The first relevant observation is that the D peak intensity is practically null in all spectra acquired on the blister. This indicates that the swelling of the graphite layers does not introduce structural defects on the surface. This finding confirms the previous results obtained through AFM-Raman correlation and demonstrates that, despite the significant morphological deformation associated with blister formation, the graphite lattice remains structurally intact. This result is particularly important, as it shows that strain and defect formation are not intrinsically coupled in this system: the deformation induced by the internal gas pressure is accommodated through elastic strain and interlayer rearrangement, without leading to lattice damage.

The second important result concerns the G peak position. In all three spectra, the G peak exhibits a clear redshift relative to the basal-plane position. This shift is consistent with the results discussed in Chapter 5, obtained through conventional

Raman measurements, confirming that the swelling process induces a tensile strain in the upper layers of graphite.

Panel c) reports a detailed view of the G peak region, allowing a more accurate visualization of this spectral shift. As already visible in panel b), all three TERS spectra exhibit an evident redshift of the G peak; however, it is possible to notice that the peak position slightly varies among the three spectra. This is not a random fluctuation. Although only three representative spectra are shown in Figure 6.5, a total of eleven spectra were actually acquired over ten distinct points across the blister surface. The variability of the G peak position across all measurements is represented by the grey shaded area plotted in panel c). The average G peak position measured on the blister surface is 1575.5 cm^{-1} , whereas the corresponding value measured on the basal plane is 1581.7 cm^{-1} , resulting in an average redshift of approximately 6.2 cm^{-1} .

This result is particularly relevant because it confirms that, on top of the blister, a consistent redshift of the G peak is observed, in full agreement with the conclusions drawn in Chapter 5. Furthermore, thanks to the high spatial resolution of TERS, it is possible to detect a local variability in the G peak position, always shifted with respect to the basal plane but not homogeneous over the blister surface.

This non-uniformity indicates that the strain distribution is not uniform but exhibits significant variations at the nanometric scale, depending on the local morphology and curvature of the surface. While conventional Raman measurements provide an average response over the entire blister, TERS allows direct access to this local strain distribution, revealing spatial variations that would otherwise remain unresolved.

At the same time, the consistency between TERS and AFM-Raman results confirms that these local variations develop around a well-defined average strain state, ensuring that the nanoscale heterogeneity does not contradict, but rather refines, the macroscopic picture obtained from far-field measurements.

The results obtained in area B (Figure 6.4) represent the most significant outcome achieved with TERS measurements on blisters, as they provide a clear answer to the key question regarding how the G peak shifts at the blister border. In panel a) the STM image of the blister edge is shown, together with the positions where the TERS spectra were acquired, as reported in panel b). Each spectrum was collected along a single line, with a spatial separation of 40 nm between consecutive acquisition points. The third spectrum corresponds exactly to the position where the upper graphite layers detach from the underlying, non-swollen region. Starting

from this point, two spectra were recorded outside the blister, on the adjacent basal plane namely spectra 1 and 2 at distances of 80 nm and 40 nm from point 3, respectively. In the same way, two additional spectra were acquired inside the blister, spectra 4 and 5, positioned 40 nm and 80 nm away from point 3. This configuration allows a direct and continuous investigation across the blister border, from the basal plane to the interior of the swollen region.

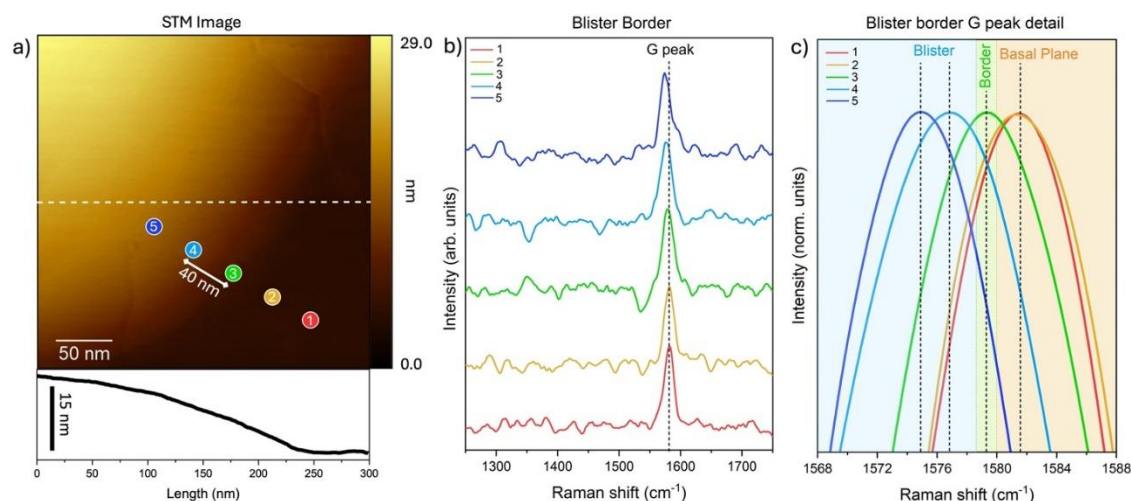


Figure 6.6: **a)** $300 \times 300 \text{ nm}^2$ STM image of the border of the blister, the number in the circle indicates the position of the respective TERS spectra, in panel b and c. Each circle has the dimension of the TERS spatial resolution, $V_{\text{bias}} = 0.1 \text{ V}$, $I_T = 900 \text{ pA}$. **b)** full TERS spectra of the 5 point indicate in panel a. **c)** Detail of the G peak presented in panel b, I divided the spectral region in background colour in order to have a better view of the different regions where the spectra were acquired. The diameter of the circles marking each acquisition point corresponds to the estimated lateral spatial resolution of the TERS measurement.

Panel b) reports the complete TERS spectra collected in the selected positions. As previously observed for the centre of the blister, no D peak is visible in any of the spectra, indicating that even the blister border can be considered a nearly defect-free surface. This result confirms that the swelling process itself does not introduce lattice defects, in agreement with the interpretation already proposed for the central region of the blister. In order to better visualize the variation of the G peak position, panel c) shows a magnified view of the G band region for all spectra. The spectra acquired in positions 1 and 2 exhibit the same G peak frequency, located at 1581.7 cm^{-1} , which corresponds to the typical value measured on the pristine basal plane. This result is consistent with the theoretical interpretation, since the regions of the basal plane adjacent to the blister are not affected by any significant deformation or strain.

When the surface begins to rise at the blister edge, corresponding to spectrum 3, a clear redshift of the G peak becomes visible. The G peak position in this case is 1579.5 cm^{-1} , indicating a shift of approximately 2.2 cm^{-1} with respect to the basal plane. This demonstrates that as soon as the graphite surface starts to swell, the C–C bonds experience stretching due to the pressure exerted by the gas trapped underneath.

Moving further inside the blister, the G peak continues to shift towards lower frequencies: the measured values are 1576.8 cm^{-1} for spectrum 4 and 1574.8 cm^{-1} for spectrum 5. It is important to highlight that these two spectra were acquired only a few tens of nanometres away from the blister edge, showing that the strain associated with the stretching of C–C bonds, showing the high resolution reachable with TERS. Overall, these results indicate a progressive evolution of the G peak redshift starting at the blister border and increasing over the first tens of nanometres inside the swollen region. This behaviour suggests that the strain distribution develops gradually from the edge toward the interior, further emphasizing the nanoscale spatial resolution achievable with TERS.

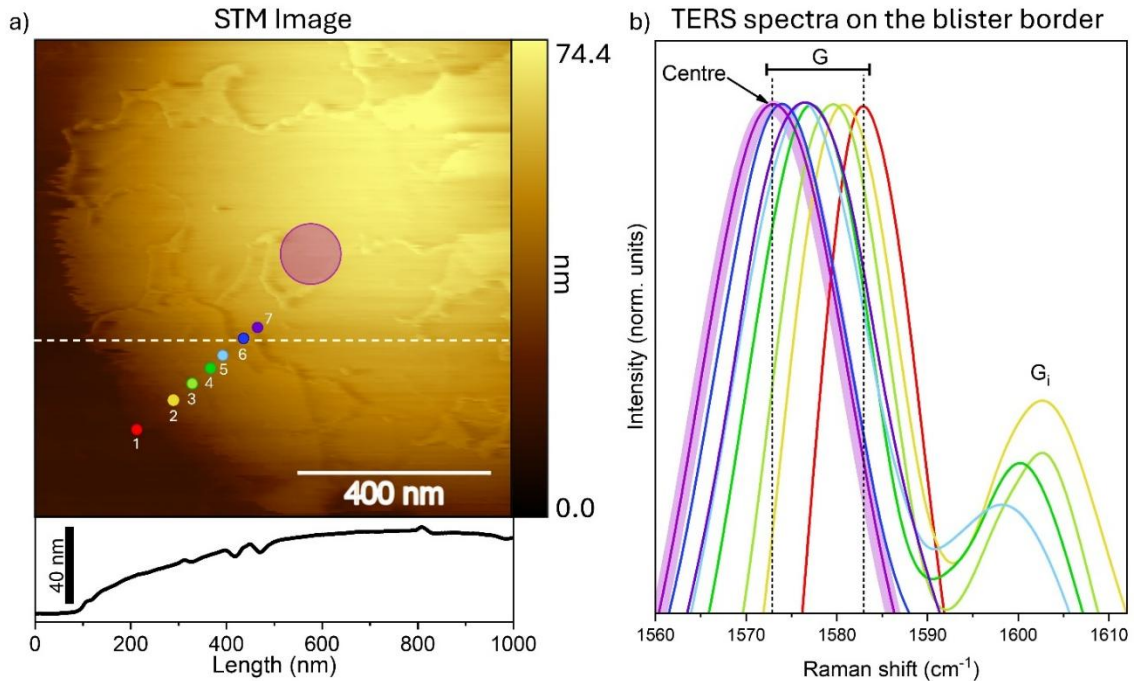


Figure 6.7: **a)** $1000 \times 1000 \text{ nm}^2$ STM image of the border of the another blister, the number in the circle indicates the position of the respective TERS spectra. Each circle has the dimension of the TERS spatial resolution $V_{\text{bias}} = 0.1 \text{ V}$, $I_T = 850 \text{ pA}$. **b)** Detail TERS spectra, focusing on the G and G_i peak of the 7 point indicate in panel a and the average acquired in the large purple spectrum, considered as the centre of the blisters. Each spectra has the colour of the corresponding circle in panel a. The diameter of the circles marking each acquisition point corresponds to the estimated lateral spatial resolution of the TERS measurement.

In Figure 6.7, another example is presented in which TERS spectra were acquired along a path extending from the blister border toward its centre. Panel (a) shows the corresponding STM image of the blister edge. The coloured circles indicate the specific positions at which the TERS spectra were recorded, and their diameter represents the approximate spatial resolution of each measurement. The larger purple circle non-numbered marks the region where four independent spectra were collected; the averaged spectrum from this set was used as the representative TERS signal for the blister centre. Panel (b) reports the detailed evolution of the G and G_i peaks for all spectra acquired at the positions highlighted in panel (a), with colours matching the corresponding markers.

In this particular blister, the maximum redshift comparable to the value measured at the blister centre is reached at a distance of approximately 300 nm from the edge, as shown by spectrum 6. The G_i peak appears exclusively in spectra acquired within ~200 nm of the blister edge (spectra 2–5) and is absent in both the pristine basal-plane region and the blister centre. This peak does not appear in the spectra collected on the pristine basal plane nor in those corresponding to the blister centre. Considering that the vertical resolution of TERS is better than 2 nm, the appearance of the G_i peak in this confined region indicates that intercalated ions can penetrate into the upper graphitic layers forming the blister shell.

The restricted spatial occurrence of the G_i feature present only in a narrow annular region and absent at the blister centre could not be conclusively explained. It is plausible that this behaviour reflects a specific or anomalous configuration of this particular blister rather than a general trend. Nevertheless, the detection of the G_i peak provides strong support for the interpretation of the G-band redshift observed along the blister surface, demonstrating that such spatially resolved spectroscopic information would be inaccessible using conventional Raman microscopy.

Overall, this analysis highlights the unique capability of TERS to probe correlations between local morphology, intercalation phenomena, and strain distribution at the nanometre scale, offering insights into the internal structure of graphite blisters that extend well beyond the reach of far-field techniques.

Last results is a Map on a blister border. In panel a is shown a 3D STM image of a portion of a blister is shown, starting from the basal plane throw the centre, in white is highlighted the blister border. In panel B is shown the variation of the G peak maximum position on the same area of the STM image, the map was acquired thanks to the TERS technique.

The spectra of the map are affected from noise, due to the lack of integration time employed, in order to avoid mechanical drift, and also is important to remember that the system is acquiring both the far field and the near field, without having the possibility of divide them. In any case despite this problem it is possible to appreciate important Raman shift as soon as the surface is swelled, and when we are 200 nm inside the blister surface, is possible to observe an even higher shift, confirming that the shift is present already as soon the surface is swelled.

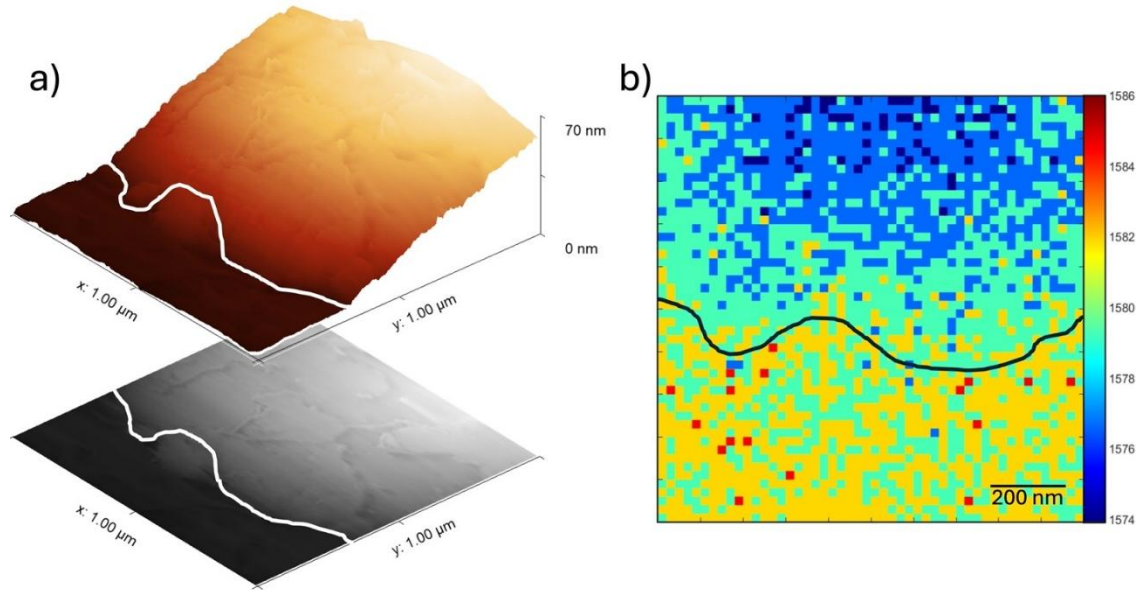


Figure 6.8: a) $1000 \times 1000 \text{ nm}^2$ STM 3D Image of the border of the blister, the whit line highlight the border of the blister, $V_{\text{bias}} = 0.1 \text{ V}$, $I_T = 850 \text{ pA}$. b) TERS map of the G shift from the same area presented in panel a, the black line highlight the blister border.

In literature different works has found that the local strain distribution in blister formed by 2D materials is not uniforms [155,159–164]. Several have demonstrated that the strain distribution is different in the centre respect the edge [165]. This is due the fact that blister the strain distribution depends by two components, one radial, ε_r , and one circumferential, ε_θ . This two components depends on the variable r normalize respect the radius of the bubble a . In the centre of the blisters, where $r/a = 0$, (e.g. $r = 0$), the two components has the same values, and the strain is biaxial. Instead at the border of the blister, where $r/a = 1$, (e.g. $r = a$), the circumferential component is 0, resulting in a uniaxial strain distribution. The strain components, normalized by the $\sqrt{\Gamma/E_{2D}}$, are plotted in Figure 6.9 as a function of r/a , where Γ is the adhesion energy and E_{2D} is the 2D young modulus for monolayer graphene [165]. While the circumferential strain depends linearly by r/a , the radial strain is nonlinear, with a minimum close where $r/a = 0.5$, and a

non-null value at the border of the blister. A similar result obtained with TERS was obtained on a graphene bilayer wrinkle, where the Raman shift increases from the border reaching a maximum in a large area in the centre of the wrinkle [166].

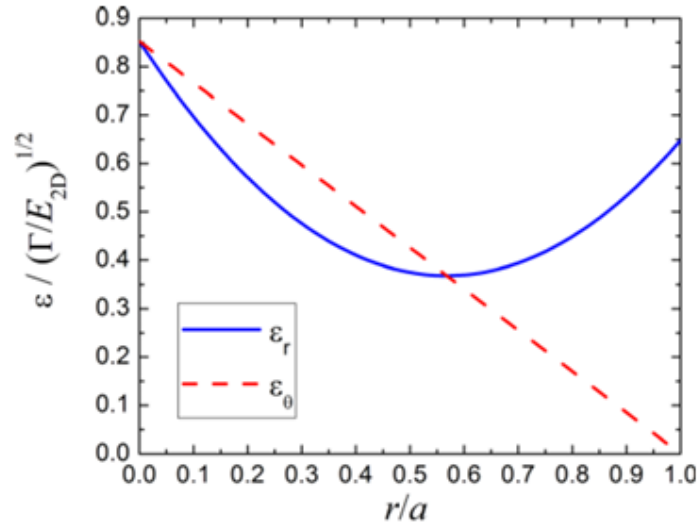


Figure 6.9: Variation of the radial and circumferential strain components in a graphene bubble calculate by Yule *et al.* [165]

It is worth emphasising that the spherical-cap model, despite its simplicity, represents an effective and reliable approach for estimating the average strain in bubble- or blister-like structures [7]. The strain values obtained using this model are in good agreement with experimental results reported in the literature that follow procedures similar to those adopted in Chapter 5 of this thesis. However, this model is not sufficient when the local strain distribution within the blister is considered, as required for a direct comparison with TERS results.

In this context, the TERS results presented in this chapter provide direct experimental evidence that the strain within HOPG blisters is intrinsically non-uniform and develops over nanometric length scales.

At the same time, the absence of defect-related Raman features across all investigated regions confirms that even locally enhanced strain does not lead to structural degradation of the graphite lattice. These findings demonstrate that TERS enables the separation of strain distribution and structural integrity, offering a level of insight that cannot be achieved through far-field techniques alone.

6.4 Raman shift and strain correlation

As a final validation of the consistency between the TERS measurements and those obtained through the AFM–Raman correlation approach, I compared the G-band frequency shift measured at the centre of each blister with the corresponding strain values. In this way, it is possible to verify whether the strain inferred from the nanoscale spectroscopic probe agrees with the trend previously extracted from the statistically averaged measurements. For the strain estimation I used the spherical cup approximation, calculate an average radius and average height from the STM image, in order to be coherent for the comparison with the data presented in chapter 5.

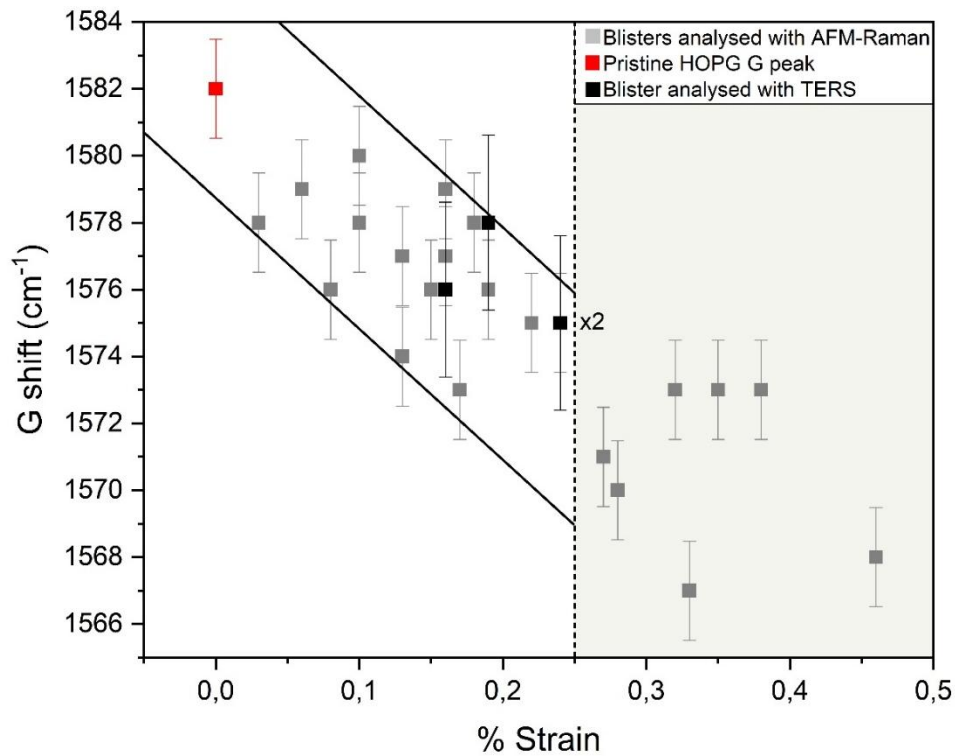


Figure 6.10: G peak Raman shift of 26 blisters spectrums as function of the calculated % strain, the downshift of the G peak in few layer graphite is linear dependent on the blister strain for a value less than 0.25%. The black square represent the results obtained on the blisters analysed with TERS, the grey ones represent the blister analysed in chapter 5. For the confidence interval I considered the blister that has a strain less then 0.25 %. G peak confidence interval slope $m = (-39,3 \pm 0,2) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+1582 \pm 4) \text{ cm}^{-1}$.

In Figure 6.10, the three TERS-derived strain–shift data points (black symbols) are superimposed on the statistical distribution presented in Chapter 5 (grey points). Although only two representative blisters are discussed in detail in the preceding figures, three independent blisters were analysed for the quantitative

strain comparison. Again, all the discussion regarding the loss of linearity relation when the strain is higher than 0.25% is still valid.

The inclusion of the three TERS data points does not alter the confidence interval of the statistical distribution, which remains essentially unchanged with respect to that reported in Figure 6b. This result clearly indicates that the two characterization methods are mutually consistent and that TERS, despite probing a significantly smaller interaction volume, provides Raman-shift values that are fully compatible with those obtained from correlative AFM–Raman mapping. As reminder the values of the confidence interval are: G peak confidence interval slope $m = (-39,3 \pm 0,2) \text{ cm}^{-1}/\%$, confidence interval intercept $q = (+1582 \pm 4) \text{ cm}^{-1}$.

Finally, the results concerning the 2D peak are not presented here due to a technical limitation encountered during the acquisition process. In order to achieve an efficient electromagnetic field enhancement with the gold tips, a laser source with a wavelength of 633 nm was employed. Under these excitation conditions, the 2D peak of graphite is expected to appear around 764 nm. However, at this specific wavelength, when using a 300 lines/mm diffraction grating, the recorded spectra are strongly affected by optical interference effects generated inside the CCD detector. In particular, the silicon layer of the back-illuminated CCD behaves as a thin Fabry–Perot cavity, giving rise to the so-called *etalon effect* [167,168].

Through this mechanism, a fraction of the near-infrared light penetrates the silicon substrate and undergoes multiple internal reflections between its parallel surfaces. The superposition of the forward- and backward-propagating waves produces stationary interference fringes that manifest as periodic ripples in the acquired spectra. These artificial modulations, which become especially pronounced in the spectral region around 760–800 nm where the silicon is partially transparent, significantly distort the true Raman profile and alter the shape and relative intensity of the 2D components.

Because of this interference artifact, it was not possible to perform a reliable analysis of the 2D band, nor to accurately evaluate the intensity ratio between its sub-components. As a consequence, any quantitative estimation of the number of graphene layers constituting the outer shell of the blister could not be obtained. This limitation, although purely instrumental, highlights the intrinsic sensitivity of the 2D peak to experimental setup parameters and confirms the necessity of optimizing both the spectral configuration and the detector architecture in order to enable precise layer-thickness determination in future measurements.

6.5 Estimation the strain from theoretical model

In conclusion, following the results presented in chapter 5 and 6, I estimate the internal pressure and the adhesion energy of the blisters and compare with values found in literature of some 2D material blister.

For the pressure estimation the equation presented by Yule and coworker is used, for blister with a radius (a) much higher than the height (h), that's the case of the HOPG blisters [165].

$$\Delta p = \frac{E_{2D}h^3}{\phi a^4} \quad (6.1)$$

Where h and a are respectively the height and the radius of the blister, considering my statistics the average values are $h = 80$ nm, $a = 1.6$ μm . ϕ is a constant that depends on the Poisson ratio ν . The expression of ϕ vs ν given by the model propose by Yule is the following:

$$\phi = \frac{75(1-\nu^2)}{8(23+18\nu-3\nu^2)} \quad (6.2)$$

If for the Poisson ration I consider the value for the graphene, 0.16, and this gives a value for $\phi = 0.353$

E_{2d} is the two dimensional Young modulus for the blister skin. Its values depends by the Young modulus of the Graphene (E) and by the thickness (t) of the 2D material taken in account.

$$E_{2D} = Et \quad (6.3)$$

For E the usual values found in literature is equal to 1 TPa, however more recently lee and coauthors combining Raman measurements and finite element analysis, they found a value equal to 2.4 TPa for the single layer and 2 TPa for the bilayer graphene [169]. I'll will use the latter value for the internal pressure estimation.

As explained in chapter for is difficult to estimate the exact number of layers that form the blister skin, however following the consideration over the number of layer I would estimate that the average number of layer of my statistics is between 5 and 10. So t has a range between $(1.68 \div 3.35)$ nm.

Following all these considerations the average pressure inside the blister is

$$\Delta p = (0.74 \div 1.48) \text{ MPa} \quad (6.4)$$

This range is compatible with some other values calculate for graphene blister with similar dimensions [3]. It is instructive to compare this value with the results obtained in Chapter 4. In particular, Figure 4.1b reports a representative nanoindentation curve acquired on a blistered region, for which the maximum applied force is approximately 0.3 nN. Assuming a tip apex radius of about 10 nm, this force corresponds to an estimated applied pressure on the order of 1 MPa. This value is consistent with the internal pressure of the blister, since, in order for nanoindentation to occur, the externally applied pressure must exceed the internal pressure sustaining the swollen membrane.

It is possible from the pressure calculate the strain, following the model proposed by Yule.

At first the volume is calculated from equation 6.5:

$$V = \frac{\pi}{2} a^2 h \quad (6.5)$$

Considering the gas inside the bubble as an ideal one, from equation 5.6, it is possible to calculate the number of particles inside the bubble, substituting the expression found in equations 6.1 and 6.5 inside the noble gas equation:

$$N = \frac{pV}{kT} = \frac{\pi}{2kT} \left(\frac{E_{2D} h^4}{\phi a^2} + p_0 a^2 h \right) \quad (6.6)$$

where k is the Boltzman constant and T is the temperature. Assuming $p \gg p_0$, and considering the number of particles N fixed, is it possible to find the potential energy inside the bubble as a function of the bubble radius. The relation is approximately given by equation 6.7:

$$\Pi(a, N) = \frac{NkT}{4} - NkT \ln \left(\frac{p_0 a^{5/2}}{(NkT)^{3/4} \cdot E_{2D}^{1/4}} \right) \quad (6.7)$$

The first term is the strain energy, that is independent from the radius bubble, the second term is the potential energy of the gas, in ambient condition. As the radius of the bubble increases the potential energy decreases, at the same time the interfacial energy increase, since more part of the layer material is detached from the substrate. The equilibrium is reached when the potential energy of the blister balances the adhesion energy Γ .

$$\left(\frac{\partial \Pi}{\partial a} \right)_N = -2\pi a \Gamma \quad (6.8)$$

From equation 6.8 is possible to find the expression for the adhesion energy:

$$\Gamma = \frac{5NkT}{4\pi a^2} = \frac{5E_{2D}h^4}{8\phi a^4} \quad (6.9)$$

From equation 6.9, I estimate the following range for the adhesion energy:

$$\Gamma = (0.037 \div 0.074) J/m^2 \quad (6.10)$$

That is in accordance with values provided by [7], and in agreement with the graphene blister over hBN [3].

As a final consideration, Yule and coauthors provides the relation between the adhesion energy and the average strain along the whole diameter of the blister.

$$\varepsilon_r^{la} = 0.508 \sqrt{\Gamma/E_{2D}} \quad (6.11)$$

It is important to notice that with the relation in 5.11, since Γ is directly proportional to E_{2D} , the average strain does not depends from the Young modulus and so, as the two dimensional Young modulus has been defined, does not depends from the layers.

If the average radius and height of blister of the statistics presented in this work is substitute in the equation 6.11, the average strain is:

$$\varepsilon = 0.17\% \quad (6.12)$$

This averages theoretical strain values is compatible with the average strain of the statistics extrapolated from AFM measurements, e.g. 0.20 %.

It is important to notice that with this model it has not taken in account the shear stress between the layers, that creates interlayer sliding, and so the top layers are has a less strain respect the layers below. To improve this model is not so straight forward, an possible idea is to modify the expression of the constant ϕ , in order to take in account the shear stress by the layers, like suggested by Wang for the bilayer case [142].

7

Conclusions and perspectives

7.1 Objectives and framework of the present work

This thesis addresses the study of blisters formed in highly oriented pyrolytic graphite (HOPG) as a consequence of electrochemical intercalation processes, with the specific aim of moving beyond a purely morphological description of these structures. Rather than being treated as incidental surface features, blisters are investigated here as confined physical systems characterized by a well-defined mechanical and spectroscopic response. In this context, a broader motivation of the work is the potential use of such structures as platforms for strain-related phenomena and, more generally, for prospective applications in strain engineering.

Historically, blisters observed on intercalated graphite surfaces have been interpreted primarily as manifestations of oxidation reactions and gas evolution associated with anion intercalation, and therefore regarded mainly as sources of crystal degradation. In contrast, the present work adopts a different perspective, in which blisters are described as locally delaminated multilayer membranes, inflated by pressurized gases and characterized by a non-uniform strain distribution. This description closely parallels the physical framework that has been successfully applied to bubbles and blisters in two-dimensional materials. A crucial distinction, however, lies in the mechanism of formation: while blisters in transferred two-dimensional materials originate from a not perfect transfer on the substrate, intercalation-induced blisters in HOPG arise from gas generation between graphite layers during electrochemical reactions. As a result, both the blister and the surrounding substrate consist of the same material, a feature that confers a markedly enhanced structural stability and long-term persistence to these systems.

Within this framework, the primary objective of the thesis is the experimental characterization of HOPG blisters as mechanically active entities. This is achieved

through a multiscale approach that integrates atomic force microscopy, correlative AFM–Raman spectroscopy, and high-resolution Raman spectroscopy based on tip-enhanced techniques. The purpose is not limited to the extraction of isolated quantitative parameters, but rather to the construction of a coherent physical picture capable of linking morphology, elastic response, and vibrational properties within a unified interpretation.

7.2 Mechanical response of blisters

A central outcome of this work concerns the direct mechanical characterization of blisters by means of AFM-based nanoindentation experiments. By exploiting the AFM not only as an imaging tool but as an active mechanical probe, force–distance curves were acquired on individual blisters and systematically compared with those obtained on the non-intercalated HOPG basal plane.

The analysis of these measurements reveals that blisters exhibit a mechanical response that is drastically different from that of bulk graphite. While the basal plane of HOPG displays a stiff response consistent with Young’s modulus values reported in the literature ($E \approx 100$ GPa), blistered regions behave as compliant membranes, characterized by effective elastic moduli several orders of magnitude lower, in the range $E \approx 1\text{--}10$ MPa. This result provides clear experimental evidence that the mechanical behaviour of blisters cannot be interpreted as that of chemically degraded graphite, but must instead be attributed to a finite-thickness system that is locally delaminated and subjected to an internal pressure.

An additional aspect emerging from the measurements is the dependence of the mechanical response on the electrochemical history of the sample, in particular on the number of cyclic voltammetry cycles used to generate the blisters. This observation indicates that electrochemical evolution influences not only blister size and density, but also their internal mechanical state, plausibly through variations in gas pressure and in the effective number of layers involved in the delamination process.

Beyond indentation experiments, the AFM tip was also employed to mechanically manipulate the blisters and investigate their response under large applied loads. When a controlled vertical displacement was imposed across the entire diameter of a blister, the swollen layers were observed to recompact onto the underlying graphite without leaving permanent traces, becoming indistinguishable from the surrounding basal plane. At higher applied pressures, it was possible to

generate a groove in the graphite surface, effectively cutting through the blister. In this case, the blister skin collapsed completely onto the underlying layers, again without leaving evidence of the prior delamination. These observations demonstrate the reversible nature of the blister deformation and the absence of irreversible damage to the graphite lattice.

Overall, these results are consistent with the qualitative framework proposed by Murray and co-workers, in which blisters are described as superficial graphite layers lifted by the pressure of gas generated during intercalation and oxidation. The present measurements extend this framework by providing direct experimental access to the mechanical response of blisters, thereby enabling their description in terms of elastic membranes rather than as simple structural defects.

7.3 Correlation between morphology, mechanics, and Raman response

Alongside mechanical probing, vibrational spectroscopy plays a fundamental role in this work, particularly through the combined use of AFM and Raman spectroscopy. Initial *in situ* analyses reveal clear correlations between Raman spectra, optical contrast, and AFM images. Regions displaying distinct optical contrast were found to spatially coincide with areas characterized by pronounced variations in the AFM phase signal, and in these same regions an enhancement of the Raman G-band intensity was observed. While the correlation between optical contrast and phase contrast can be qualitatively attributed to local physico-chemical modifications, a definitive explanation for the observed increase in G-band intensity could not be established at this stage.

A second important result concerns the influence of intercalation-related Raman features on the second-order Raman response. In particular, the appearance of the G_i band indicates the presence of intercalated ions between graphite layers, which locally modify the interlayer spacing. The concurrent emergence of the D band, associated with defect density, reflects a structural modification of the lattice and consequently a change in its vibrational properties. Together, these effects lead to a modification of the 2D-band lineshape, which is known to be sensitive to both interlayer coupling and crystal structure.

When the analysis is restricted to blistered regions, a statistical approach enables a well-defined spectroscopic characterization of these structures. One of the most significant observations is an increase in the G-band intensity in areas

corresponding to blisters, which coincide with regions exhibiting a softer mechanical response and a modified AFM phase contrast. This behaviour can be rationalized by analogy with few-layer graphene systems, where the G-band intensity is known to depend strongly on the number of layers involved.

In parallel, shifts in the Raman peak frequencies provide direct evidence of strain associated with blister formation. A linear dependence of the redshift of the G and 2D bands on strain is observed, in agreement with established results for biaxial strain in few-layer graphene. These findings highlight Raman spectroscopy as a highly sensitive probe of the mechanical state of the system, whose full interpretative power emerges only when combined with the morphological and mechanical information provided by AFM.

More generally, the observed correlation establishes a direct experimental link between the mechanical description of pressurised multilayer membranes and their Raman spectroscopic response. In this framework, Raman spectroscopy emerges as a quantitative probe of strain in confined graphitic systems, enabling a direct correlation between morphology, mechanics, and vibrational properties.

7.4 Nanoscale resolution and contribution of TERS

To overcome the spatial averaging inherent to conventional Raman spectroscopy and to access a more local description of strain and defectivity, tip-enhanced Raman spectroscopy (TERS) was employed. This technique enables the acquisition of Raman spectra with nanometric spatial resolution, allowing spatial variations of the vibrational response within individual blisters to be resolved.

TERS measurements demonstrate, first, that the Raman spectrum acquired directly on the blister surface does not exhibit a detectable D band, indicating that blister swelling does not act as a source of defects and that the graphite lattice remains structurally intact. This observation represents a key result, as it demonstrates that the strain induced by blister formation does not intrinsically lead to lattice degradation. In this sense, blisters provide a model system for strain engineering in graphite in which mechanical deformation can be decoupled from defect generation.

In addition, TERS reveals that strain within the blister is not uniformly distributed. In agreement with theoretical models, the strain field can be decomposed into radial and circumferential components, which are equivalent at

the centre of the blister but differ near its perimeter, where the circumferential component vanishes.

This behaviour is experimentally evidenced by a localized redshift of the G band detected at the blister edge. Moving away from the perimeter toward the interior of the blister, the G-band position progressively shifts to lower energies, reaching a maximum redshift at a distance of several hundreds of nanometres from the edge, as predicted by continuum models. Such insights are made accessible exclusively by the nanometric resolution of TERS and could not be obtained through far-field spectroscopic techniques.

From a methodological standpoint, the application of TERS to the study of blisters in intercalated HOPG represents a significant element of originality of this work. It opens the way to future investigations in which the mechanical and vibrational properties of confined systems can be explored with a level of spatial resolution that has so far been largely restricted to isolated two-dimensional materials.

7.5 Refined Interpretative Framework for Graphite Blisters

Taken together, the results presented in this thesis contribute to a refinement of the blister model originally introduced in section 3.4. When evaluated against the six assumptions underlying the simplified interpretative framework, the experimental findings demonstrate that the model provides an accurate and physically consistent description of the blistered system.

Within this regime, the simplified model captures the dominant physics of the system, yielding an effective description in which the complex interplay of intercalation, gas evolution, and possible chemical modifications is reduced to a mechanically driven membrane response.

The experimental evidence indicates that individual blisters can be treated, to a very good approximation, as single, isolated swollen regions with a rounded geometry. The possible influence of neighbouring blisters or of underlying blistered structures appears to be negligible within the spatial and mechanical resolution of the present measurements. Blister swelling is driven by pressurised gaseous species generated during the intercalation process, which induce both out-of-plane deformation and in-plane strain in the graphene layers forming the blister skin.

Spectroscopic analyses confirm that the blister skin consists of a finite number of graphene layers, typically ranging between two and fifteen. Importantly, blister

formation does not act as a source of lattice defects: Raman and TERS measurements show no systematic increase of defect-related bands. Crystallographic steps, when present, are found to deform together with the basal plane and do not significantly affect the mechanical response of the blister.

The presence of intercalated ions between the layers forming the blister skin is observed in some cases, but not systematically across all blisters. Moreover, TERS measurements indicate that, when present, the ionic species do not exhibit strong spatial localisation within the blistered region. Importantly, no measurable influence of intercalated ions on the mechanical behaviour of the blisters is detected. In addition, the presence of graphite oxide beneath the blister skin is not evidenced within the sensitivity limits of Raman spectroscopy.

On the basis of these results, the interpretative model and the experimental findings presented in this work support a redefinition of the role of blisters in bulk layered materials. Rather than being regarded as undesirable by-products of electrochemical intercalation, blisters emerge as complex physical systems in which mechanical deformation, structural modification, and lattice vibrations are intrinsically coupled.

From an experimental perspective, this thesis demonstrates the effectiveness of an integrated approach based on AFM, Raman spectroscopy, and TERS, in which each technique provides complementary and indispensable information. From a physical standpoint, blisters in HOPG can be viewed as a conceptual bridge between two-dimensional materials and bulk layered systems, illustrating how concepts such as pressurised membranes, strain engineering, and confinement can be extended beyond strictly two-dimensional crystals.

7.6 Limitations and future perspectives

A natural extension of the present work concerns the experimental determination of the number of graphene layers forming the blister skin. This parameter is expected to play a crucial role in governing the mechanical response of the system, as the bending rigidity, strain distribution, and adhesion energy of multilayer graphene depend sensitively on the number of stacked layers. Within the framework of this thesis, however, instrumental limitations associated with optical interference effects—specifically the etalon effect arising from the back-illuminated EMCCD detector—prevented a detailed and fully reliable analysis of the Raman 2D band by TERS, thereby hindering a possible determination of the number of graphene layers with sufficient precision at the single-blister level.

From a methodological perspective, this limitation is essentially technical rather than conceptual. Future experimental developments could overcome it by exploiting low-energy Raman modes below 100 cm^{-1} , such as shear and layer-breathing modes, which provide a more direct fingerprint of the number of graphene layers and are intrinsically less sensitive to interference effects. These modes could be investigated by combining low-frequency Raman spectroscopy with AFM-based morphological characterization, enabling a direct correlation between vibrational signatures and local blister geometry. In parallel, low-energy Raman modes could also be accessed by TERS, provided that sufficient low-frequency spectral resolution and effective suppression of background contributions can be achieved, thus allowing a direct comparison between far-field and near-field spectroscopic information. In parallel, the replication of the TERS experiments on the 2D band using a front-illuminated EMCCD detector would represent a straightforward instrumental upgrade to mitigate etalon-related artefacts, potentially enabling a more robust spectroscopic analysis of multilayer graphene within blistered regions [170,171].

Access to an independent and reliable determination of the number of graphene layers would enable a more quantitative assessment of the relationship between blister thickness and key physical parameters, including internal pressure, strain distribution across the membrane, effective adhesion energy at the graphene–substrate interface, and the overall blister geometry, such as possible correlations between the number of layers and the blister lateral radius and out-of-plane height. Importantly, such refinements would not alter the physical interpretation developed in this work, but rather complete it by providing more direct experimental constraints on parameters that are already indirectly supported by the combined mechanical and spectroscopic evidence presented throughout the thesis.

More generally, the results presented in this thesis indicate that the membrane-based description remains valid within a well-defined deformation regime. Deviations observed at higher strain suggest the onset of interlayer sliding and stacking perturbation, marking the breakdown of the continuum approximation.

Beyond the specific issue of layer-number determination, the results presented in this thesis indicate that electrochemically induced blisters in HOPG provide a controlled and reproducible experimental platform in which local mechanical deformation and vibrational response can be systematically correlated. In this context, blisters may be used as well-defined testbeds to probe the effects of curvature and strain gradients on Raman signatures, or to investigate how substrate-induced strain influences the vibrational properties of thin molecular or two-dimensional films deposited on graphite. Importantly, these perspectives rely

on the experimental framework established in the present work, rather than on the introduction of new physical assumptions or interpretative models.

7.7 Final considerations

In conclusion, this thesis demonstrates that blisters formed in electrochemically intercalated HOPG can be regarded as confined multilayer membrane systems, characterized by a well-defined mechanical and spectroscopic response governed by general principles of elasticity. Through a multiscale experimental approach, it was possible to establish direct and quantitative links between morphology, mechanical response, and lattice vibrations, providing a unified physical description of these structures.

Beyond advancing the understanding of electrochemical intercalation in graphite, the present work redefines the role of blisters in bulk layered materials, showing that they can be exploited as stable and controllable platforms to investigate strain-related phenomena. In this perspective, blisters represent a bridge between bulk graphite and two-dimensional materials, opening new opportunities for the study of confined mechanical systems and for future developments in strain engineering.

Bibliography

- [1] D.A. Sanchez, Z. Dai, N. Lu, 2D Material Bubbles: Fabrication, Characterization, and Applications, 2021.
- [2] E. Khestanova, F. Guinea, L. Fumagalli, A.K. Geim, I. V. Grigorieva, Universal shape and pressure inside bubbles appearing in van der Waals heterostructures, *Nat. Commun.* 7 (2016). <https://doi.org/10.1038/ncomms12587>.
- [3] W. Wang, X. Ma, Z. Dai, S. Zhang, Y. Hou, G. Wang, Q. Li, Z. Zhang, Y. Wei, L. Liu, Mechanical Behavior of Blisters Spontaneously Formed by Multilayer 2D Materials, *Adv. Mater. Interfaces* 9 (2022). <https://doi.org/10.1002/admi.202101939>.
- [4] M. Pandey, R. Kumar, Polymer curing assisted formation of optically visible sub-micron blisters of multilayer graphene for local strain engineering, *Journal of Physics Condensed Matter* 34 (2022). <https://doi.org/10.1088/1361-648X/ac61b4>.
- [5] M. Pandey, G. Wang, G. Singh, R. Ahuja, R. Kumar, Instabilities in the blistering of two-dimensional materials, *2d Mater.* 12 (2025). <https://doi.org/10.1088/2053-1583/adba75>.
- [6] E. Khestanova, F. Guinea, L. Fumagalli, A.K. Geim, I. V. Grigorieva, Graphene bubbles on a substrate: Universal shape and van der Waals pressure, (2016). <https://doi.org/10.1038/ncomms12587>.
- [7] T. Georgiou, L. Britnell, P. Blake, R. V. Gorbachev, A. Gholinia, A.K. Geim, C. Casiraghi, K.S. Novoselov, Graphene bubbles with controllable curvature, *Appl. Phys. Lett.* 99 (2011). <https://doi.org/10.1063/1.3631632>.
- [8] N.G. Boddeti, S.P. Koenig, R. Long, J. Xiao, J.S. Bunch, M.L. Dunn, Mechanics of adhered, pressurized graphene blisters, *Journal of Applied Mechanics, Transactions ASME* 80 (2013). <https://doi.org/10.1115/1.4024255>.
- [9] J. Zabel, R.R. Nair, A. Ott, T. Georgiou, A.K. Geim, K.S. Novoselov, C. Casiraghi, Raman spectroscopy of graphene and bilayer under biaxial strain: Bubbles and balloons, *Nano Lett.* 12 (2012) 617–621. <https://doi.org/10.1021/nl203359n>.

- [10] G. Bussetti, R. Yivlialin, F. Ciccacci, L. Duó, A. Podestá, Blistering at the solid-liquid interface: The graphite case-study, in: *Encyclopedia of Solid-Liquid Interfaces*, Elsevier, 2024: pp. 380–390. <https://doi.org/10.1016/B978-0-323-85669-0.00063-5>.
- [11] D. Alliata, R. Kötz, Investigation of Nanoscale intercalation into graphite and carbon materials by in situ Scanning Probe Microscopy, n.d.
- [12] R. Yivlialin, G. Bussetti, L. Magagnin, F. Ciccacci, L. Duò, Temporal analysis of blister evolution during anion intercalation in graphite, *Physical Chemistry Chemical Physics* 19 (2017) 13855–13859. <https://doi.org/10.1039/c7cp00481h>.
- [13] C.A. Goss, J.C. Brumfield, E.A. Irene, R.W. Murray, R.W. Langmuir,) Besenhard, UTC, 1993. <https://pubs.acs.org/sharingguidelines>.
- [14] K.W. Hathcock, J.C. Brumfield, C.A. Goss, E.A. Irene, R.W. Murray, R.L. J Electrochem, b) Fong, R; Sacken, U. von; Dahn, John Wiley & Sons, 1995. <https://pubs.acs.org/sharingguidelines>.
- [15] S. De Rosa, P. Branchini, R. Yivlialin, L. Duò, G. Bussetti, L. Tortora, Disclosing the Graphite Surface Chemistry in Acid Solutions for Anion Intercalation, *ACS Appl. Nano Mater.* 3 (2020) 691–698. <https://doi.org/10.1021/acsanm.9b02220>.
- [16] S. De Rosa, P. Branchini, V. Spampinato, A. Franquet, R. Yivlialin, L. Duò, G. Bussetti, L. Tortora, Stratigraphic analysis of intercalated graphite electrodes in aqueous inorganic acid solutions, *Nano Res.* 15 (2022) 1120–1127. <https://doi.org/10.1007/s12274-021-3614-6>.
- [17] M. Aoki, H. Amawashi, Dependence of band structures on stacking and field in layered graphene, *Solid State Commun.* 142 (2007) 123–127. <https://doi.org/10.1016/j.ssc.2007.02.013>.
- [18] P. TRUCANO, R. CHEN, Structure of graphite by neutron diffraction, *Nature* 258 (1975) 136–137. <https://doi.org/10.1038/258136a0>.
- [19] The structure of graphite, *Proceedings of the Royal Society of London. Series A, Containing Papers of a Mathematical and Physical Character* 106 (1924) 749–773. <https://doi.org/10.1098/rspa.1924.0101>.
- [20] XXIX cycle POLITECNICO DI MILANO DEPARTMENT OF PHYSICS
DOCTORAL PROGRAMME IN PHYSICS
_____ Anion intercalation in graphite: a combined Electrochemical Atomic Force and Scanning Tunneling Microscopy investigation, n.d.

- [21] P.N.B.J.L. Richard C. Alkire, *Electrochemical Engineering Across Scales: From Molecules to Processes*, 2015.
- [22] S. Reich, C. Thomsen, Raman spectroscopy of graphite, *Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences* 362 (2004) 2271–2288. <https://doi.org/10.1098/rsta.2004.1454>.
- [23] Data obtained from © Optigraph GmbH Web page, (n.d.).
- [24] L. Spanu, S. Sorella, G. Galli, Nature and Strength of Interlayer Binding in Graphite, *Phys. Rev. Lett.* 103 (2009) 196401. <https://doi.org/10.1103/PhysRevLett.103.196401>.
- [25] C. Lee, X. Wei, J.W. Kysar, J. Hone, Measurement of the Elastic Properties and Intrinsic Strength of Monolayer Graphene, *Science* (1979). 321 (2008) 385–388. <https://doi.org/10.1126/science.1157996>.
- [26] D. Tománek, S.G. Louie, First-principles calculation of highly asymmetric structure in scanning-tunneling-microscopy images of graphite, *Phys. Rev. B* 37 (1988) 8327–8336. <https://doi.org/10.1103/PhysRevB.37.8327>.
- [27] B. Jayasena, S. Subbiah, A novel mechanical cleavage method for synthesizing few-layer graphenes, *Nanoscale Res. Lett.* 6 (2011) 95. <https://doi.org/10.1186/1556-276X-6-95>.
- [28] R. V. Lapshin, Automatic lateral calibration of tunneling microscope scanners, *Review of Scientific Instruments* 69 (1998) 3268–3276. <https://doi.org/10.1063/1.1149091>.
- [29] G. Binnig, C.F. Quate, Ch. Gerber, Atomic Force Microscope, *Phys. Rev. Lett.* 56 (1986) 930–933. <https://doi.org/10.1103/PhysRevLett.56.930>.
- [30] R.L. McCreery, *Carbon electrodes: structural effects on electron transfer kinetics*, 1991.
- [31] R.L. McCreery, Advanced Carbon Electrode Materials for Molecular Electrochemistry, *Chem. Rev.* 108 (2008) 2646–2687. <https://doi.org/10.1021/cr068076m>.
- [32] J. Xia, F. Chen, J. Li, N. Tao, Measurement of the quantum capacitance of graphene, *Nat. Nanotechnol.* 4 (2009) 505–509. <https://doi.org/10.1038/nnano.2009.177>.
- [33] J.-H. Zhong, J.-Y. Liu, Q. Li, M.-G. Li, Z.-C. Zeng, S. Hu, D.-Y. Wu, W. Cai, B. Ren, Interfacial capacitance of graphene: Correlated differential capacitance and in situ electrochemical Raman spectroscopy study,

- Electrochim. Acta 110 (2013) 754–761.
<https://doi.org/10.1016/j.electacta.2013.04.004>.
- [34] N.M.P. and R.L.M. Ronald J. Rice, Quantitative Correlations of Heterogeneous Electron-Transfer Kinetics with Surface Properties of Glassy Carbon Electrodes, *J. Am. Chem. Soc.* 112 (1990) 4617–4622.
- [35] V. Nicolosi, M. Chhowalla, M.G. Kanatzidis, M.S. Strano, J.N. Coleman, Liquid Exfoliation of Layered Materials, *Science* (1979). 340 (2013).
<https://doi.org/10.1126/science.1226419>.
- [36] C.-J. Shih, A. Vijayaraghavan, R. Krishnan, R. Sharma, J.-H. Han, M.-H. Ham, Z. Jin, S. Lin, G.L.C. Paulus, N.F. Reuel, Q.H. Wang, D. Blankschtein, M.S. Strano, Bi- and trilayer graphene solutions, *Nat. Nanotechnol.* 6 (2011) 439–445. <https://doi.org/10.1038/nnano.2011.94>.
- [37] G. Eda, H. Yamaguchi, D. Voiry, T. Fujita, M. Chen, M. Chhowalla, Photoluminescence from Chemically Exfoliated MoS₂, *Nano Lett.* 11 (2011) 5111–5116. <https://doi.org/10.1021/nl201874w>.
- [38] Z.Y. Xia, S. Pezzini, E. Treossi, G. Giambastiani, F. Corticelli, V. Morandi, A. Zanelli, V. Bellani, V. Palermo, The Exfoliation of Graphene in Liquids by Electrochemical, Chemical, and Sonication-Assisted Techniques: A Nanoscale Study, *Adv. Funct. Mater.* 23 (2013) 4684–4693.
<https://doi.org/10.1002/adfm.201203686>.
- [39] J.O. Besenhard, H.P. Fritz, The Electrochemistry of Black Carbons, *Angewandte Chemie International Edition in English* 22 (1983) 950–975.
<https://doi.org/10.1002/anie.198309501>.
- [40] K. Kinoshita, *Carbon: Electrochemical and Physicochemical Properties*, New York, 1988.
- [41] M.S.E.M. Dresselhaus, “Graphite Intercalation CompoundsII,” Berlin, 1992.
- [42] F. Beck, H. Krohn, Reversible electrochemical intercalation of anions from aqueous solutions in polymer bound graphite electrodes, *Synth. Met.* 7 (1983) 193–199. [https://doi.org/10.1016/0379-6779\(83\)90047-4](https://doi.org/10.1016/0379-6779(83)90047-4).
- [43] M.S. Jagadeesh, A. Calloni, I. Denti, C. Goletti, F. Ciccacci, L. Duò, G. Bussetti, The effect of cyclic voltammetry speed on anion intercalation in HOPG, *Surf. Sci.* 681 (2019) 111–115.
<https://doi.org/10.1016/j.susc.2018.11.009>.
- [44] P. Samorì, Scanning probe microscopies beyond imaging, *J. Mater. Chem.* 14 (2004) 1353–1366. <https://doi.org/10.1039/B314626J>.

- [45] R. Yivlialin, L. Brambilla, A. Accogli, E. Gibertini, M. Tommasini, C. Goletti, M. Leone, L. Duò, L. Magagnin, C. Castiglioni, G. Bussetti, Evidence of graphite blister evolution during the anion de-intercalation process in the cathodic regime, *Appl. Surf. Sci.* 504 (2020). <https://doi.org/10.1016/j.apsusc.2019.144440>.
- [46] D. Alliata, R. Kötz, O. Haas, H. Siegenthaler, In situ AFM study of interlayer spacing during anion intercalation into HOPG in aqueous electrolyte, *Langmuir* 15 (1999) 8483–8489. <https://doi.org/10.1021/la990402o>.
- [47] G. Bussetti, R. Yivlialin, D. Alliata, A. Li Bassi, C. Castiglioni, M. Tommasini, C.S. Casari, M. Passoni, P. Biagioni, F. Ciccacci, L. Duò, Disclosing the Early Stages of Electrochemical Anion Intercalation in Graphite by a Combined Atomic Force Microscopy/Scanning Tunneling Microscopy Approach, *Journal of Physical Chemistry C* 120 (2016) 6088–6093. <https://doi.org/10.1021/acs.jpcc.6b00407>.
- [48] R. Yivlialin, L. Magagnin, L. Duò, G. Bussetti, Blister evolution time invariance at very low electrolyte pH: H₂SO₄/graphite system investigated by electrochemical atomic force microscopy, *Electrochim. Acta* 276 (2018) 352–361. <https://doi.org/10.1016/j.electacta.2018.04.204>.
- [49] R. Yivlialin, G. Pace, G. Bussetti, M. Caironi, L. Duò, Contact potential and scanning Kelvin force microscopy measurements on sulphate-anion intercalated graphite, *Electrochim. Acta* 267 (2018) 20–23. <https://doi.org/10.1016/j.electacta.2018.02.056>.
- [50] G. Bussetti, M. Campione, A. Bossi, R. Yivlialin, L. Duò, F. Ciccacci, Anion intercalated graphite: a combined electrochemical and tribological investigation by in situ AFM, *J. Microsc.* 280 (2020) 222–228. <https://doi.org/10.1111/jmi.12927>.
- [51] G. Bussetti, M. Menegazzo, S. Mitko, C. Castiglioni, M. Tommasini, A. Lucotti, L. Magagnin, V. Russo, A. Li Bassi, M. Siena, A. Guadagnini, S. Grillo, D. Del Curto, L. Duò, A Combined Raman Spectroscopy and Atomic Force Microscopy System for In Situ and Real-Time Measures in Electrochemical Cells, *Materials* 16 (2023). <https://doi.org/10.3390/ma16062239>.
- [52] D.C. Alsmeyer, R.L. McCreery, In Situ Raman Monitoring of Electrochemical Graphite Intercalation and Lattice Damage in Mild Aqueous Acids, 1992. <https://pubs.acs.org/sharingguidelines>.

- [53] A. Merlen, J.G. Buijnsters, C. Pardanaud, A guide to and review of the use of multiwavelength Raman spectroscopy for characterizing defective aromatic carbon solids: From graphene to amorphous carbons, *Coatings* 7 (2017). <https://doi.org/10.3390/coatings7100153>.
- [54] C. Ma, X. Yang, Y. Chen, J. Chu, Mechanical Mapping of Nanoblister Confined by Two-Dimensional Materials Reveals Complex Ridge Patterns, *Langmuir* 40 (2024) 8409–8417. <https://doi.org/10.1021/acs.langmuir.3c03879>.
- [55] D. Fotiadis, S. Scheuring, S.A.M. Èller, A. Engel, D.J.M. Èller, M.E. Mu, Imaging and manipulation of biological structures with the AFM, n.d. www.elsevier.com/locate/micron.
- [56] R.M. Stockle, Y.D. Suh, V. Deckert, R. Zenobi, S. Switzerland, Nanoscale chemical analysis by tip-enhanced Raman spectroscopy, 2000. www.elsevier.nl/locate/rscpllett.
- [57] T.-X. Huang, X. Cong, S.-S. Wu, J.-B. Wu, Y.-F. Bao, M.-F. Cao, L. Wu, M.-L. Lin, X. Wang, P.-H. Tan, B. Ren, Visualizing the structural evolution of individual active sites in MoS₂ during electrocatalytic hydrogen evolution reaction, *Nat. Catal.* 7 (2024) 646–654. <https://doi.org/10.1038/s41929-024-01148-x>.
- [58] T. Touzalin, S. Joiret, I.T. Lucas, E. Maisonhaute, Electrochemical tip-enhanced Raman spectroscopy imaging with 8 nm lateral resolution, *Electrochem. Commun.* 108 (2019) 106557. <https://doi.org/10.1016/j.elecom.2019.106557>.
- [59] A.J. Bard, F.-R. Fan, *Introductory Lecture Studies of the Liquid/Solid Interface by Scanning Tunnelling Microscopy and Scanning Electrochemical Microscopy*, 1992.
- [60] V.S. Bagotsky, *Fundamentals of Electrochemistry*, Wiley, 2005. <https://doi.org/10.1002/047174199X>.
- [61] S. Otles, ed., *Handbook of Food Analysis Instruments*, CRC Press, 2016. <https://doi.org/10.1201/9781420045673>.
- [62] S.-I. Pyun, H.-C. Shin, J.-W. Lee, J.-Y. Go, *Electrochemistry of Insertion Materials for Hydrogen and Lithium*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2012. <https://doi.org/10.1007/978-3-642-29464-8>.
- [63] P.T. Kissinger, W.R. Heineman, Cyclic voltammetry, *J. Chem. Educ.* 60 (1983) 702. <https://doi.org/10.1021/ed060p702>.

- [64] J. Heinze, *Electrochemistry, Principles, Methods, and Applications*. Von C. M. A. Brett und A. M. O. Brett. Oxford University Press, Oxford, 1993. 427 S., Broschur 25.00 £. – ISBN 0-19-855388-9, *Angewandte Chemie* 106 (1994) 2441–2442. <https://doi.org/10.1002/ange.19941062240>.
- [65] H. Reiss, A. Heller, The absolute potential of the standard hydrogen electrode: a new estimate, *J. Phys. Chem.* 89 (1985) 4207–4213. <https://doi.org/10.1021/j100266a013>.
- [66] <https://eureka.patsnap.com/blog/what-is-standard-hydrogen-electrode/>, (n.d.).
- [67] H.A. Kooijman, A Modification of the Stokes–Einstein Equation for Diffusivities in Dilute Binary Mixtures, *Ind. Eng. Chem. Res.* 41 (2002) 3326–3328. <https://doi.org/10.1021/ie010690v>.
- [68] G. Inzelt, A. Lewenstam, F. Scholz, eds., *Handbook of Reference Electrodes*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2013. <https://doi.org/10.1007/978-3-642-36188-3>.
- [69] D. Alliata, R. Kötz, Investigation of Nanoscale intercalation into graphite and carbon materials by in situ Scanning Probe Microscopy, n.d.
- [70] A. Fick, On liquid diffusion, *J. Memb. Sci.* 100 (1995) 33–38. [https://doi.org/10.1016/0376-7388\(94\)00230-V](https://doi.org/10.1016/0376-7388(94)00230-V).
- [71] H.H., C.G.D., O.J.E. Bauer, *Analisi strumentale* , 1985.
- [72] D.A.C. Brownson, C.E. Banks, Introduction to Graphene, in: *The Handbook of Graphene Electrochemistry*, Springer London, London, 2014: pp. 1–22. https://doi.org/10.1007/978-1-4471-6428-9_1.
- [73] H. Wang, S.Y. Sayed, E.J. Lubner, B.C. Olsen, S.M. Shirurkar, S. Venkatakrishnan, U.M. Tefashe, A.K. Farquhar, E.S. Smotkin, R.L. McCreery, J.M. Buriak, Redox Flow Batteries: How to Determine Electrochemical Kinetic Parameters, *ACS Nano* 14 (2020) 2575–2584. <https://doi.org/10.1021/acsnano.0c01281>.
- [74] V.L. Mironov, The textbook for students of the senior courses of higher educational institutions *Fundamentals of Scanning Probe Microscopy* THE RUSSIAN ACADEMY OF SCIENCES INSTITUTE OF PHYSICS OF MICROSTRUCTURES Nizhniy Novgorod, 2004.
- [75] Y.S. Barash, *Van der Waals forces*, Nauka, Moscow, 1988.
- [76] M. Saint Jean, S. Hudlet, C. Guthmann, J. Berger, Van der Waals and capacitive forces in atomic force microscopies, *J. Appl. Phys.* 86 (1999) 5245–5248. <https://doi.org/10.1063/1.371506>.

- [77] R.S. Bradley, LXXIX. *The cohesive force between solid surfaces and the surface energy of solids*, The London, Edinburgh, and Dublin Philosophical Magazine and Journal of Science 13 (1932) 853–862. <https://doi.org/10.1080/14786449209461990>.
- [78] B.V. Derjaguin, V.M. Muller, Yu.P. Toporov, Effect of contact deformations on the adhesion of particles, J. Colloid Interface Sci. 53 (1975) 314–326. [https://doi.org/10.1016/0021-9797\(75\)90018-1](https://doi.org/10.1016/0021-9797(75)90018-1).
- [79] Surface energy and the contact of elastic solids, Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences 324 (1971) 301–313. <https://doi.org/10.1098/rspa.1971.0141>.
- [80] C.A.J. Putman, K.O. Van der Werf, B.G. De Groot, N.F. Van Hulst, J. Greve, Tapping mode atomic force microscopy in liquid, Appl. Phys. Lett. 64 (1994) 2454–2456. <https://doi.org/10.1063/1.111597>.
- [81] Bruker AFM Manual, n.d.
- [82] G. Binnig, H. Rohrer, C. Gerber, E. Weibel, Tunneling through a controllable vacuum gap, Appl. Phys. Lett. 40 (1982) 178–180. <https://doi.org/10.1063/1.92999>.
- [83] G. Binnig, H. Rohrer, Scanning tunneling microscopy, Surf. Sci. 126 (1983) 236–244. [https://doi.org/10.1016/0039-6028\(83\)90716-1](https://doi.org/10.1016/0039-6028(83)90716-1).
- [84] J. Frommer, Scanning Tunneling Microscopy and Atomic Force Microscopy in Organic Chemistry, Angewandte Chemie International Edition in English 31 (1992) 1298–1328. <https://doi.org/10.1002/anie.199212981>.
- [85] N. Byrne, J.M. Pringle, C. Tiyapiboonchaiya, D.R. MacFarlane, M. Forsyth, The additive effect of zwitterion and nano-particles on ion dissociation in polyelectrolytes, Electrochim. Acta 50 (2005) 2733–2738. <https://doi.org/10.1016/j.electacta.2004.11.050>.
- [86] A.J. Bard, F.-R. Fan, Introductory lecture. Studies of the liquid/solid interface by scanning tunnelling microscopy and scanning electrochemical microscopy, Faraday Discuss. 94 (1992) 1. <https://doi.org/10.1039/fd9929400001>.
- [87] R. Sonnenfeld, P.K. Hansma, Atomic-Resolution Microscopy in Water, Science (1979). 232 (1986) 211–213. <https://doi.org/10.1126/science.232.4747.211>.
- [88] P.K. Hansma, J. Tersoff, Scanning tunneling microscopy, J. Appl. Phys. 61 (1987) R1–R24. <https://doi.org/10.1063/1.338189>.

- [89] P.K. Hansma, V.B. Elings, O. Marti, C.E. Bracker, Scanning Tunneling Microscopy and Atomic Force Microscopy: Application to Biology and Technology, *Science* (1979). 242 (1988) 209–216. <https://doi.org/10.1126/science.3051380>.
- [90] K. Itaya, E. Tomita, Scanning tunneling microscope for electrochemistry - a new concept for the in situ scanning tunneling microscope in electrolyte solutions, *Surf. Sci.* 201 (1988) L507–L512. [https://doi.org/10.1016/0039-6028\(88\)90489-X](https://doi.org/10.1016/0039-6028(88)90489-X).
- [91] K. Itaya, In situ scanning tunneling microscopy in electrolyte solutions, *Prog. Surf. Sci.* 58 (1998) 121–247. [https://doi.org/10.1016/S0079-6816\(98\)00022-7](https://doi.org/10.1016/S0079-6816(98)00022-7).
- [92] V.M. Hallmark, S. Chiang, J.F. Rabolt, J.D. Swalen, R.J. Wilson, Observation of Atomic Corrugation on Au(111) by Scanning Tunneling Microscopy, *Phys. Rev. Lett.* 59 (1987) 2879–2882. <https://doi.org/10.1103/PhysRevLett.59.2879>.
- [93] G. Zhang, R. Zhang, G. Gu, &title>Real-time map building for a mobile robot</title>, in: G.R. Gerhart, B.A. Abbott (Eds.), 1998: pp. 189–194. <https://doi.org/10.1117/12.317544>.
- [94] J. Tersoff, D.R. Hamann, Theory and Application for the Scanning Tunneling Microscope, *Phys. Rev. Lett.* 50 (1983) 1998–2001. <https://doi.org/10.1103/PhysRevLett.50.1998>.
- [95] K. Oura, M. Katayama, A. V. Zotov, V.G. Lifshits, A.A. Saranin, *Surface Science*, Springer Berlin Heidelberg, Berlin, Heidelberg, 2003. <https://doi.org/10.1007/978-3-662-05179-5>.
- [96] W. Auwärter, K. Seufert, F. Bischoff, D. Eciya, S. Vijayaraghavan, S. Joshi, F. Klappenberger, N. Samudrala, J. V. Barth, A surface-anchored molecular four-level conductance switch based on single proton transfer, *Nat. Nanotechnol.* 7 (2012) 41–46. <https://doi.org/10.1038/nnano.2011.211>.
- [97] J. Tersoff, D.R. Hamann, Theory of the scanning tunneling microscope, *Phys. Rev. B* 31 (1985) 805–813. <https://doi.org/10.1103/PhysRevB.31.805>.
- [98] S.-W. Hla, Scanning tunneling microscopy single atom/molecule manipulation and its application to nanoscience and technology, *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures Processing, Measurement, and Phenomena* 23 (2005) 1351–1360. <https://doi.org/10.1116/1.1990161>.

- [99] R.J. Brook, A. Cheetham, A. Heuer, P. Hirsch, T.J. Marks, D.G. Pettifor, M. Ruhle, J. Silcox, A.P. Sutton, M. V Tirrell, V. Vitek, MONOGRAPHS ON THE PHYSICS AND CHEMISTRY OF MATERIALS General Editors, n.d.
- [100] R. Koch, J.J. Schulz, K.H. Rieder, Scanning tunneling microscopy artifact and real structure: Steps of Ag(110), 1999.
- [101] A. Smekal, Zur Quantentheorie der Dispersion, *Naturwissenschaften* 11 (1923) 873–875. <https://doi.org/10.1007/BF01576902>.
- [102] F. Justicia, M.C. Pichardo, F. Cano, A.B.G. Berbén, J. De la Fuente, The Revised Two-Factor Study Process Questionnaire (R-SPQ-2F): Exploratory and confirmatory factor analyses at item level, *European Journal of Psychology of Education* 23 (2008) 355–372. <https://doi.org/10.1007/BF03173004>.
- [103] *Infrared and Raman Spectroscopy*, Elsevier, 2011. <https://doi.org/10.1016/C2010-0-68479-3>.
- [104] E. Smith, G. Dent, *Modern Raman Spectroscopy – A Practical Approach*, Wiley, 2004. <https://doi.org/10.1002/0470011831>.
- [105] *Introductory Raman Spectroscopy*, Elsevier, 2003. <https://doi.org/10.1016/B978-0-12-254105-6.X5000-8>.
- [106] Z. Ni, Y. Wang, T. Yu, Z. Shen, Raman spectroscopy and imaging of graphene, *Nano Res.* 1 (2008) 273–291. <https://doi.org/10.1007/s12274-008-8036-1>.
- [107] T.A.O. Jafer, H.A.A. Abdelbagi, A. Sulyok, G.Z. Radnóczy, K. Tőkési, J.B. Malherbe, Highly oriented pyrolytic graphite chemical bonding structure after gallium implantation, *Sci. Rep.* 15 (2025). <https://doi.org/10.1038/s41598-025-20624-3>.
- [108] M. Menegazzo, L. Marfori, R. Yivlialin, A. Podestà, F. Ciccacci, L. Duò, V. Russo, M. Campione, G. Bussetti, Stiffness and mechanical manipulation of blisters grown on electrochemically intercalated graphite, *Electrochim. Acta* 488 (2024). <https://doi.org/10.1016/j.electacta.2024.144201>.
- [109] Nt-mdt, *Performing Measurements Instruction Manual AFM and STM Measurements, Spectroscopy, Many-Pass Techniques, Litography*, n.d. www.ntmdt-si.com.
- [110] M.V. Shinnur, M. Menegazzo, G. Bussetti, L. Duò, M.P. Pedferri, M.V. Diamanti, Studying the Photoactivity of Ag-Decorated TiO₂ Nanotubes with

- Combined AFM and Raman Spectroscopy, *Surfaces* 7 (2024) 938–950. <https://doi.org/10.3390/surfaces7040061>.
- [111] E. Mazzeletti, Statistical analysis of blisters in electrochemically intercalated HOPG by innovative combined AFM-Raman investigation *Tesi di Laurea Magistrale in Engineering physics-Ingegneria fisica*, n.d.
- [112] C. Höppener, J. Aizpurua, H. Chen, S. Gräfe, A. Jorio, S. Kupfer, Z. Zhang, V. Deckert, Tip-enhanced Raman scattering, *Nature Reviews Methods Primers* 4 (2024) 47. <https://doi.org/10.1038/s43586-024-00323-5>.
- [113] P. Verma, Tip-Enhanced Raman Spectroscopy: Technique and Recent Advances, *Chem. Rev.* 117 (2017) 6447–6466. <https://doi.org/10.1021/acs.chemrev.6b00821>.
- [114] L. Gao, H. Zhao, T. Li, P. Huo, D. Chen, B. Liu, Atomic Force Microscopy Based Tip-Enhanced Raman Spectroscopy in Biology, *Int. J. Mol. Sci.* 19 (2018) 1193. <https://doi.org/10.3390/ijms19041193>.
- [115] T.-X. Huang, S.-C. Huang, M.-H. Li, Z.-C. Zeng, X. Wang, B. Ren, Tip-enhanced Raman spectroscopy: tip-related issues, *Anal. Bioanal. Chem.* 407 (2015) 8177–8195. <https://doi.org/10.1007/s00216-015-8968-8>.
- [116] T. Itoh, M. Procházka, Z.-C. Dong, W. Ji, Y.S. Yamamoto, Y. Zhang, Y. Ozaki, Toward a New Era of SERS and TERS at the Nanometer Scale: From Fundamentals to Innovative Applications, *Chem. Rev.* 123 (2023) 1552–1634. <https://doi.org/10.1021/acs.chemrev.2c00316>.
- [117] L. Xia, M. Chen, X. Zhao, Z. Zhang, J. Xia, H. Xu, M. Sun, Visualized method of chemical enhancement mechanism on SERS and TERS, *Journal of Raman Spectroscopy* 45 (2014) 533–540. <https://doi.org/10.1002/jrs.4504>.
- [118] B. Pettinger, K.F. Domke, D. Zhang, G. Picardi, R. Schuster, Tip-enhanced Raman scattering: Influence of the tip-surface geometry on optical resonance and enhancement, *Surf. Sci.* 603 (2009) 1335–1341. <https://doi.org/10.1016/j.susc.2008.08.033>.
- [119] T. Touzalin, J. Landoulsi, L. Fillaud, I.T. Lucas, E. Maisonhaute, Electroactive molecular layers produced by reduction of an aryldiazonium salt scrutinized by Tip Enhanced Raman Spectroscopy, *Opt. Commun.* 589 (2025) 132034. <https://doi.org/10.1016/j.optcom.2025.132034>.
- [120] B. Pettinger, G. Picardi, R. Schuster, G. Ertl, Surface-enhanced and STM-tip-enhanced Raman Spectroscopy at Metal Surfaces, *Single Molecules* 3 (2002) 285–294. [https://doi.org/10.1002/1438-5171\(200211\)3:5/6<285::AID-SIMO285>3.0.CO;2-X](https://doi.org/10.1002/1438-5171(200211)3:5/6<285::AID-SIMO285>3.0.CO;2-X).

- [121] B. Ren, G. Picardi, B. Pettinger, Preparation of gold tips suitable for tip-enhanced Raman spectroscopy and light emission by electrochemical etching, *Review of Scientific Instruments* 75 (2004) 837–841. <https://doi.org/10.1063/1.1688442>.
- [122] P. Alice Fiocco, M. Breton Tony, M. Noël Jean-Marc, Development of electrochemical tip-enhanced Raman spectroscopy Towards the characterization of electroactive molecular architectures, n.d.
- [123] T. Touzalin, S. Joiret, E. Maisonhaute, I.T. Lucas, Complex Electron Transfer Pathway at a Microelectrode Captured by *in Situ* Nanospectroscopy, *Anal. Chem.* 89 (2017) 8974–8980. <https://doi.org/10.1021/acs.analchem.7b01542>.
- [124] V. Wang, D.C. Alsmeyer, R.L. McCreery, Raman Spectroscopy of Carbon Materials: Structural Basis of Observed Spectra, 1990. <https://pubs.acs.org/sharingguidelines>.
- [125] A.C. Ferrari, Raman spectroscopy of graphene and graphite: Disorder, electron-phonon coupling, doping and nonadiabatic effects, *Solid State Commun.* 143 (2007) 47–57. <https://doi.org/10.1016/j.ssc.2007.03.052>.
- [126] L.M. Malard, M.A. Pimenta, G. Dresselhaus, M.S. Dresselhaus, Raman spectroscopy in graphene, *Phys. Rep.* 473 (2009) 51–87. <https://doi.org/10.1016/j.physrep.2009.02.003>.
- [127] L.G. Cançado, A. Reina, J. Kong, M.S. Dresselhaus, Geometrical approach for the study of G' band in the Raman spectrum of monolayer graphene, bilayer graphene, and bulk graphite, *Phys. Rev. B Condens. Matter Mater. Phys.* 77 (2008). <https://doi.org/10.1103/PhysRevB.77.245408>.
- [128] C. Castiglioni, F. Negri, M. Rigolio, G. Zerbi, Raman activation in disordered graphites of the A'1 symmetry forbidden $k \neq 0$ phonon: The origin of the D line, *Journal of Chemical Physics* 115 (2001) 3769–3778. <https://doi.org/10.1063/1.1381529>.
- [129] A.C. Ferrari, J.C. Meyer, V. Scardaci, C. Casiraghi, M. Lazzeri, F. Mauri, S. Piscanec, D. Jiang, K.S. Novoselov, S. Roth, A.K. Geim, Raman spectrum of graphene and graphene layers, *Phys. Rev. Lett.* 97 (2006). <https://doi.org/10.1103/PhysRevLett.97.187401>.
- [130] C. Filoni, B. Shirzadi, M. Menegazzo, E. Martinelli, C. Di Natale, A. Li Bassi, L. Magagnin, L. Duò, G. Bussetti, Compared EC-AFM analysis of laser-induced graphene and graphite electrodes in sulfuric acid electrolyte, *Molecules* 26 (2021). <https://doi.org/10.3390/molecules26237333>.

- [131] G.G. Hoffmann, G. De With, J. Loos, Micro-Raman and tip-enhanced Raman spectroscopy of carbon allotropes, in: *Macromol. Symp.*, 2008: pp. 1–11. <https://doi.org/10.1002/masy.200850501>.
- [132] M. Bayle, N. Reckinger, A. Felten, P. Landois, O. Lancry, B. Dutertre, J.F. Colomer, A.A. Zahab, L. Henrard, J.L. Sauvajol, M. Paillet, Determining the number of layers in few-layer graphene by combining Raman spectroscopy and optical contrast, *Journal of Raman Spectroscopy* 49 (2018) 36–45. <https://doi.org/10.1002/jrs.5279>.
- [133] H. Lee, J. Il Choi, J. Park, S.S. Jang, S.W. Lee, Role of anions on electrochemical exfoliation of graphite into graphene in aqueous acids, *Carbon* N. Y. 167 (2020) 816–825. <https://doi.org/10.1016/j.carbon.2020.06.044>.
- [134] L. Puricelli, M. Galluzzi, C. Schulte, A. Podestà, P. Milani, Nanomechanical and topographical imaging of living cells by atomic force microscopy with colloidal probes, *Review of Scientific Instruments* 86 (2015). <https://doi.org/10.1063/1.4915896>.
- [135] H.-J. Butt, B. Cappella, M. Kappl, Force measurements with the atomic force microscope: Technique, interpretation and applications, *Surf. Sci. Rep.* 59 (2005) 1–152. <https://doi.org/10.1016/j.surfrep.2005.08.003>.
- [136] H. Hertz, Ueber die Berührung fester elastischer Körper., *Crll* 1882 (1882) 156–171. <https://doi.org/10.1515/crll.1882.92.156>.
- [137] B. Anczykowski, B. Gotsmann, H. Fuchs, J.P. Cleveland, V.B. Elings, How to measure energy dissipation in dynamic mode atomic force microscopy, *Appl. Surf. Sci.* 140 (1999) 376–382. [https://doi.org/10.1016/S0169-4332\(98\)00558-3](https://doi.org/10.1016/S0169-4332(98)00558-3).
- [138] D. Metten, F. Federspiel, M. Romeo, S. Berciaud, All-Optical Blister Test of Suspended Graphene Using Micro-Raman Spectroscopy, *Phys. Rev. Appl.* 2 (2014). <https://doi.org/10.1103/PhysRevApplied.2.054008>.
- [139] Y.Y. Wang, Z.H. Ni, Z.X. Shen, H.M. Wang, Y.H. Wu, Interference enhancement of Raman signal of graphene, *Appl. Phys. Lett.* 92 (2008). <https://doi.org/10.1063/1.2838745>.
- [140] D.L. Silva, J.L.E. Campos, T.F.D. Fernandes, J.N. Rocha, L.R.P. Machado, E.M. Soares, D.R. Miquita, H. Miranda, C. Rabelo, O.P. Vilela Neto, A. Jorio, L.G. Cançado, Raman spectroscopy analysis of number of layers in mass-produced graphene flakes, *Carbon* N. Y. 161 (2020) 181–189. <https://doi.org/10.1016/j.carbon.2020.01.050>.

- [141] D. Yoon, H. Moon, H. Cheong, J. Choi, J. Choi, B. Park, Variations in the Raman Spectrum as a Function of the Number of Graphene Layers, *Journal of the Korean Physical Society* 55 (2009) 1299–1303. <https://doi.org/10.3938/jkps.55.1299>.
- [142] G. Wang, Z. Dai, Y. Wang, P. Tan, L. Liu, Z. Xu, Y. Wei, R. Huang, Z. Zhang, Measuring interlayer shear stress in bilayer graphene, *Phys. Rev. Lett.* 119 (2017). <https://doi.org/10.1103/PhysRevLett.119.036101>.
- [143] T.M.G. Mohiuddin, A. Lombardo, R.R. Nair, A. Bonetti, G. Savini, R. Jalil, N. Bonini, D.M. Basko, C. Galiotis, N. Marzari, K.S. Novoselov, A.K. Geim, A.C. Ferrari, Uniaxial strain in graphene by Raman spectroscopy: G peak splitting, Grüneisen parameters, and sample orientation, *Phys. Rev. B Condens. Matter Mater. Phys.* 79 (2009). <https://doi.org/10.1103/PhysRevB.79.205433>.
- [144] I. Polyzos, M. Bianchi, L. Rizzi, E.N. Koukaras, J. Parthenios, K. Papagelis, R. Sordan, C. Galiotis, Suspended monolayer graphene under true uniaxial deformation, *Nanoscale* 7 (2015) 13033–13042. <https://doi.org/10.1039/c5nr03072b>.
- [145] V.M. Pereira, A.H. Castro Neto, N.M.R. Peres, Tight-binding approach to uniaxial strain in graphene, *Phys. Rev. B Condens. Matter Mater. Phys.* 80 (2009). <https://doi.org/10.1103/PhysRevB.80.045401>.
- [146] Z.H. Ni, T. Yu, Y.H. Lu, Y.Y. Wang, Y.P. Feng, Z.X. Shen, Uniaxial strain on graphene: Raman spectroscopy study and band-gap opening, *ACS Nano* 2 (2008) 2301–2305. <https://doi.org/10.1021/nn800459e>.
- [147] D. Graf, F. Molitor, K. Ensslin, C. Stampfer, A. Jungen, C. Hierold, L. Wirtz, Spatially resolved raman spectroscopy of single- and few-layer graphene, *Nano Lett.* 7 (2007) 238–242. <https://doi.org/10.1021/nl061702a>.
- [148] Z. Peng, X. Chen, Y. Fan, D.J. Srolovitz, D. Lei, Strain engineering of 2D semiconductors and graphene: from strain fields to band-structure tuning and photonic applications, *Light Sci. Appl.* 9 (2020). <https://doi.org/10.1038/s41377-020-00421-5>.
- [149] Y.C. Cheng, Z.Y. Zhu, G.S. Huang, U. Schwingenschlögl, Grüneisen parameter of the G mode of strained monolayer graphene, *Phys. Rev. B Condens. Matter Mater. Phys.* 83 (2011). <https://doi.org/10.1103/PhysRevB.83.115449>.

- [150] R.J. Young, L. Gong, I.A. Kinloch, I. Riaz, R. Jalil, K.S. Novoselov, Strain mapping in a graphene monolayer nanocomposite, *ACS Nano* 5 (2011) 3079–3084. <https://doi.org/10.1021/nn2002079>.
- [151] C. Metzger, S. Rémi, M. Liu, S. V. Kusminskiy, A.H. Castro Neto, A.K. Swan, B.B. Goldberg, Biaxial strain in graphene adhered to shallow depressions, *Nano Lett.* 10 (2010) 6–10. <https://doi.org/10.1021/nl901625v>.
- [152] C. Androulidakis, E.N. Koukaras, J. Parthenios, G. Kalosakas, K. Papagelis, C. Galiotis, Graphene flakes under controlled biaxial deformation, *Sci. Rep.* 5 (2015). <https://doi.org/10.1038/srep18219>.
- [153] R. Beams, Tip-enhanced Raman scattering of graphene, *Journal of Raman Spectroscopy* 49 (2018) 157–167. <https://doi.org/10.1002/jrs.5211>.
- [154] S. Vantasin, I. Tanabe, Y. Tanaka, T. Itoh, T. Suzuki, Y. Kutsuma, K. Ashida, T. Kaneko, Y. Ozaki, Tip-enhanced raman scattering of the local nanostructure of epitaxial graphene grown on 4H-SiC (0001⁻), *Journal of Physical Chemistry C* 118 (2014) 25809–25815. <https://doi.org/10.1021/jp508730y>.
- [155] M.V. Balois-Oguchi, N. Hayazawa, S. Yasuda, K. Ikeda, T.Q. Nguyen, M.C. Escaño, T. Tanaka, Probing Strain and Doping along a Graphene Wrinkle Using Tip-Enhanced Raman Spectroscopy, *Journal of Physical Chemistry C* 127 (2023) 5982–5990. <https://doi.org/10.1021/acs.jpcc.2c08529>.
- [156] A.C. Gadelha, D.A.A. Ohlberg, C. Rabelo, E.G.S. Neto, T.L. Vasconcelos, J.L. Campos, J.S. Lemos, V. Ornelas, D. Miranda, R. Nadas, F.C. Santana, K. Watanabe, T. Taniguchi, B. van Troeye, M. Lamparski, V. Meunier, V.H. Nguyen, D. Paszko, J.C. Charlier, L.C. Campos, L.G. Cançado, G. Medeiros-Ribeiro, A. Jorio, Localization of lattice dynamics in low-angle twisted bilayer graphene, *Nature* 590 (2021) 405–409. <https://doi.org/10.1038/s41586-021-03252-5>.
- [157] R.H. Rickman, P.R. Dunstan, Enhancement of lattice defect signatures in graphene and ultrathin graphite using tip-enhanced Raman spectroscopy, *Journal of Raman Spectroscopy* 45 (2014) 15–21. <https://doi.org/10.1002/jrs.4416>.
- [158] Y.F. Bao, M.Y. Zhu, X.J. Zhao, H.X. Chen, X. Wang, B. Ren, Nanoscale chemical characterization of materials and interfaces by tip-enhanced Raman spectroscopy, *Chem. Soc. Rev.* 53 (2024) 10044–10079. <https://doi.org/10.1039/d4cs00588k>.

- [159] T.P. Darlington, C. Carmesin, M. Florian, E. Yanev, O. Ajayi, J. Ardelean, D.A. Rhodes, A. Ghiotto, A. Krayev, K. Watanabe, T. Taniguchi, J.W. Kysar, A.N. Pasupathy, J.C. Hone, F. Jahnke, N.J. Borys, P.J. Schuck, Imaging strain-localized excitons in nanoscale bubbles of monolayer WSe₂ at room temperature, *Nat. Nanotechnol.* 15 (2020) 854–860. <https://doi.org/10.1038/s41565-020-0730-5>.
- [160] J. Shao, W. Su, Tip-enhanced nanoscopy of two-dimensional transition metal dichalcogenides: progress and perspectives, *Nanoscale* 14 (2022) 17119–17133. <https://doi.org/10.1039/d2nr04864g>.
- [161] J. Chaste, A. Missaoui, A. Saadani, D. Garcia-Sanchez, D. Pierucci, Z. Ben Aziza, A. Ouerghi, Nanomechanical Strain Concentration on a Two-Dimensional Nanobridge within a Large Suspended Bilayer Graphene for Molecular Mass Detection, *ACS Appl. Nano Mater.* 1 (2018) 6752–6759. <https://doi.org/10.1021/acsanm.8b01555>.
- [162] C. Carmesin, M. Lorke, M. Florian, D. Erben, A. Schulz, T.O. Wehling, F. Jahnke, Quantum-Dot-Like States in Molybdenum Disulfide Nanostructures Due to the Interplay of Local Surface Wrinkling, Strain, and Dielectric Confinement, *Nano Lett.* 19 (2019) 3182–3186. <https://doi.org/10.1021/acs.nanolett.9b00641>.
- [163] G. Soares, R.R. Barreto, R. Nadas, K. Watanabe, T. Taniguchi, L.C. Campos, L.G. Cançado, A. Malachias, Anisotropic Strain Observation in Naturally Occurring Buckling on Twisted Bilayer Graphene: A Nano-Raman Study, *Journal of Physical Chemistry C* 129 (2025) 7508–7519. <https://doi.org/10.1021/acs.jpcc.5c00506>.
- [164] R. Beams, L.G. Cançado, A. Jorio, A.N. Vamivakas, L. Novotny, Tip-enhanced Raman mapping of local strain in graphene, *Nanotechnology* 26 (2015) 1–6. <https://doi.org/10.1088/0957-4484/26/17/175702>.
- [165] K. Yue, W. Gao, R. Huang, K.M. Liechti, Analytical methods for the mechanics of graphene bubbles, *J. Appl. Phys.* 112 (2012). <https://doi.org/10.1063/1.4759146>.
- [166] G. Soares, R.R. Barreto, R. Nadas, K. Watanabe, T. Taniguchi, L.C. Campos, L.G. Cançado, A. Malachias, Anisotropic Strain Observation in Naturally Occurring Buckling on Twisted Bilayer Graphene: A Nano-Raman Study, *Journal of Physical Chemistry C* 129 (2025) 7508–7519. <https://doi.org/10.1021/acs.jpcc.5c00506>.

- [167] M. Whitehead, G. Parry, P. Wheatley, P. Wheatley, Investigation of etalon effects in GaAs-AlGaAs multiple quantum well modulators, *IEE Proceedings J Optoelectronics* 136 (1989) 52. <https://doi.org/10.1049/ip-j.1989.0012>.
- [168] J.H. McElroy, G. Schiffner, R.S. Reynolds, Temperature-Dependent Etalon Effects in Laser Systems, *Appl. Opt.* 10 (1971) 2065. <https://doi.org/10.1364/AO.10.002065>.
- [169] J.U. Lee, D. Yoon, H. Cheong, Estimation of young's modulus of graphene by Raman spectroscopy, *Nano Lett.* 12 (2012) 4444–4448. <https://doi.org/10.1021/nl301073q>.
- [170] S. Fang, S. Duan, X. Wang, S. Chen, L. Li, H. Li, B. Jiang, C. Liu, N. Wang, L. Zhang, X. Wen, Y. Yao, J. Zhang, D. Xie, Y. Luo, W. Xu, Direct characterization of shear phonons in layered materials by mechano-Raman spectroscopy, *Nat. Photonics* 17 (2023) 531–537. <https://doi.org/10.1038/s41566-023-01181-5>.
- [171] P.H. Tan, W.P. Han, W.J. Zhao, Z.H. Wu, K. Chang, H. Wang, Y.F. Wang, N. Bonini, N. Marzari, N. Pugno, G. Savini, A. Lombardo, A.C. Ferrari, The shear mode of multilayer graphene, *Nat. Mater.* 11 (2012) 294–300. <https://doi.org/10.1038/nmat3245>.

APPENDIX: Blister Morphology and Spectroscopic Dataset

In this appendix it are shown some complementary data used for the statistical Analysis of blister presented in section 5.2. of this thesis. At first are shown the AFM morphology and the Raman spectra of each blister, used for the statistics analysis in chapter 5.2. It is then presented the main reference used for the analysis of the 2D peak. At the end it is shown the distribution of the blister radius and the blister height.

For each blister it is presented the AFM morphology image and the spectrum after the subtraction of the basal plane. The black square in the morphology images, indicates the position of the acquired spectrum in the left of each image, like for image 4

BLISTER 1

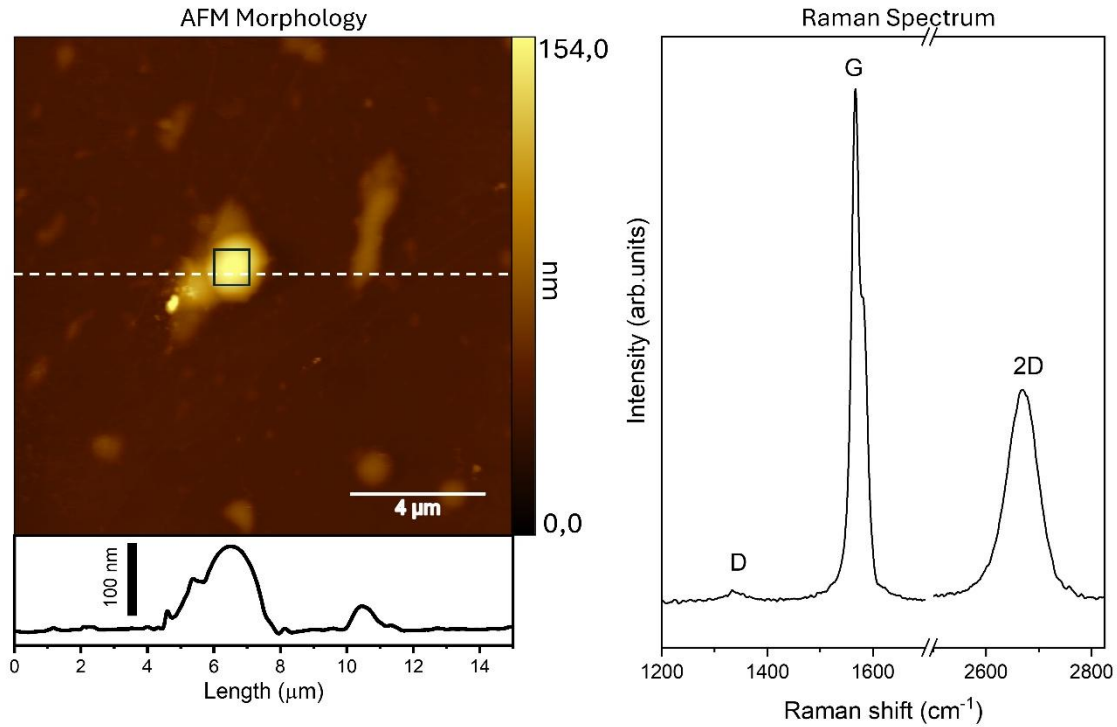


Figure A 1: AFM morphology and Raman spectrum of blister #1

BLISTER 2

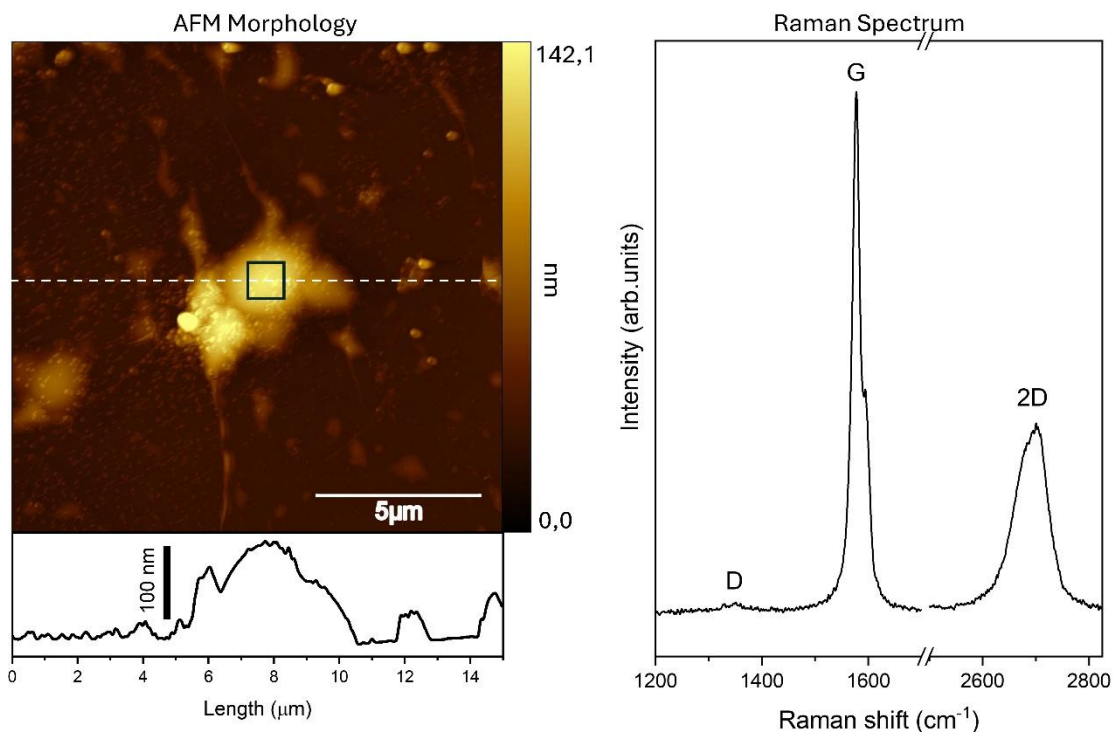


Figure A 2: AFM morphology and Raman spectrum of blister #2

BLISTER 3

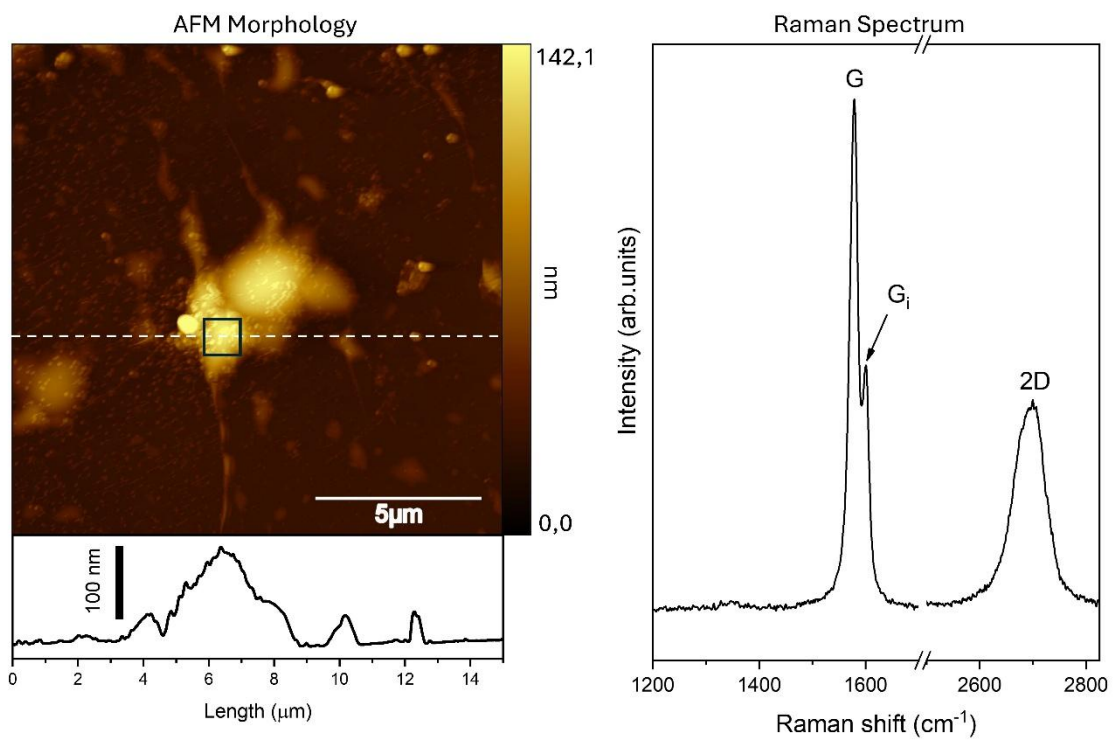


Figure A 3: AFM morphology and Raman spectrum of blister #3

BLISTER 4

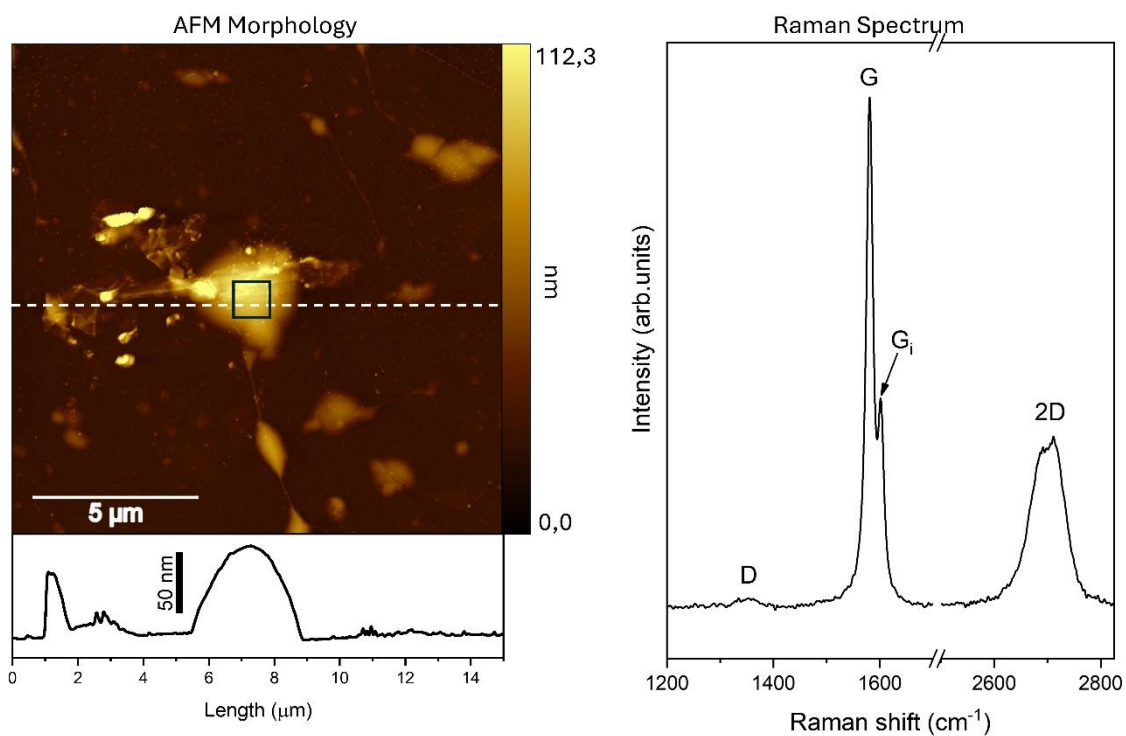


Figure A 4: AFM morphology and Raman spectrum of blister #4

BLISTER 5

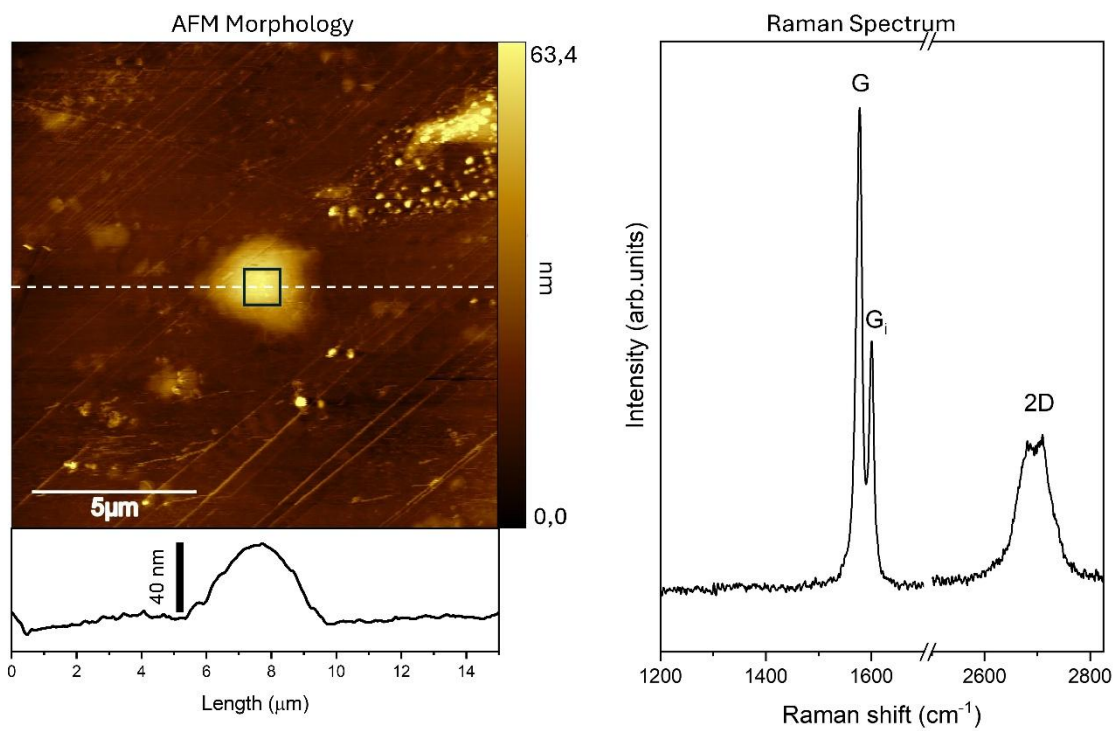


Figure A 5: AFM morphology and Raman spectrum of blister #5

BLISTER 6

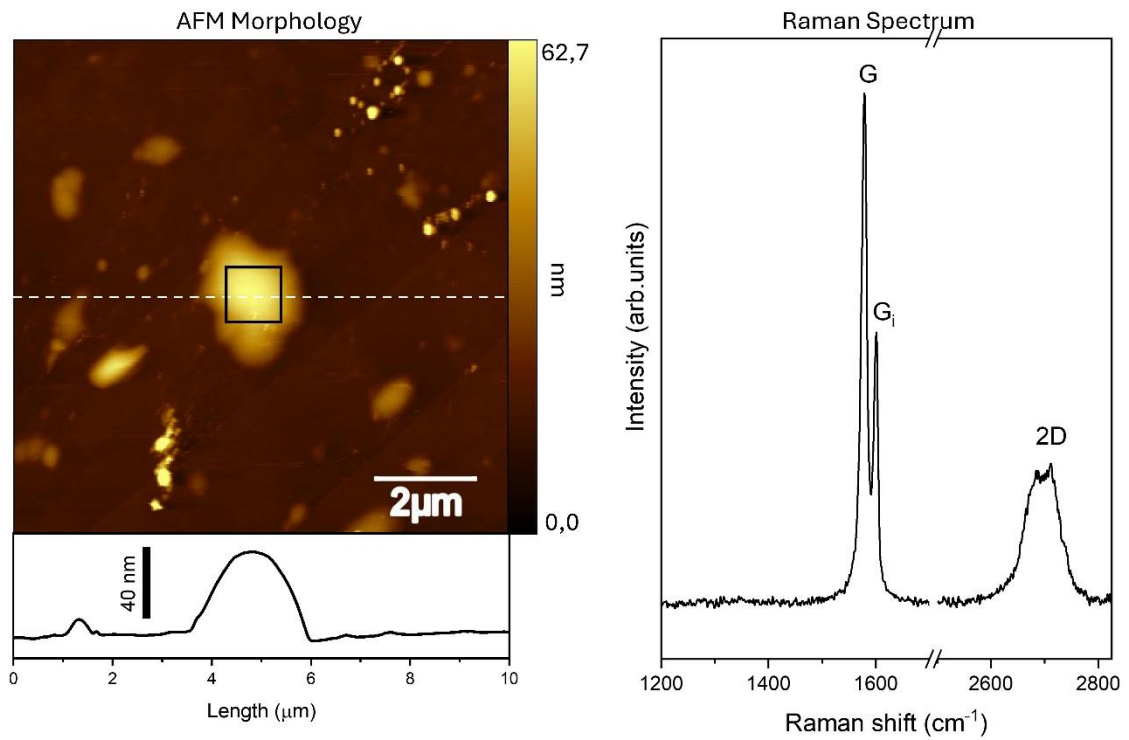


Figure A 6: AFM morphology and Raman spectrum of blister #6

BLISTER 7

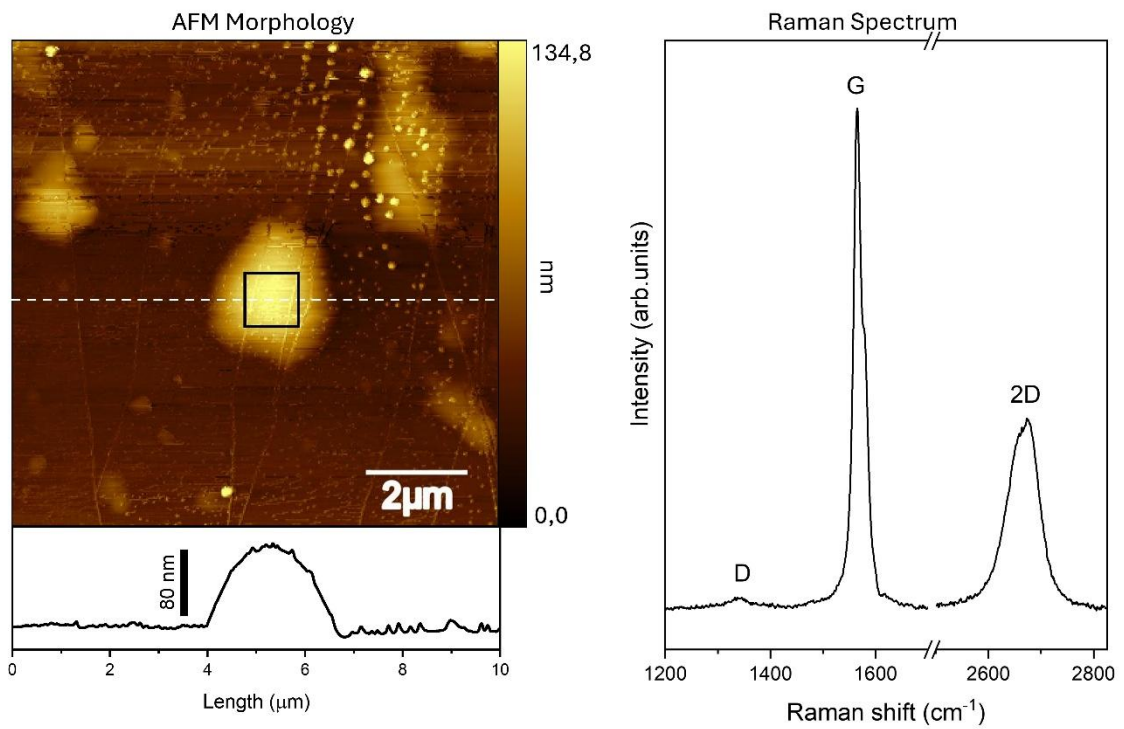


Figure A 7: AFM morphology and Raman spectrum of blister #7

BLISTER 8

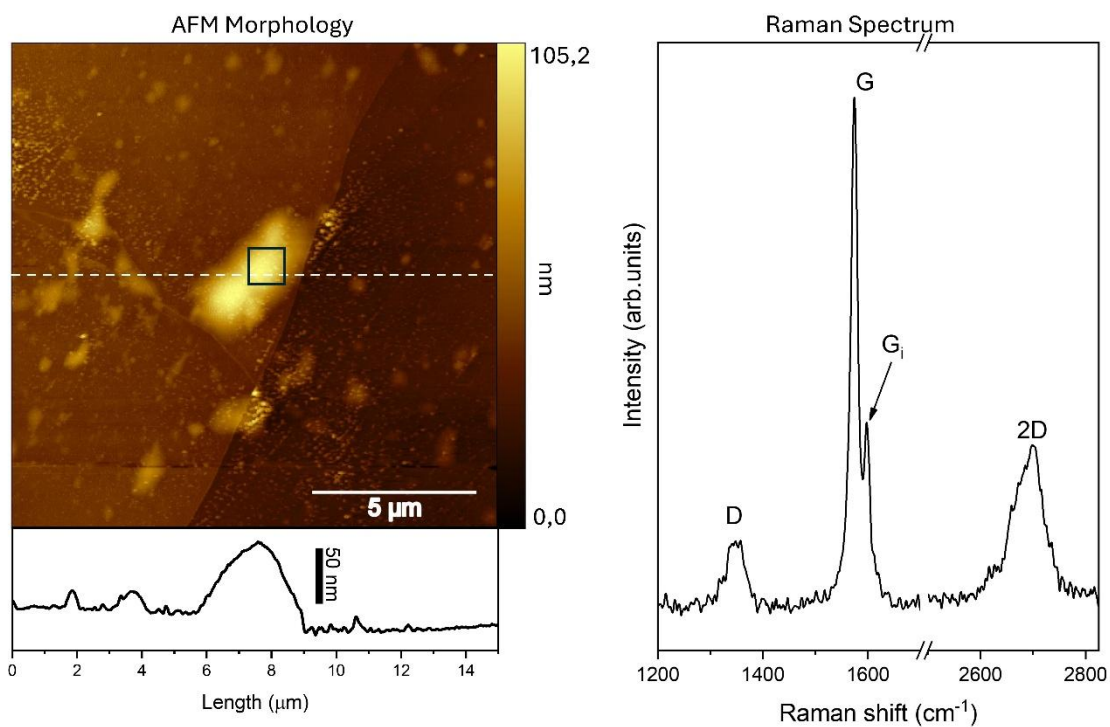


Figure A 8: AFM morphology and Raman spectrum of blister #8

BLISTER 9

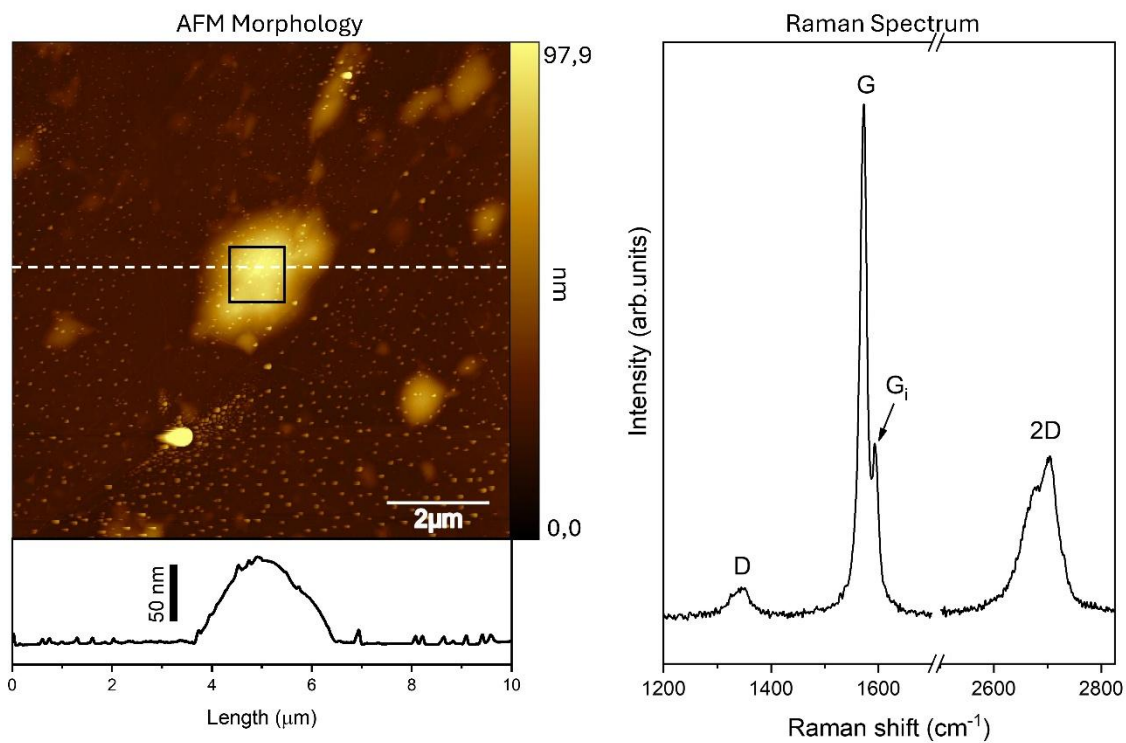


Figure A 9: AFM morphology and Raman spectrum of blister #9

BLISTER 10

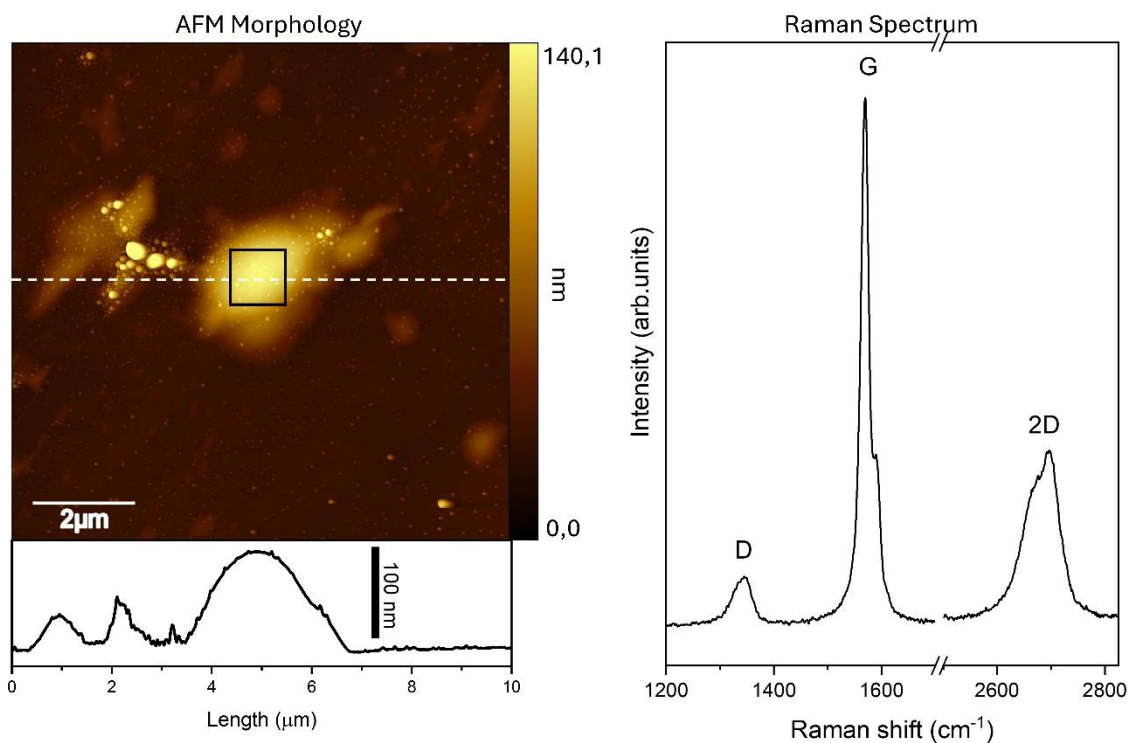


Figure A 10: AFM morphology and Raman spectrum of blister #10

BLISTER 11

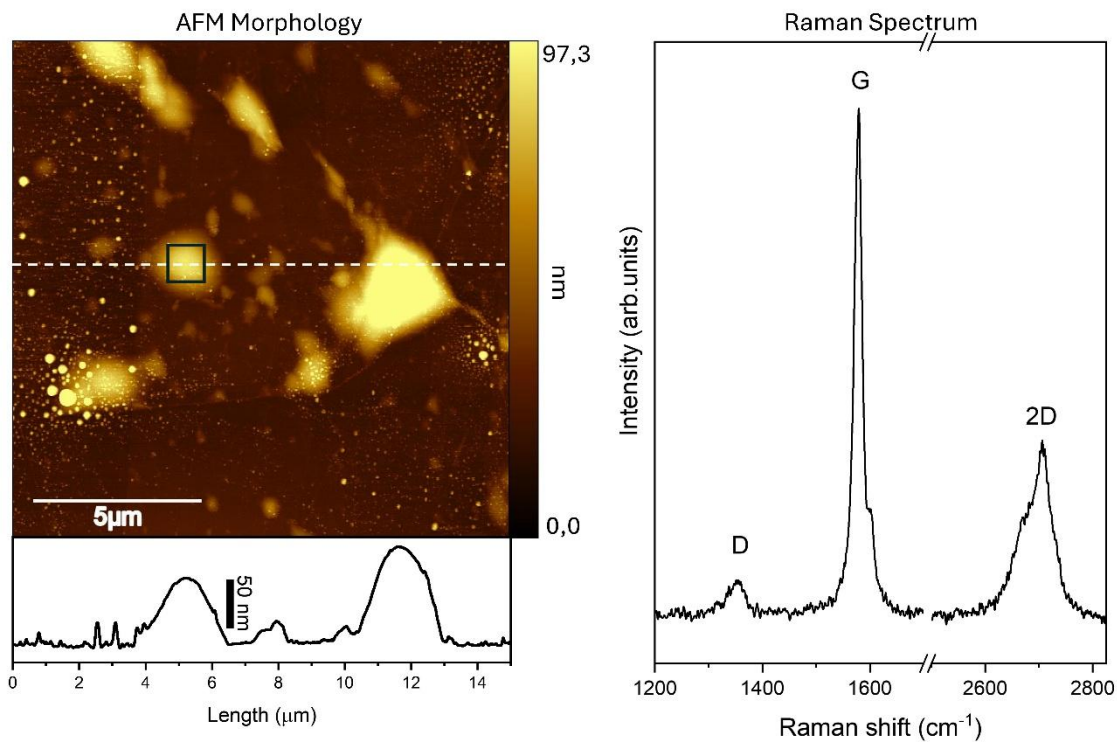


Figure A 11: AFM morphology and Raman spectrum of blister #11

BLISTER 12

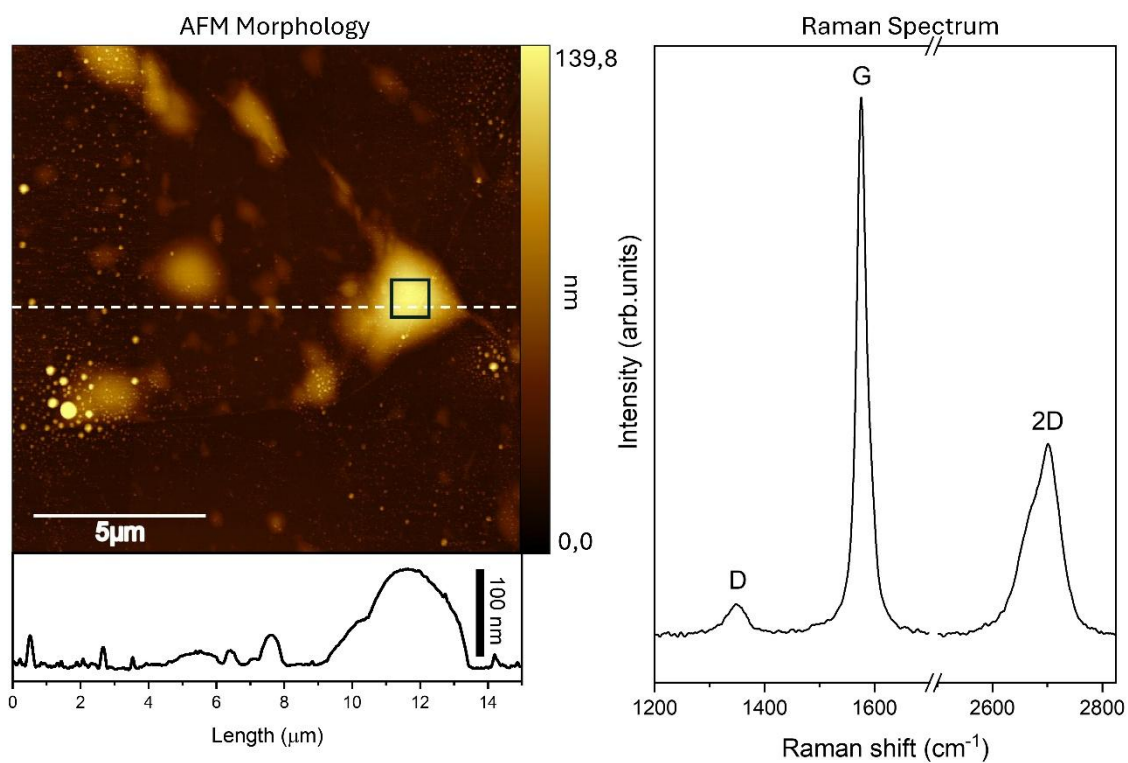


Figure A 12: AFM morphology and Raman spectrum of blister #12

BLISTER 13

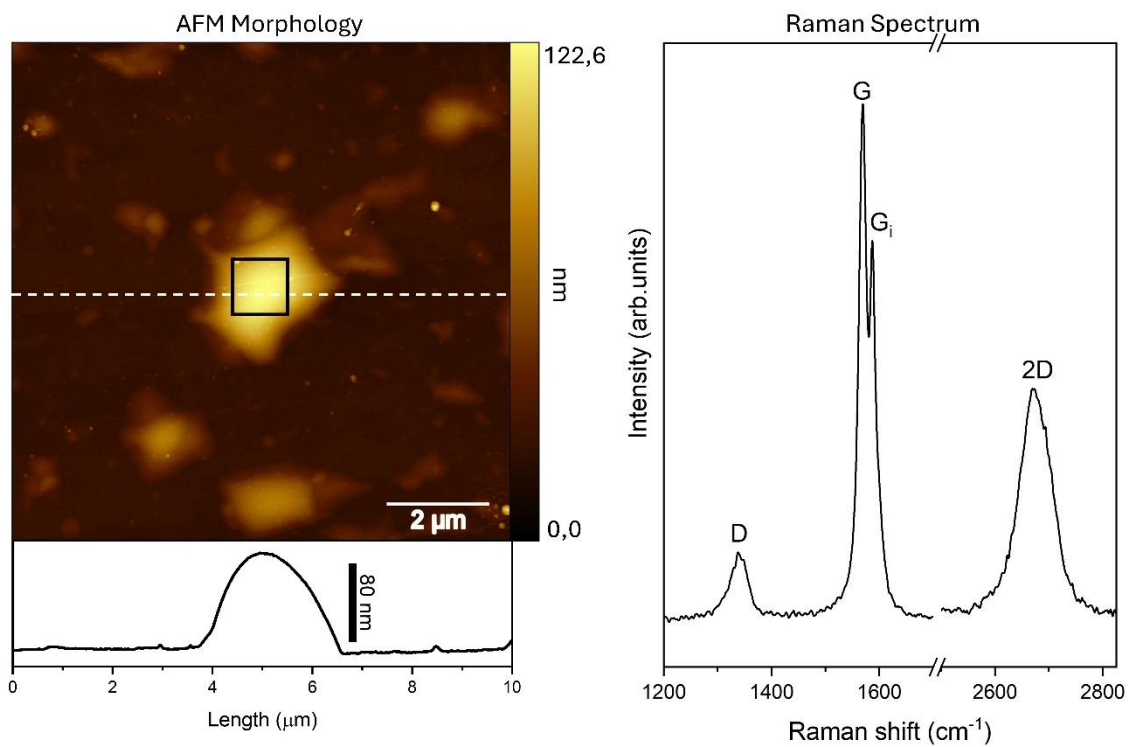


Figure A 13: AFM morphology and Raman spectrum of blister #13

BLISTER 14

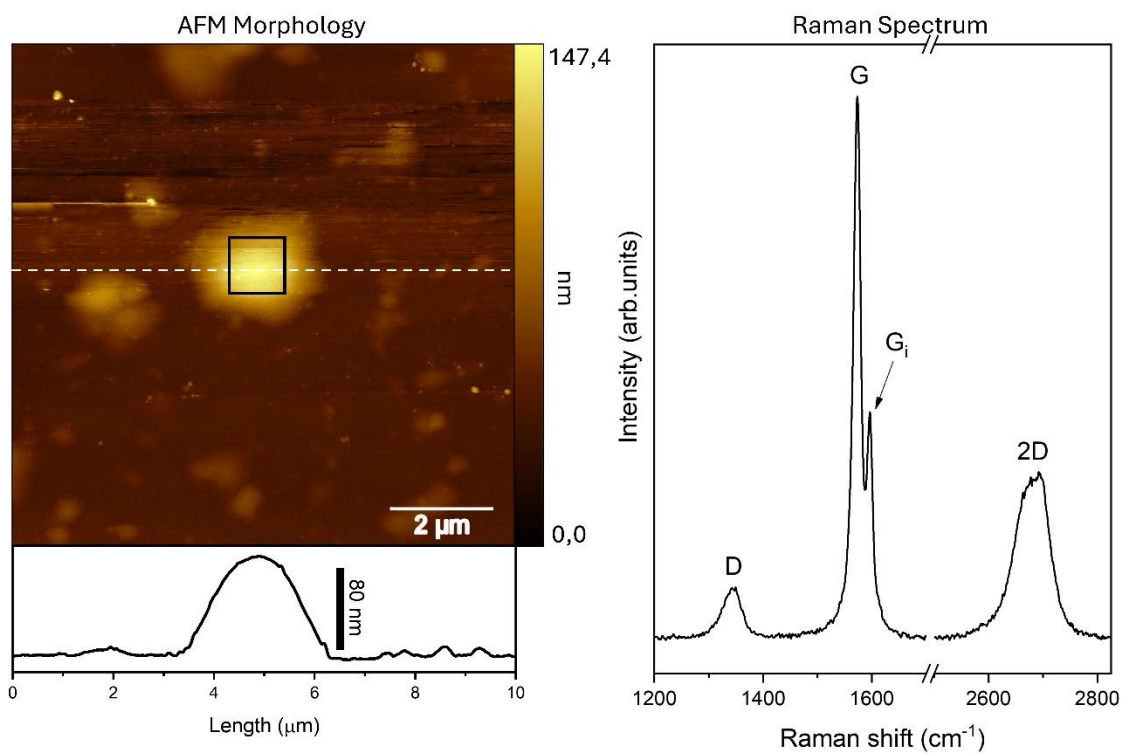


Figure A 14: AFM morphology and Raman spectrum of blister #14

BLISTER 15

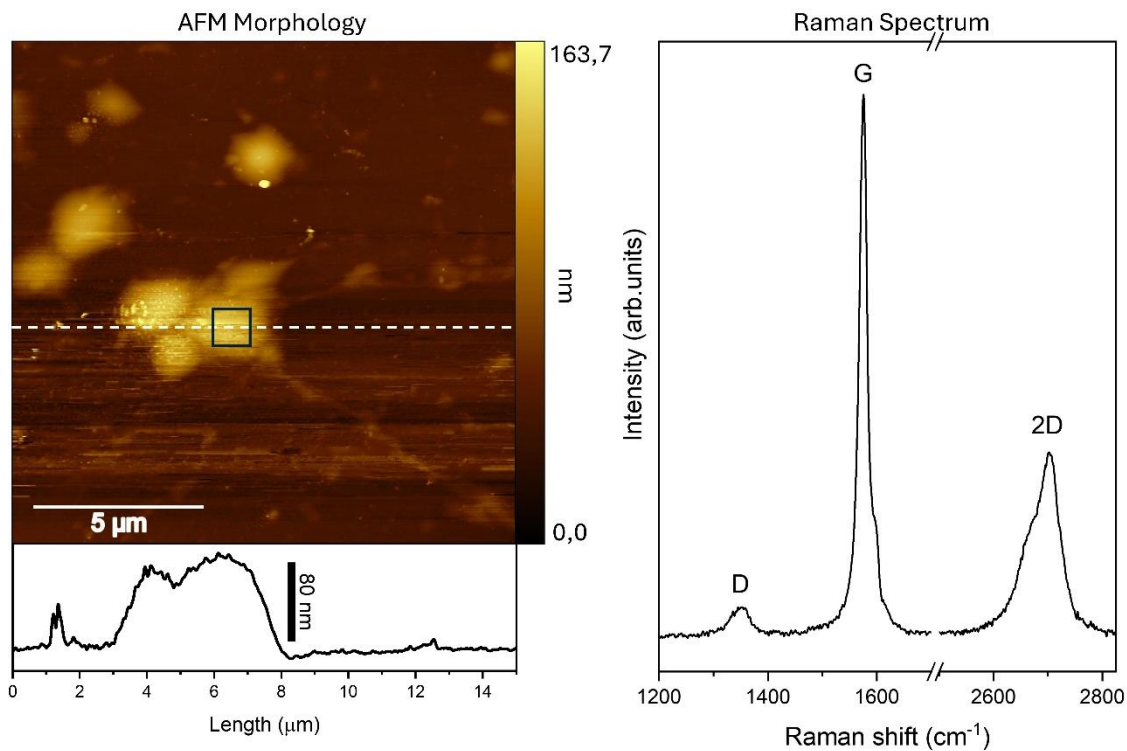


Figure A 15: AFM morphology and Raman spectrum of blister #15

BLISTER 16

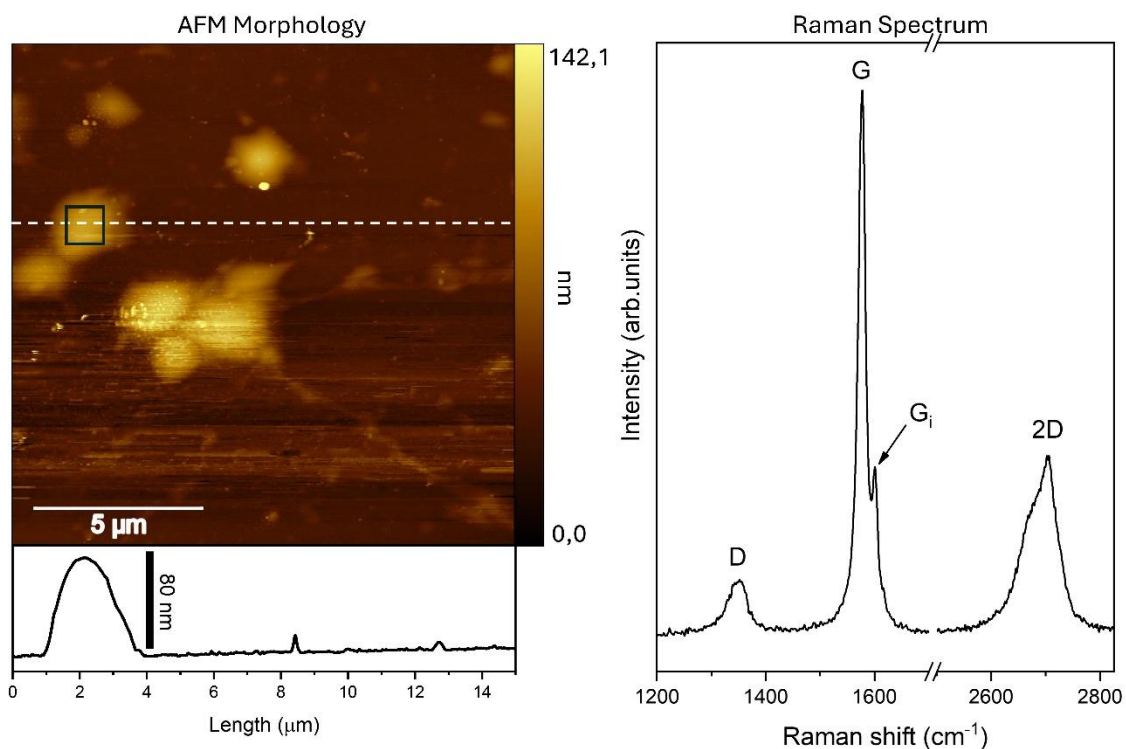


Figure A 16: AFM morphology and Raman spectrum of blister #16

BLISTER 17

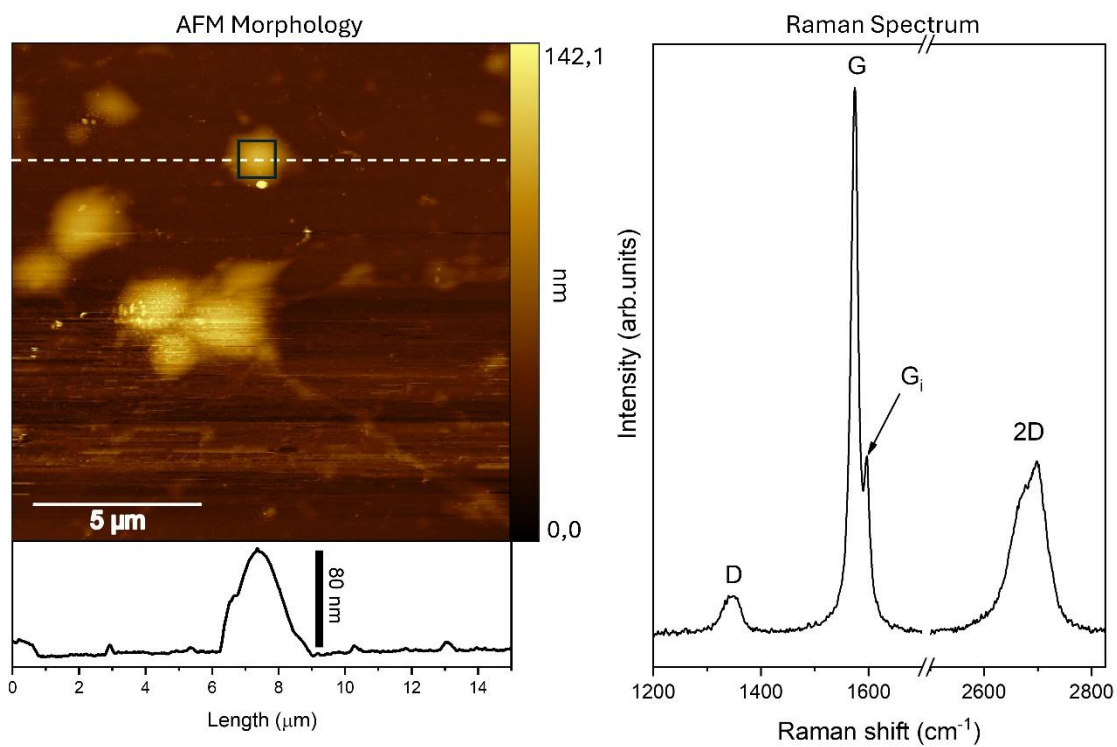


Figure A 17: AFM morphology and Raman spectrum of blister #17

BLISTER 18

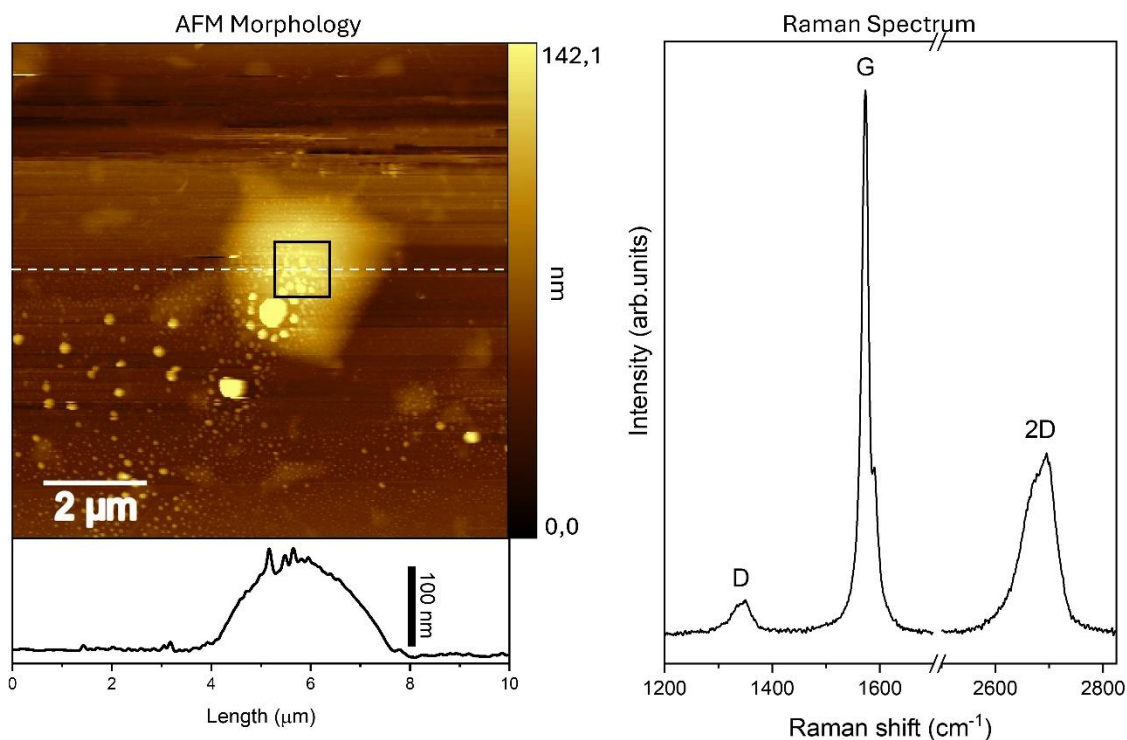


Figure A 18: AFM morphology and Raman spectrum of blister #18

BLISTER 19

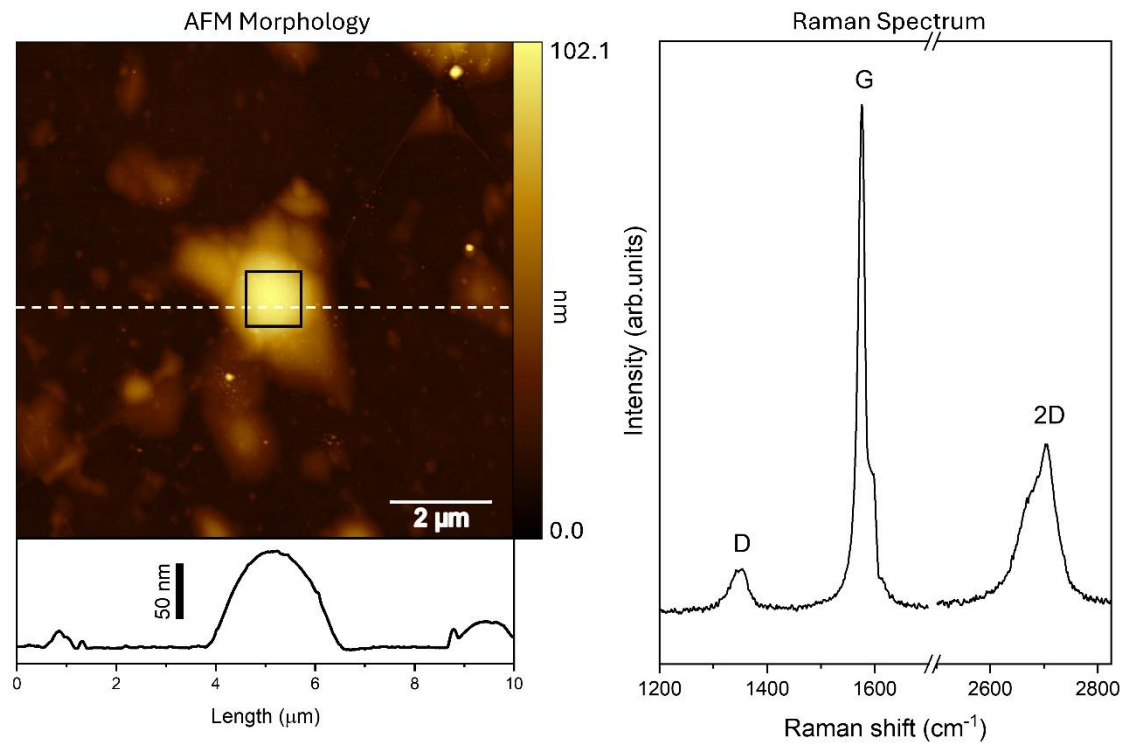


Figure A 19: AFM morphology and Raman spectrum of blister #19

BLISTER 20

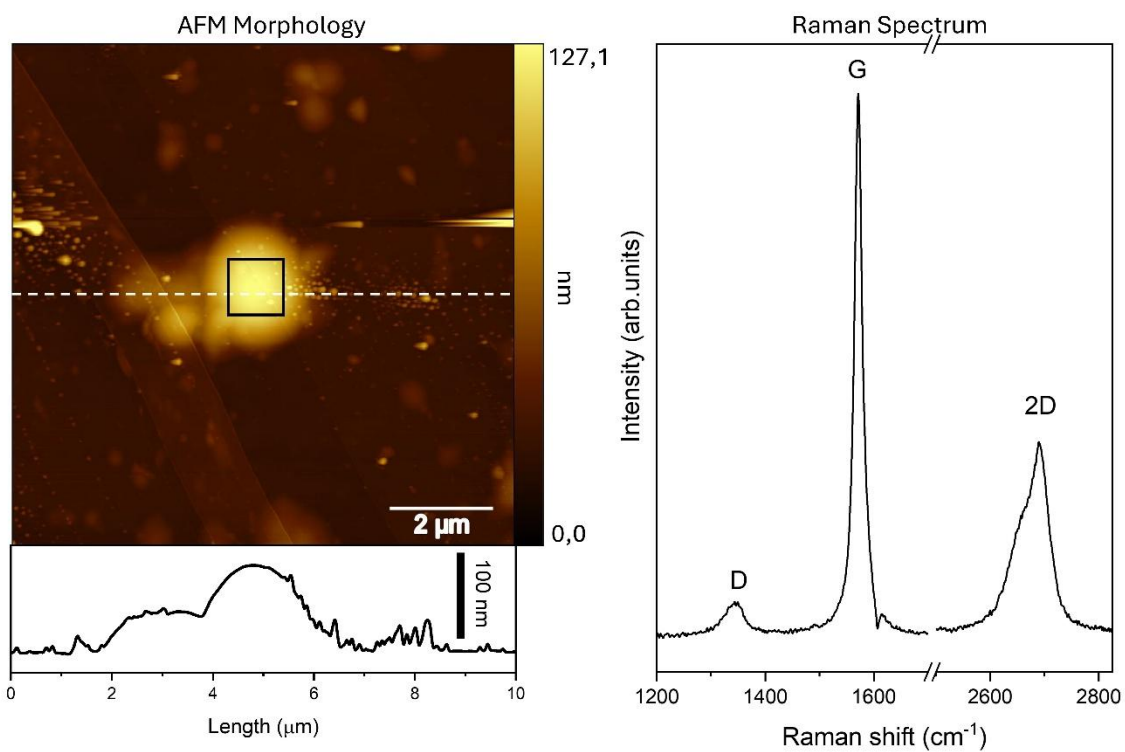


Figure A 20: AFM morphology and Raman spectrum of blister #20

BLISTER 21

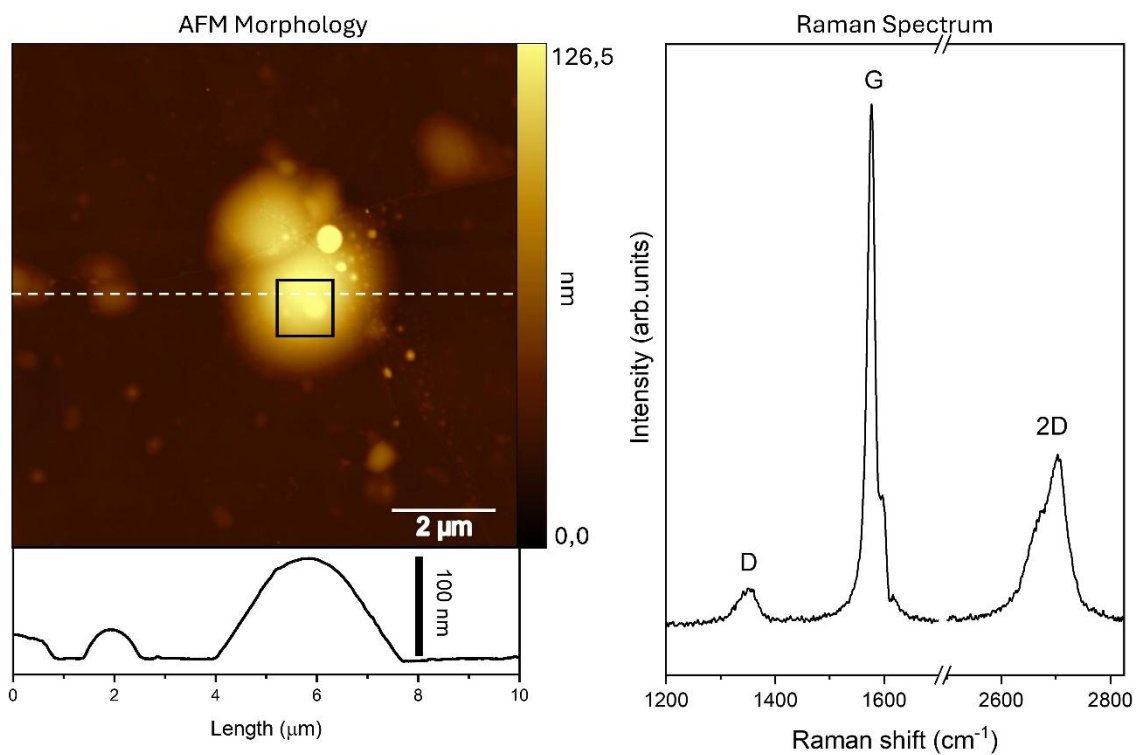


Figure A 21: AFM morphology and Raman spectrum of blister #21

BLISTER 22

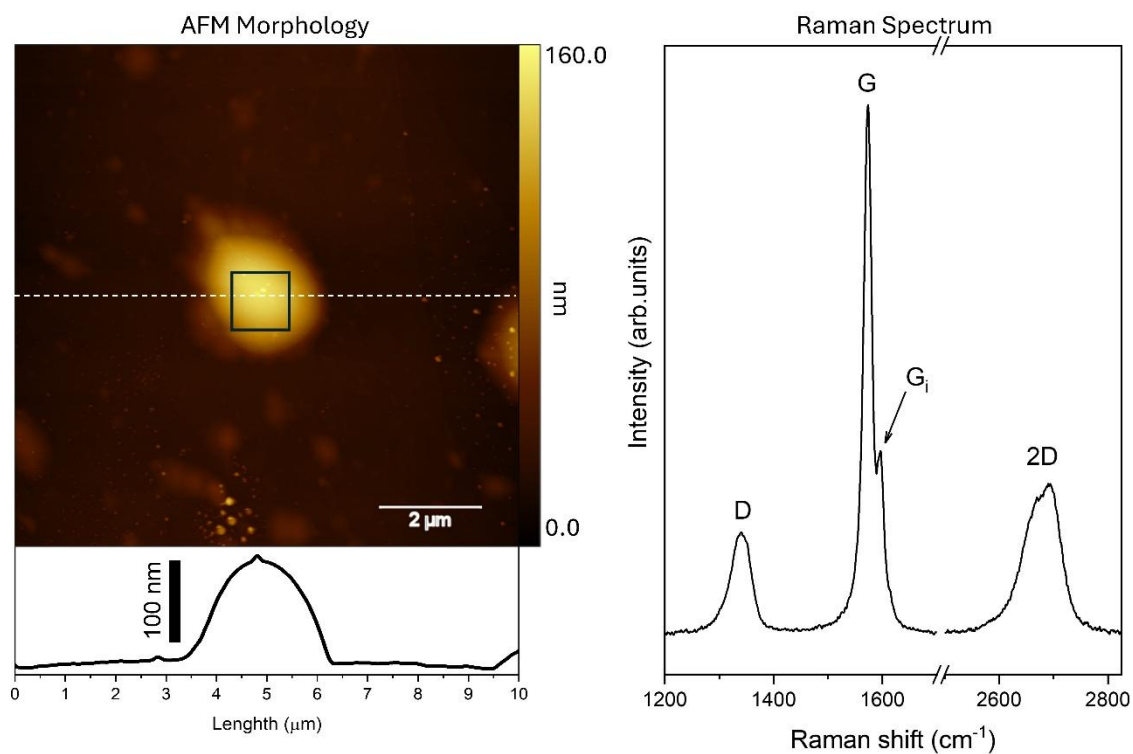


Figure A 22: AFM morphology and Raman spectrum of blister #22

BLISTER 23

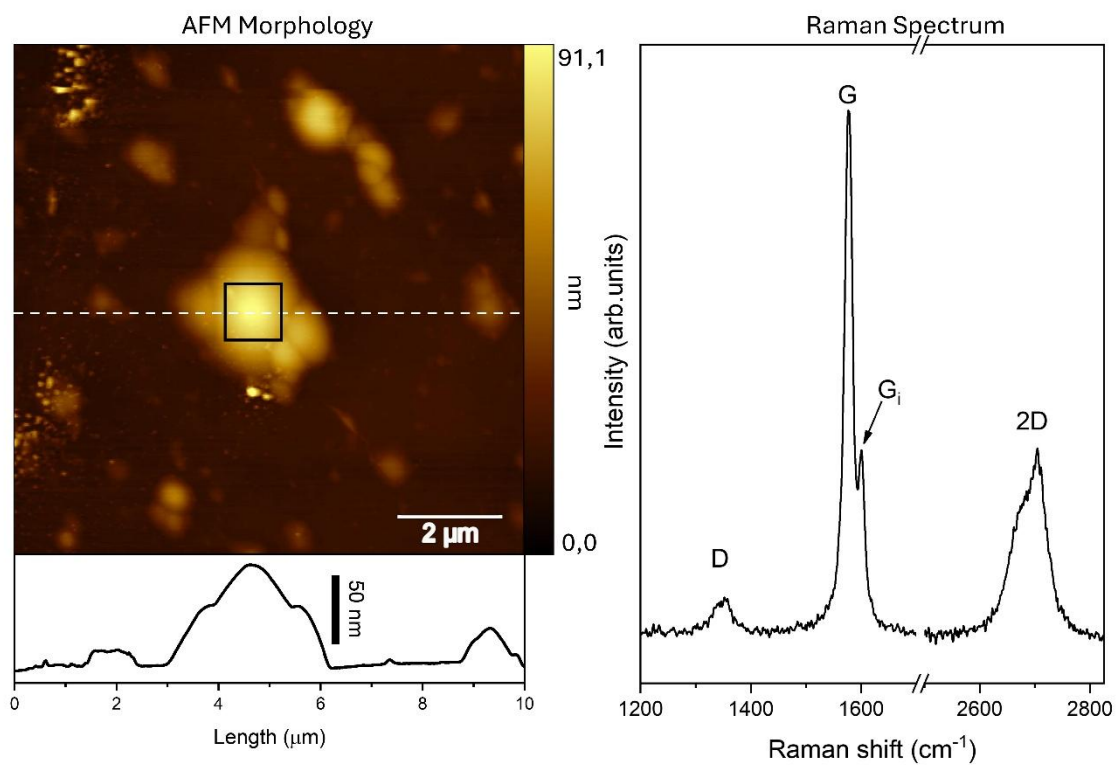


Figure A 23: AFM morphology and Raman spectrum of blister #23

The dependence of the 2D peak layer dependence on few layer graphene, acquired with laser wavelength 532 nm [140] is presented in the image below

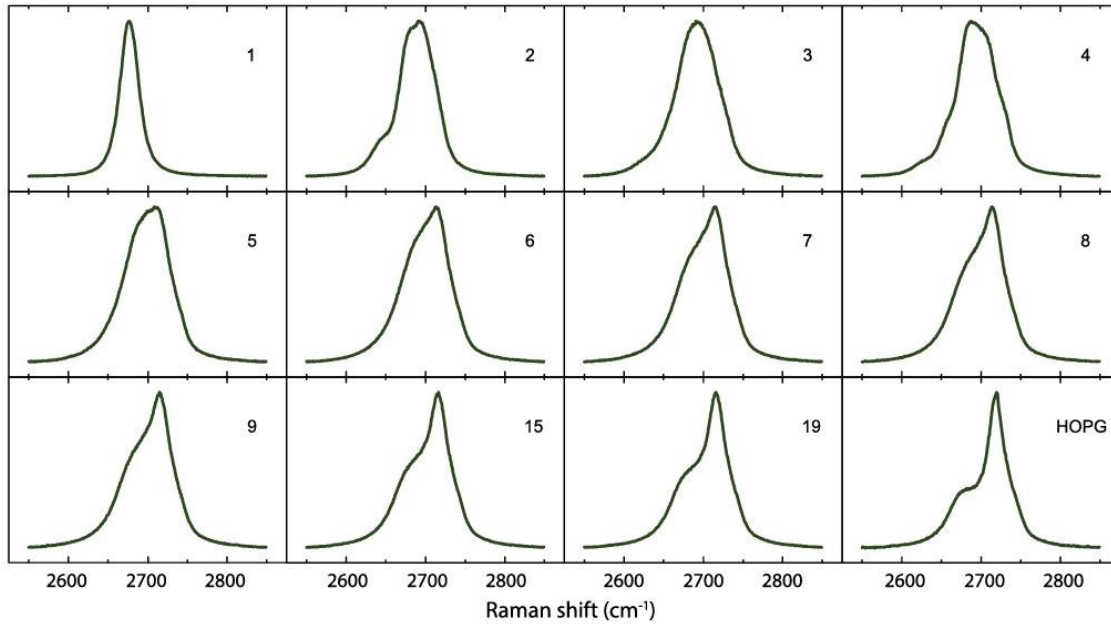


Figure A 24: 2D band of different graphene sample, in each panel is indicated the number of layer of the respective sample [133].

As well known in the literature the shape of the 2D peak is strictly dependent on the number of graphene layers. It is possible to see that the one layer graphene has just only one component, than the complexity of the peak shape increase for 2, 3 and for graphene layers. When the number of layers is equal or higher than 5 it is possible to fit with two components, has it usually done for the pristine graphite. When the number of layer reach 19 layers, the 2D peak is almost undistinguishable respect the graphite ones

As final point of this appendix the distribution of the geometrical properties of the blisters is analysed.

The selection of the analysed blisters was performed by considering structures with a diameter larger than 1 μm (corresponding to a radius $R > 0,5 \mu\text{m}$). This criteria was selected due to the Raman lateral resolution constrain, in order to have a representative Raman spectrum of a single blister. No constraints were imposed on the blister height.

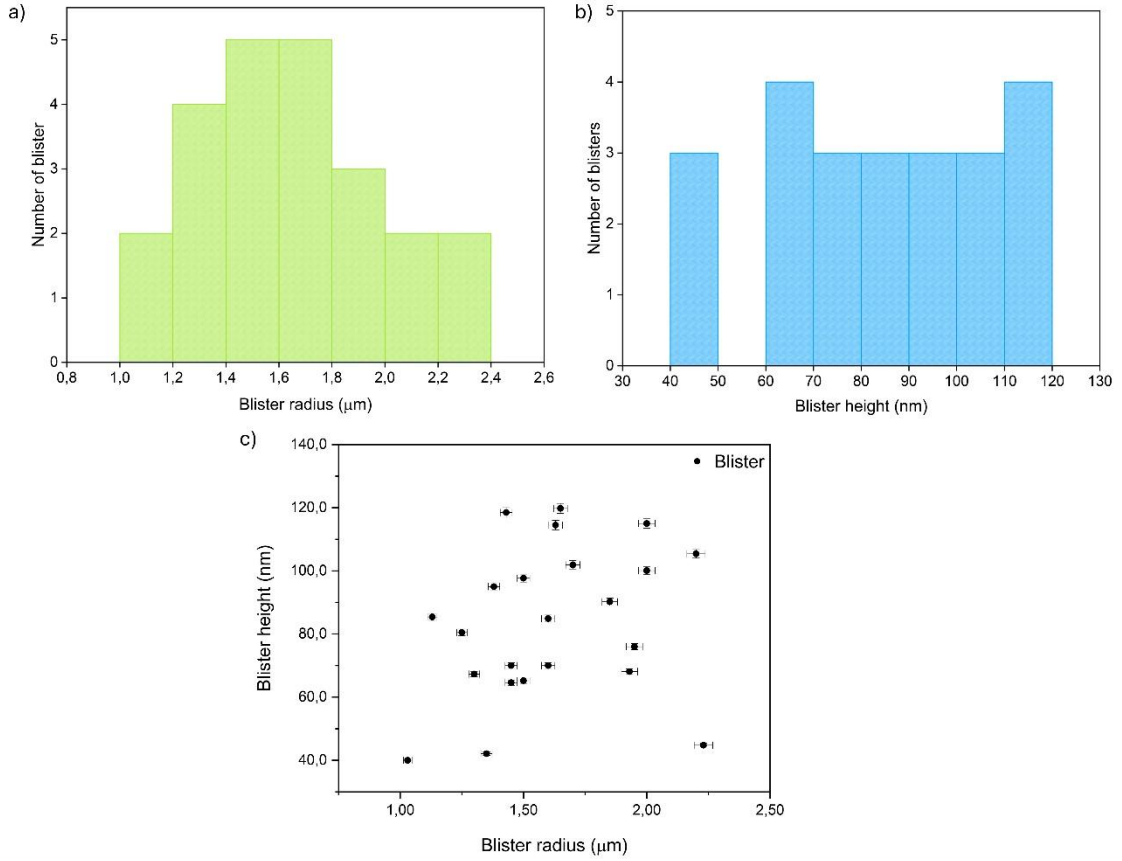


Figure A 25: a) Blister radius distribution. b) Blister height distribution. c) Correlation between the blister radius and blister height.

Figure A 25: shows the distribution of the blister radius and height, together with the scatter plot of h as a function of R . The radius distribution is centred in the range 1–2.5 μm , while the height spans a broad interval between approximately 40 and 120 nm.

Importantly, no clear correlation between blister radius and height can be identified. Blisters with comparable radii may exhibit significantly different heights, and vice versa. This observation indicates that the blister geometry is not governed by a simple proportional scaling between lateral extension and vertical swelling.

Such behaviour is consistent with the energetic framework introduced in section 3.4, where the equilibrium configuration of a blister results from the balance between elastic energy stored in the multilayer membrane, internal gas pressure, and effective interlayer adhesion. Since both the internal pressure and the elastic response depend on the effective thickness of the blister skin which is not known with precision the absence of a direct R – h correlation is physically expected.

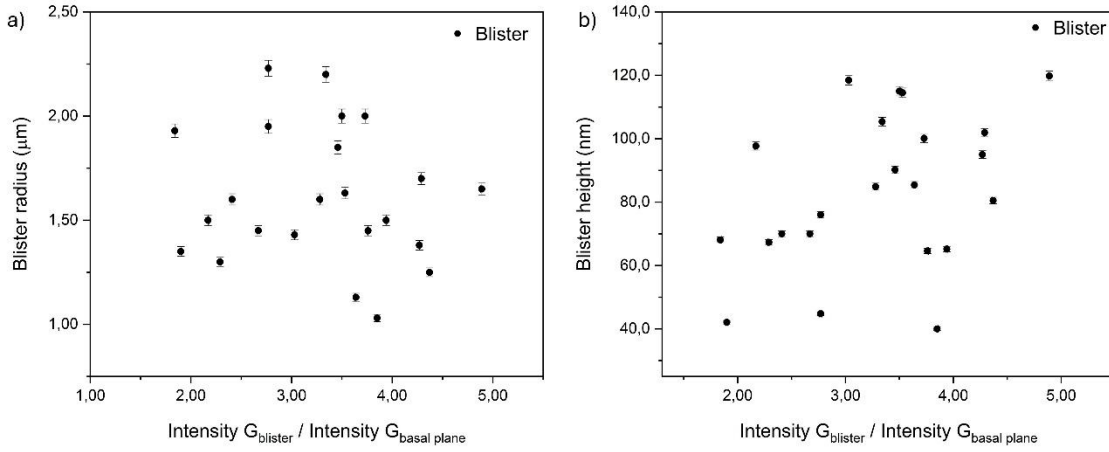


Figure A26: a) Blister radius as a function of the G-band intensity ratio $I_G^{\text{blister}}/I_G^{\text{basal plane}}$. b) Blister height as a function of the same intensity ratio.

To further evaluate whether the Raman intensity enhancement is related to simple geometrical parameters, the blister radius and height were plotted as a function of the G-band intensity ratio $I_G^{\text{blister}}/I_G^{\text{basal plane}}$ (Figure A26). In both cases, no systematic dependence can be identified. Blisters exhibiting similar intensity ratios may present markedly different radii, and likewise, structures with comparable G-band enhancement display a broad dispersion in height values.

This observation confirms that neither the lateral extension nor the vertical swelling alone governs the Raman intensity increase. The absence of a clear dependence on R or h is consistent with the results discussed in the main text, where strain — derived from the combined geometrical parameters — emerges as the only morphological quantity showing a robust relationship with Raman peak shifts.