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GRADO EN INGENIERÍA EN TECNOLOGÍA INDUSTRIAL

TRABAJO FIN DE GRADO

ATOMIC FORCE MICROSCOPY: SETTING UP THE PEAKFORCE TAPPING AND CARRYING OUT AN INITIAL RUN-THROUGH

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1. TRILINGUAL ABSTRACT

ABSTRACT

The use of Atomic Force Microscopy (AFM) has been a major step forward in the field of material characterization. Optical microscopy could only provide us two-dimensional images, whereas AFM brought us the next step: obtaining three-dimensional images of material topography, at a nanometric scale.

Besides topography, it is also important to know about the characterization of a material. Among the modules that can be used in AFM, the PeakForce Tapping module is the one that brings us closer to this goal. This module has been recently set in the Group in Science and Engineering of Polymeric Biomaterials (ZIBIO) research group, the one the student is working with.

Therefore, the objective of this project is setting up the PeakForce Tapping mode, aiming to carry out an initial run-through.

RESUMEN

El uso del Microscopio de Fuerzas Atómicas (Atomic Force Microscopy, AFM) ha supuesto un gran avance en el campo de la caracterización de materiales. Anteriormente, los métodos ópticos de microscopía solo permitían obtener imágenes en dos dimensiones. El AFM ha permitido dar un paso más: con él podemos conseguir imágenes tridimensionales de la topografía de un material a escala nanométrica.

En un material, además de la topografía, conviene conocer también sus características mecánicas. Entre los distintos modos de los que dispone el AFM, el módulo PeakForce Tapping es aquel que mejor permite alcanzar este objetivo. Este módulo se acaba de incorporar al Grupo de

Investigación en Ciencia e Ingeniería de Biomateriales Poliméricos (ZIBIO) con el que colabora el estudiante.

Este proyecto pretende, por tanto, poner a punto el modo PeakForce Tapping, para utilizarlo posteriormente en el análisis de polímeros.

LABURPENA

Indar Atomikoen Mikroskopia (AFM) aurrera pauso handia izan da materialen karakterizazio arloan. Metodo optikoek bi dimentsioko irudiak besterik ez dituzte lortzen; aldiz, AFM-ak hiru dimentsioko irudiak lortzeko gaitasuna du, materialaren topografia eskala nanometrokoan ikustea ahalbidetuz.

Topografiaz gain, materialaren ezaugarri mekanikoak jakitea garrantzitsua da. AFMak dituen hainbat moduren artean, PeakForce Tapping modua da helburu hori lortzera gehien hurbiltzen gaituena. Modu hau Biomaterial Polimerikoen Zientzia eta Ingeniaritza Ikerketa taldera (ZIBIO) iritsi berri da, non ikaslea elkar lanean dabilen.

Proiektu honek PeakForce Tapping modua martxan jartzea du helburu, gero polimeroen zientzian aplikatzeko asmoarekin.

KEYWORDS

Atomic Force Microscopy

Peak Force Tapping

Polymer characterization

Setup

Optimization

2. LIST OF TABLES, FIGURES, EQUATIONS

FIGURES

TABLES

EQUATIONS

3. INTRODUCTION

The following is a Final Degree Project on the Degree of Industrial Technology Engineering, carried out in the Engineering School of Bilbao, with the collaboration of the Department of Mining, Metallurgy and Material Science. On the following pages, the **setup of the PeakForce Tapping module of the AFM** will be explained. Then, **the characterization of some polymeric samples** will be carried out.

With that aim, the state of the art of the Atomic Force Microscopy (AFM) will be firstly evaluated, as well as its operation and the different modules it owns. Then, the setup of a new mode will be detailed: the PeakForce Tapping mode. The advantages of this new mode will be also explained. Finally, the results obtained by examining some polymeric samples with the PeakForce Tapping mode will be discussed.

4. CONTEXT

It is important to know the properties and the structure of any material. Indeed, the knowledge of different materials offers a better framework to engineering, in order to make up for various applications and uses for them.

Throughout history, material characterization has been improving. In olden days, it was enough to determine some basic properties of the material, obtained by means of tests such as the tensile test, the compression test or the impact test. This was considered enough due to the thought that the sample was homogeneous. Later on, as the consideration of "perfect sample" was rejected, new methods were applied. Among them, the best known were those regarding optic microscopy and electronic microscopy, which allowed us to watch the surface structure of the material. **[1]**

However, two-dimensional analysis poses some limitations that have to be solved in order to improve the characterization of the material. It is in this field where the AFM becomes relevant. The AFM allows us to visualize the three-dimensional topography of a material at nanometric scale, while its mechanical properties can also be calculated. **[2]**

The AFM has two basic operating modes. The 'Contact' mode and the 'Tapping' mode. **[3]** However, nowadays another mode is available, the PeakForce Tapping mode, which allows us, besides analyzing the topography of the material, to obtain new information about its mechanical characteristics in each part of the material.

So far, the University has worked with the Contact and Tapping mode. The undeniable advantages of the PeakForce Tapping mode are the key to improve in the field of material characterization. Thus, the setup of this new mode has to be carried out.

The application field of this mode is especially interesting with biodegradable polymeric materials. The increase of this type of materials and their application in medical uses make its precise characterization a project to bear in mind. **[4]** Most of these materials are made of such tiny crystals that the optic microscope does not let us watch them. The AFM allows us to solve this problem and get to know the micro-structure of the material. The PeakForce Tapping mode gives us valuable information about the mechanical properties of the material.

Besides, the AFM has a temperature module that allows us to set the material in the desired conditions; in the specific case of biodegradable polymers, this is a great benefit, given that they can be tested at service conditions. **[4]** For example, they can be tested in the laboratory at the human body temperature, if their operation environment is going to be that one.

5. SCOPE AND OBJECTIVES

The **main goal** of this project is to **setup the PeakForce Tapping** mode of the AFM so that it can be used afterwards to **analyze different materials**.

In addition to the setup, this project pretends to an operation manual that can be helpful for future users.

Once the setup done, the next aim will be to analyze its benefits and drawbacks regarding the other modes of the AFM in the characterization of biodegradable polymers.

The reason of getting to know deeply these polymers is that their scope belongs to a field of high interest: the biomedical engineering. If the mechanical properties of the material are known at a nanometric scale, it may be used as an alternative to prosthesis, or as a holder to medicines that need a progressive secretion to blood.

6. BENEFITS

6.1 BENEFITS OF THE PEAKFORCE TAPPING MODE

Technologic benefits

- Operating at frequencies well below the cantilever resonance, thus avoiding the filtering effect and dynamics of a resonating system.

- Combining the benefits of 'Contact' and 'Tapping' modes imaging.

- Using direct force control but avoiding of damaging lateral forces, in contrast to 'Contact' mode.

- Avoiding unwanted resonances at the turnaround points, in contrast to 'Tapping' mode.

- Possibility of execution of continuous force curves at frequencies between 1 kHz and 10 kHz.

- Possibility of using ScanAsyst, while in 'Tapping' mode an experienced user is needed, given that the cantilever works at resonant frequency, where the dynamics are relatively complicated.

Scientific benefits

- Increasing the knowledge of material characterization in the AFM

- Making the specific analysis needed by each material, using a suitable method.

Economic benefits

- Being able to take full advantage of the possibilities the AFM offers, instead of reducing ourselves to 'Contact' and 'Tapping' modes.

- Amortizing the buy of the PeakForce Tapping module.

6.2 BENEFITS OF THE CHARACTERIZATION OF BIODEGRADABLE POLYMERS

Technologic benefits

- Getting to know the microstructure of these materials, their crystallization at a nanometric scale.

Social benefits

- Improving the living standards of people, using better materials, less harmful for the human body and with better properties.

Scientific benefits

- Increasing the knowledge of these polymers, key in the field of the engineering for medical uses.

- Making the scientific branch of medicine and engineering compatible, proving material science as link between them.

Economic benefits

- Maximizing the application of materials, given certain characteristics. The better the properties of polymers are known, a specific material will be chosen with a better criteria, instead of choosing a generic one.

- Amortizing the investment in the AFM, applying it to the analysis of commercial products that can be sold to gain benefits.

7. STATE OF THE ART

7.1 Atomic Force Microscopy: How does it work?

The Atomic Force Microscopy (AFM) is the surface analysis system with the higher resolution. It operates at Armstrong level (10^{-10} m). **[1]**

Figure 1 **[5]** shows the way an AFM works: a cantilever with a tip on its end scans the surface of a sample. While the topography of the sample varies, the cantilever bends. The goal is to measure the interactions between probe tip and sample.

To do so, a laser beam impacts in the cantilever and reflects to a photo-detector, as can be observed in Figure 1a. The photo-detector is like a bull's-eye **[6]**; if the cantilever is not bending, the laser beam reaches the center of the bull's-eye. While the topography changes through the sample, the cantilever bends, so the reflection is no longer reaching the center of the photo-detector: in 'mountains' of the sample, the bending angle decreases, while in 'valleys' it increases.

Therefore, during the scanning, the laser reaches different points of the photo-detector. The point where the laser makes its impact is then converted into an electric signal. This process is shown in Figure 1b. **[5]** The signal is received and analyzed by a controller (feedback loop). The controller has two tasks: firstly, it has to convert the received data into an image and represent it on the computer screen. Secondly, it has to send an electric signal to the piezoelectric scanner, in top of which the sample is set, so that it moves up or down. **[6]** This allows us to keep a constant value: the set-point. The set-point will be a different value, depending which mode the AFM is operating with.

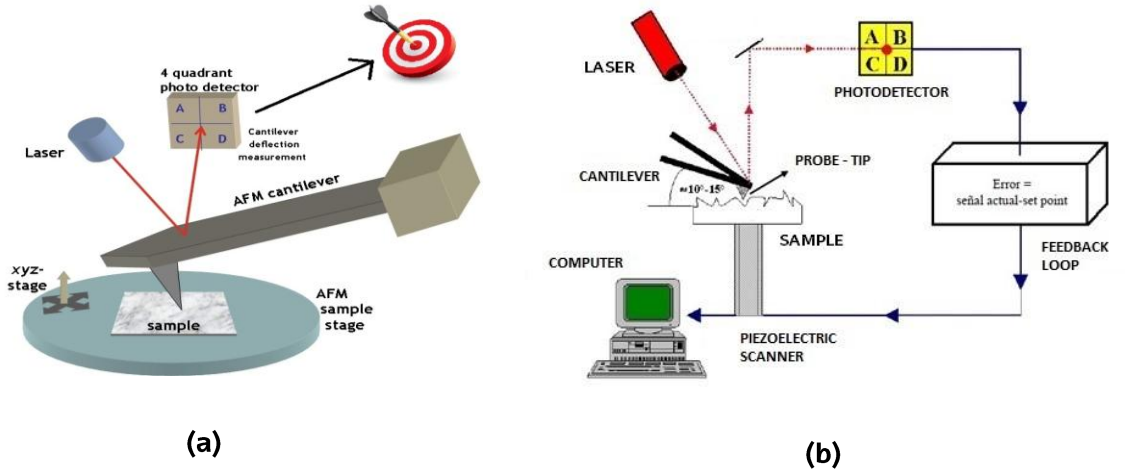


Figure 1: schemes of the AFM [5]

In contrast with the optical and electronic microscopes, the AFM is a instrument whose operation is based in mechanical feeling of the surface. Its basic characteristic is that the analysis is based in the interactive forces between sample and probe tip. Given that these forces are not dependent on the nature of the sample, the AFM can be used for the analysis of almost all materials, without needing a specific preparation of the sample.

7.2 Elements of the Atomic Force Microscopy

In this section the elements of the AFM will be described. This elements are clearly shown and labeled in Figure 1.

- **The cantilever and the probe**

The cantilever is a metallic plate, a few millimeters long and wide. In its end, there is a tip. There are several types of tips and its election is essential. it depends on the working material, as well as on the mode of operation.

The cantilever and probe tip are a key element, given that they are the ones directly interacting with the sample. The properties (flexibility, hardness...) of these elements are known. Depending on the values of these properties, the bending and torque of the cantilever will vary, and consequently, the point where the reflected laser impacts the photo-detector will do too.

- **The laser**

The most important consideration about this element is its calibration. The goal is that the laser beam impacts on the end edge of the cantilever, like Figure 2 shows. Therefore, the bending of the probe tip and the cantilever will directly influence the variations in the photo-detector, and the imaging will be accurate to the sample.

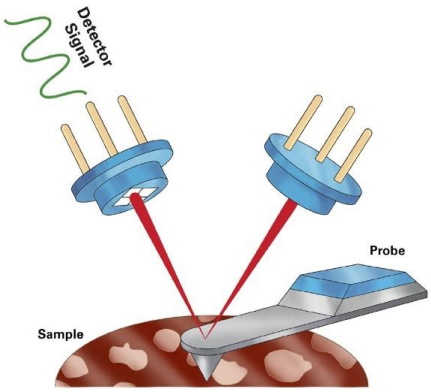


Figure 2: detailed scheme of AFM operation [3]

- **The photo-detector**

It is the element where the laser beam arrives after reflecting in the cantilever. It is calibrated so that, in the beginning, without bending in the cantilever, the laser impacts on the center of the photo-detector. The most common photo-detector has four quadrants. It measures the position where the laser beam arrives through the scanner period, and converts this position into an electric signal. The electric signal is finally sent to the controller.

- **The controller**

The controller is the main element of the AFM. It receives an electric signal from the photo-detector, which contains data about the laser beam position after reflecting in the scanning cantilever. Then, the controller amplifies the signal, analyzes it, and represents a line in the computer screen, corresponding to the sample in each specific position. While the tip scans the sample, the laser beam arrives at different points, so the controller receives a different signal and represents a different line

consequently. Therefore, the topography is represented in the screen, as well as a phase image and other characteristics of the sample, either quantitatively or qualitatively. The Figure 3 shows the controller (left), the AFM (center) and the computer (right).

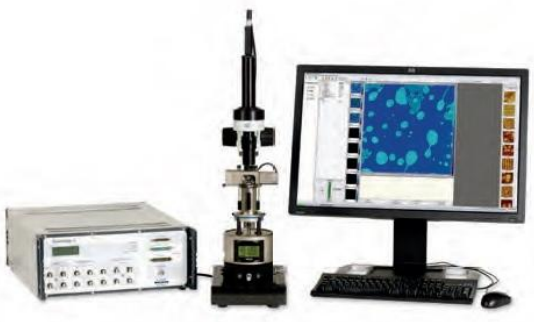
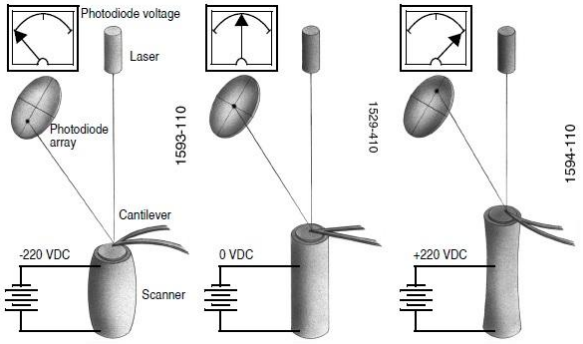


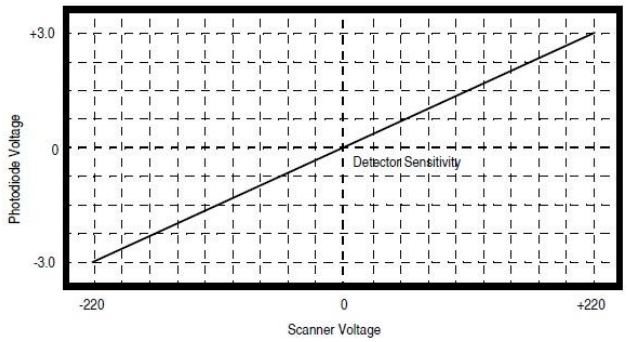
Figure 3: controller, microscope and computer of the AFM [6]

- **The piezoelectric scanner**

A piezoelectric material changes its size when it receives a potential difference, like Figure 4 shows. In the AFM, the sample is placed on top of a piezoelectric scanner. This element works to maintain a constant set-point. The setpoint will be different in each mode. For example, in 'Contact' mode, the constant value has to be the cantilever deflection. Therefore, the piezoelectric scanner receives an electric signal from the controller and increases or decreases its size in the vertical axis (moves up or down) so that the sample goes further or closer from the tip, in order to maintain the setpoint parameter constant.



(a)



(b)

Figure 4: piezoelectric scanner in AFM (a) z variation with voltage (b) sensitivity [6]

7.3 Operation Modes of Atomic Force Microscopy

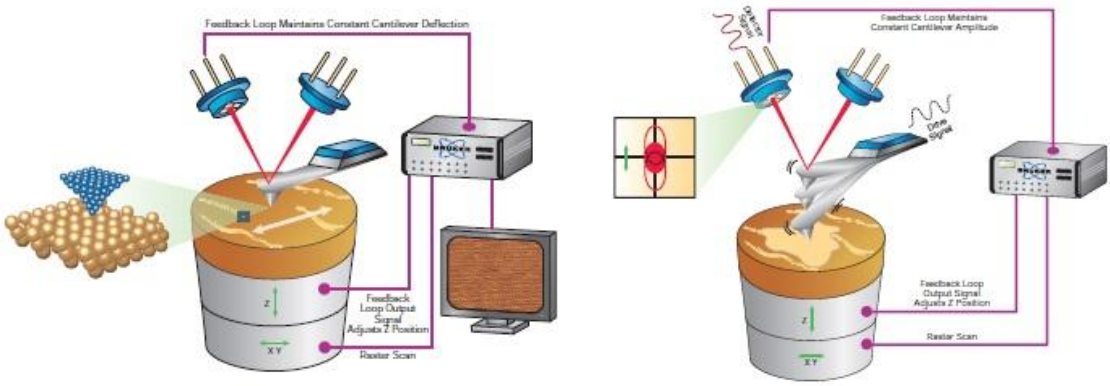
There are various modes in the AFM. Among them, the three the most relevant will be highlighted.

- **'Contact' mode**

It is the basic mode of the AFM, In this mode, the tip and the sample make always contact. The parameter to control, that is, the setpoint, is the cantilever deflection. **[6]** This mode possess many secondary modules to measure the properties of the material, such as FM, SCM, SSRM, TUNA or CAFM. Figure 5a below shows the basic scheme of this mode.

- **Tapping mode**

In this mode, the cantilever is oscillated at resonance frequency. This makes that the tip touches the sample intermittently. In this mode, the setpoint is the oscillation amplitude of the cantilever. Phase images can be obtained from the 'Tapping' mode. **[6] [7]** Figure 5b below shows the basic scheme of this mode.



(a) (b)

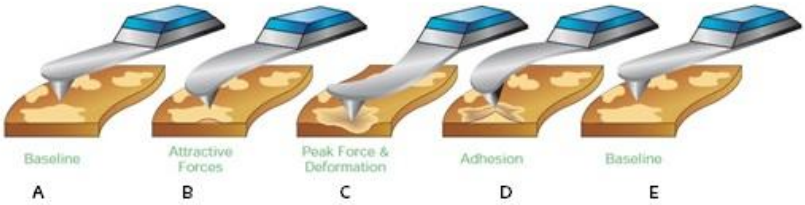
Figure 5: AFM operation modes (a) Contact Mode (b) Tapping Mode [8]

- **'PeakForce Tapping' mode**

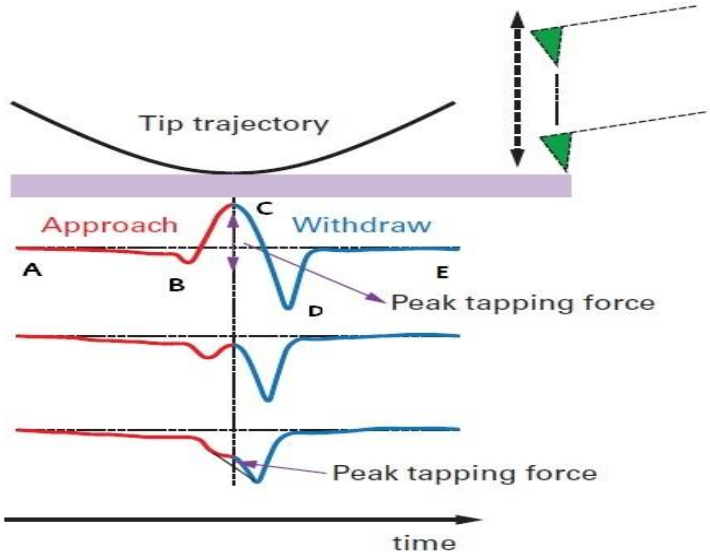
This project is about setting up this new mode of the AFM. In this mode, the cantilever oscillates too, like in 'Tapping' mode, but at a lower frequency, below resonance.

This difference offers us a major advantage. Whereas in 'Contact' and 'Tapping' mode force curves could be calculated, but by means of a tedious process, in the 'PeakForce Tapping' mode, while the tip is scanning the sample, it automatically generates a force curve for each scanning line.

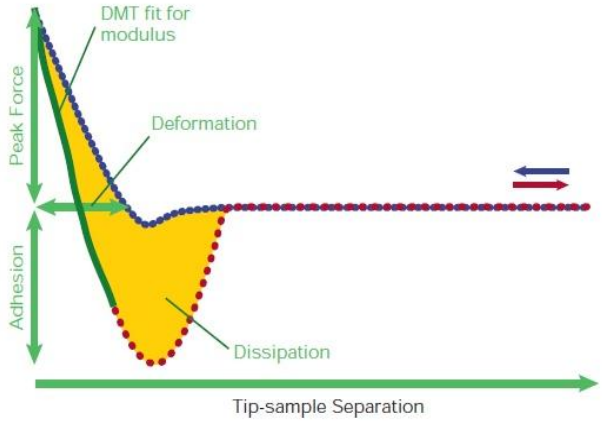
Figure 6b below shows the force curve generated at every step. Every step has five stages (Figure 6a); firstly, the tip approaches the sample. The second stage starts when attractive forces influence the tip and pull it even closer to the sample. Thirdly, the peak force is achieved and deformation occurs. Then, adhesive forces make it difficult to the tip to withdraw from the sample. The bigger these adhesive forces are, the more energy will be dissipated. This energy is the area colored in yellow in Figure 6b. Finally, the tip is pulled out of the sample and first stage starts again. The Force vs. Time display is referred to as the "heartbeat" (Figure 6c). **[3]**



(a)



(b)



(c)

Figure 6: PeakForce Tapping (a) stages (b) Force vs. time curve (c) Force vs. tip-sample separation curve [9]

The reason for this is that all interaction between tip and sample can be measured, and draw a graphic representing the force the cantilever faces in relation to the distance between tip and sample. In this mode, the setpoint is the peak of the force between tip an sample. **[3] [10]** That is why this mode is named 'PeakForce'. The operation can be carried through a range of forces far below the ones in 'Tapping' mode, and consequently, the operation regime is more stable, either in air or in fluid; furthermore, nanomecanic and nanoelectric properties can be measured in each interaction. Finally, the 'PeakForce Tapping' module includes the 'Scan-Asyst' mode, which allows an automatic management of the parameters to control while scanning, in contrast to 'Tapping' and 'Contact' modes, where an experienced user had to this.

7.4 Material used in the project for characterization with the AFM

The material that will be used for characterization with the AFM is polyhydroxyalkanoate (PHA).

The PHAs are linear polyesters produced in nature by bacteria by fermentation of sugar or lipids. They are produced by bacteria to store carbon and energy. More than 150 different monomers can be combined within this family to give materials with extremely different properties. These plastics are biodegradable and are used in the production of bioplastics. They can be thermoplastic or elastomeric materials, with melting points between 40 and 180 ° C. The mechanics and biocompatibility of PHA can also be changed by mixing, modifying the surface or combining PHA with other polymers, enzymes and inorganic materials, making possible a wider range of applications. **[11]**

At a medical level, PHAs offer interesting possibilities. The medical applications of the PHA have two main aspects. The first of these is as per se materials, since being biodegradable and biocompatible plastics, they are being used for medical implants, sutures, heart valves, etc. Its use as matrices for controlled-release of drugs is also being investigated. **[12]**

In the laboratory, the PHA is in the form of pellets. It is mixed with chloroform to obtain the desired films to analyze in the AFM.

7.5 Advantages of PeakForce Tapping over traditional Tapping method

The phase of the Tapping Mode cantilever vibration relative to the drive is a useful indication of different mechanical properties. **[9]**

Unfortunately, the phase signal reflects a mixture of material properties, depending both on dissipative and conservative forces. Since elasticity, hardness, adhesion and energy dissipation all contribute to phase shift, phase alone does not provide enough information to quantify or discriminate between all of these properties. Additionally, the phase signal depends on imaging parameters such as drive amplitude, drive frequency, and setpoint.

This makes it difficult and sometimes impossible to interpret the source of the contrast, leaving the user to conclude that there are differences in the sample without further knowledge of contributing physical factors.

Figure 7 demonstrates this difficulty with phase imaging and compares it to PeakForce QNM for a multilayer polymer sample. It is often assumed that the phase contrast is primarily caused by variations in sample modulus. Comparing (f) and (b), it is clear that this is not true in this case. By tapping harder (reducing the amplitude setpoint), one would expect to deform the sample more, increasing the contribution of the modulus to the phase. Surprisingly, in (c) the contrast does not change significantly.

The PeakForce QNM data (e) shows that the phase signal is dominated by the adhesion independent of tapping setpoint for this tip-sample interaction. It is easy to see that one must be very careful in interpreting phase results, even for qualitative use. The PeakForce QNM modulus channel, on the other hand, has unambiguous contrast that can be quantified, as shown in the trace of (f). The narrow strips have a modulus of about 300 MPa, while the wide ones have a modulus of about 100 MPa.

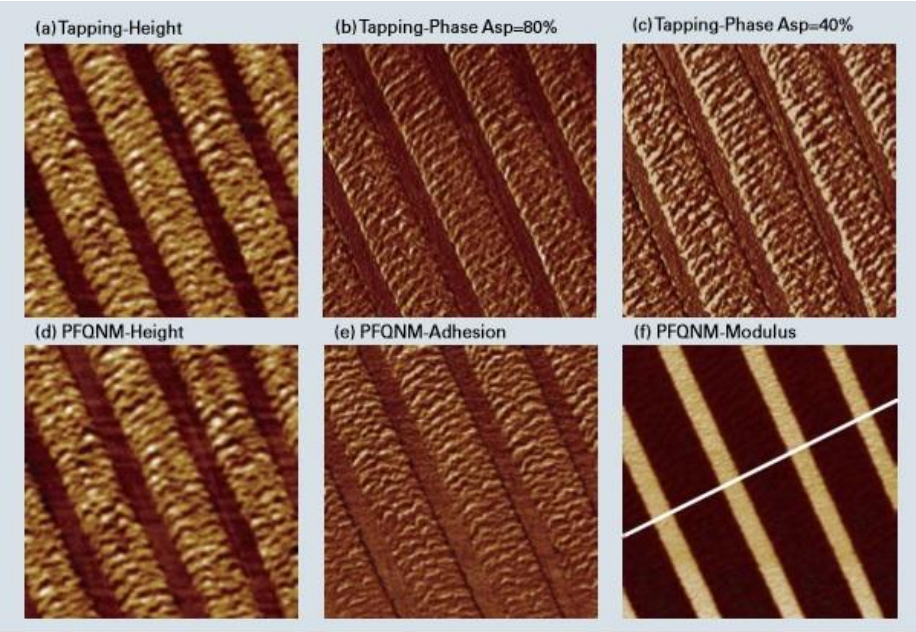


Figure 7: multilayer polymer sample images in AFM [9]

The unambiguous and quantitative modulus and adhesion data provided by PeakForce QNM can help researchers answer the critical question of what materials they are seeing in their topographic images. Additionally, it is now possible to study the variation and position of mechanical properties across a surface with ease, and at previously unattainable resolution. This imaging mode is nondestructive to both tip and sample since it directly controls the peak normal force and minimizes the lateral force on the probe. Maps of mechanical properties such as Young's modulus, adhesion and dissipation are automatically calculated at the rates and resolutions expected by advanced SPM users. Since force distance data is analyzed directly, there is no ambiguity regarding the source of image contrast, as often occurs in other techniques. Mechanical property maps are quantitative, low noise, and can span a very wide range of property values.

These capabilities of PeakForce QNM will provide researchers with critical material property information to enable better understanding of their samples at the nanoscale.

7.6 Examples of usage of PeakForce Tapping in AFM in polymer analysis

Example 1: two polymers crystallization at different temperatures

A temperature-controlled experiment done with PeakForce QNM is shown in Figure 18. In this example, a mechanical blend of syndiotactic polypropylene (sPP) and polyethylene oxide (PEO) was exposed to a sequential heating-cooling procedure with different rates of temperature change. The sample temperature was increased enough that the PEOmatrix melted, but the sPP domains stayed in the solid state to serve as reference marks in the area of interest. **[9]**

First, these steps were followed:

(a) Initial morphology

(b) Rapid heating until PEO completely melts

(c) Rapid cooling, causing quick crystallization of the PEO

One can see that, after these three stages, the PEO topography underwent minor changes (comparing (c) to (a)). This demonstrates the so-called "memory" effect of fast cooling, where the original crystallization nuclei are still active even though polymer appears to be totally melted.

Then, these steps were followed:

(d) Second heating until PEO melts

(e) Gradual temperature drop, slow crystallization

(f) Crystallization is complete

It's worth noting that, in this case, the PEO morphology becomes completely different from the original morphology in (a), indicating reorientation of the lamellae from an edge-on (a) state to a flat-on state (f).

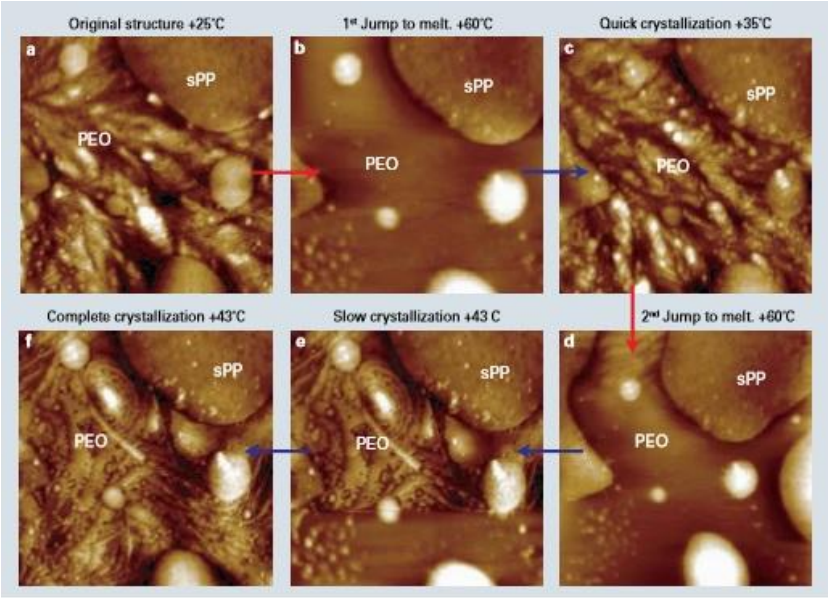


Figure 8: AFM images for PEO and sPP phases at different temperatures [9]

If this experiment were done in TappingMode, it would be necessary to adjust drive amplitude and frequency several times during each heating-cooling cycle, due to the fact that cantilever resonance frequency varies with the temperature. Since Peak Force Tapping operates far from the cantilever resonance frequency, heating induced resonance frequency drift has no effect on the feedback, so it was not necessary to adjust the drive amplitude or frequency during the entire experiment. In fact, as long as the laser reflection stays on the photodetector, the imaging can proceed continuously and without adjustment during any experiment involving sample heating and cooling.

Example 2: silver nanoparticles in polymer

Adhesion data is relevant in some examples. PeakForce QNM becomes advantageous when adhesion is important since it can measure the attractive forces with negligible lateral force and very low normal force, allowing it to be used with very delicate or weakly bound samples. **[9]**

An example is an antibacterial film consisting of poly(methyl methacrylate) (PMMA) and silver nanoparticles, shown in Figure 9. From topography alone (a), it is difficult to tell the locations of the silver nanoparticles. The

adhesion map (b), however, reveals distinct nanoparticles (indicated by smaller circles) as well as an area enriched in many particles (large circle).

Contact mode imaging would likely push the particles out of the way, making it impossible to see them. Tapping Mode Phase Imaging might be able to see the difference in the particles if the probe and imaging parameters were chosen correctly, but PeakForce QNM can see differences in adhesion, modulus and dissipation independently, making it more likely that there will be a difference in one of the data channels.

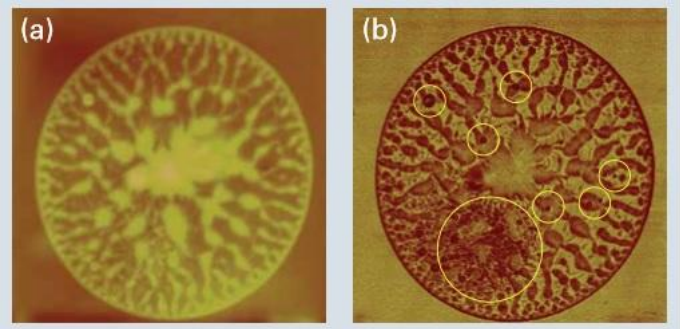


Figure 9: AFM images for PMMA (a) topography (b) adhesion [9]

8. ANALYSIS OF THE RISKS

8.1. IDENTIFICATION AND ASSESMENT OF THE RISKS

A. Breakage of the tips:

The tip is the only element directly contacting with the sample. It is an utterly little element, almost impossible to see, what makes it very fragile. It can break when inserting it into the cantilever or when approaching it to the sample. The prize of a 10 tip pack is about $250; therefore, the replacement of a tip is something to avoid.

Probability: 3/5 Effect: 2/5

B: Rupture of the controller:

The controller is the main element of the AFM; it is an electronic device which has to work perfectly so that it receives the data from the scanning and represents it accurately in the screen. Poor ventilation, alteration in the voltage arriving to the electronic diodes or dust could lead to a poor operation of this element.

Probability: 1/5 Effect:5/5

C: Vibration in the AFM:

The AFM has to be in perfect balance and stable. The scanning of the sample occurs at nanometric scale, so any alteration of the whole operation can mislead the image obtained in the screen. Vibrations in the equipment, room or even a plain travelling up the building can provoke this.

Probability: 2/5 Effect: 2/5

D: A virus in the computer:

A virus or poor operation of the computer can lead of a failure of all the AFM, which is an expensive equipment.

Probability: 1/5 Effect: 4/5

E: A dirty sample:

The AFM scans the sample in a nanometric scale. Therefore, any little dust, dirt or undesired material in the sample will appear on screen, blocking us from an accurate analysis of the material.

Probability: 3/5 Effect: 1/5

		EFFECT				
		1	2	3	4	5
PROBABILITY	1				E	B
	2		C			
	3	E	A			
	4					
	5					

Figure 10: risk analysis graphic

8.2. RISK PREVENTION AND CONTINGENCY PLANS

A. Breakage of the tips:

In the beginning, tips that had been already broken were used for practice. These tips were used to improve our skills and accuracy when introducing them into the cantilever and when approaching them to the sample. Like

this, more experience was achieved and if the tip broke, much money would not be wasted, since they were already broken.

B: Rupture of the controller:

This risk, despite being improbable to occur, actually happened. That is why it should be taken into account how to prevent it and find a proper solution to it. Ventilation is important in the controller, so reorganizing the elements of the AFM so it gets clean air to stay cool is important. Also, checking the connections to watch out the voltage arriving the electronic devices is relevant. Finally, if broken, the technical service of Bruker should be contacted, the company who sold the AFM to the University.

C: Vibration in the AFM:

Next to the University, the bus station of Bilbao was being renovated. That provoked massive works that lasted for months. Vibrations created by the works may affect the equipment. To solve this, a special balancing table was used, below the AFM. This table is a TMC CleanBench Vibration Isolation Table; a line of N_2 feeds the system.

D: A virus in the computer:

In order to prevent this risk, only the program to visualize and analyze the images from AFM was installed in the computer. No Internet connection was set, so a second and independent computer was needed if any research was aimed. Also, a correct ventilation was important.

E: A dirty sample:

A dirty sample could ruin all the operation. In order not to lose time after calibrating and scanning the sample to get no accurate result, careful operation throughout all the process was important. The polymer preparation was a full step by step process that will be explained later, and in every step latex gloves and clamps were used.

9. SUGGESTED SOLUTION

Now, the chosen solution will be shortly described.

1. Checking the connections and calibration of the AFM.

The first step is to prepare the working environment so the setup of the PeakForce Tapping mode is carried out in the best conditions. Therefore, the first thing to do is cleaning and reorganizing the equipment. Then, the scanner should be checked. In order to do that, a silicon pattern will be used.

2. Preparing the samples

A bunch of polymeric samples will be prepared. The goal is to obtain a sample that can be easily and safely scanned in the AFM, giving us the desired image. Several samples will be prepared, each of them with a different composition. Also, the thickness of the samples will be measured.

3. Choosing the probe tip criteria

Choosing the suitable tip is very important. Several catalogues are available to do so. Therefore, an analysis so that the tip is correctly selected will be made.

4. Adjusting the AFM

The first step of the setup of the PeakForce Tapping mode is adjusting the AFM and calibrating tip and sample so that the scanning can begin. This is an essential process every user of the AFM need to handle, since it is necessary for every scanned sample and every operating mode.

5. Characterization of the sample

The polymeric samples will be analyzed with the PeakForce Tappng mode. The setup and the parameters to control will be explained.

10. DESCRIPTION OF TASKS, STAGES, EQUIPMENT AND PROCEDURES

T1. Equipment, inventory and optimization

The first task in this project started in the moment to arrive to the laboratory. The Atomic Force Microscopy (AFM) lab had not been used for a while, and the project runner had no previous experience with the equipment. Therefore, a run-through the equipment was absolutely necessary. The laboratory (Figure 11) consists on a room of around 5 m long, 4 meters wide and 5 meters tall. In the beginning of this project, the laboratory had some spatial and design deficiencies. Therefore, an optimization of the space was carried out.

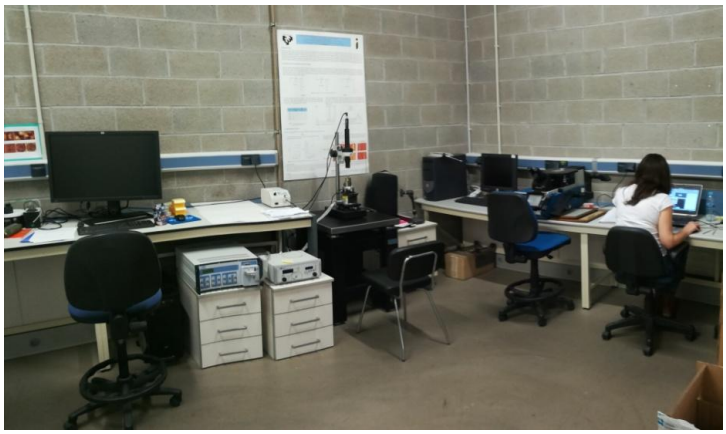


Figure 11: AFM laboratory after optimization

T2. Checking connections and calibrating the unit

When the project was initiated, the AFM had been unused for more than two years. This is problematic, since the equipment is not reliable, provided a long-term inactivity. Therefore, connections, general state and calibration of the equipment needed a check-out.

The most important element of the AFM is the controller. The controller is an electronic device, fed by 230 V from the electric line of the laboratory and connected to the microscope. Inside the controller, several diodes and electronic edges work at different voltages. In order to check that the controller was working correctly, the voltage of some spots was measured with a polymer. The connections checked are shown in Figure 12.

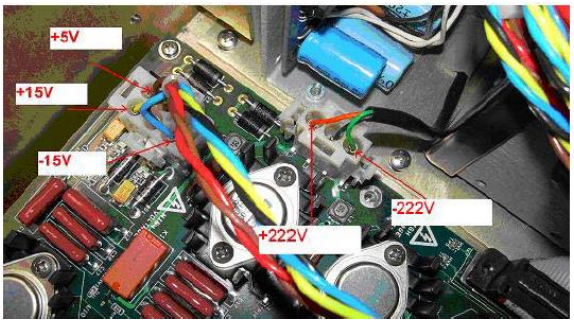


Figure 12: connections in the controller

image obtained by the technical service of Bruker after contacting with them

Before doing the measures, all connections were checked. It is important to keep safety measures in this stage, since voltages of 220V arrive to the controller and they could harm the user.

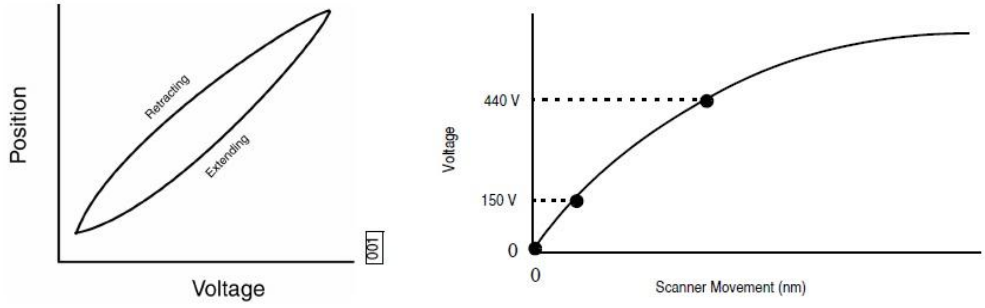
Finally, it was useful to check that the scanner of the microscope was working correctly. The AFM provider, Bruker, provides a calibration reference to do so.

Scanners typically consist of a hollow tube made of piezoelectric material. Piezo materials contract and elongate when voltage is applied, according to whether the voltage is negative or positive. Not all scanners react exactly the same to a voltage. Each scanner has a unique "personality". This personality is conveniently measured in terms of sensitivity, a ratio of piezo movement-to-piezo voltage. This non-linear relationship is determined for each scanner crystal and follows it for the life of the scanner. As the scanner ages, its sensitivity will decrease somewhat, necessitating periodic recalibration, as shown in Table 1.

Table 1: types of calibration and schedule

Calibration Routine	Time Frame	Frequency
X-Y Calibration	First year	Every 3 months
	Subsequent Years	Every 6 months
Z Calibration	First Year	Every 3 months
	Subsequent years	Every 6 months
Critical Height Measurements	All years	Monthly Z calibration

Also, piezoelectric materials exhibit hysteresis, as shown in Figure 13: hysteresis curves in piezoelectric materials, so their response to increasing voltage is not the same as their response to decreasing voltage. That is, they exhibit "memory", which causes the scanner to behave differently as voltages recede toward zero.



(a) (b)

Figure 13: hysteresis curves in piezoelectric materials [13]

Bearing in mind all this, it is necessary to precisely measure these sensitivities, then establish software parameters for controlling the scanner.

This task is accomplished with the use of a calibration reference, shown in Figure 14.

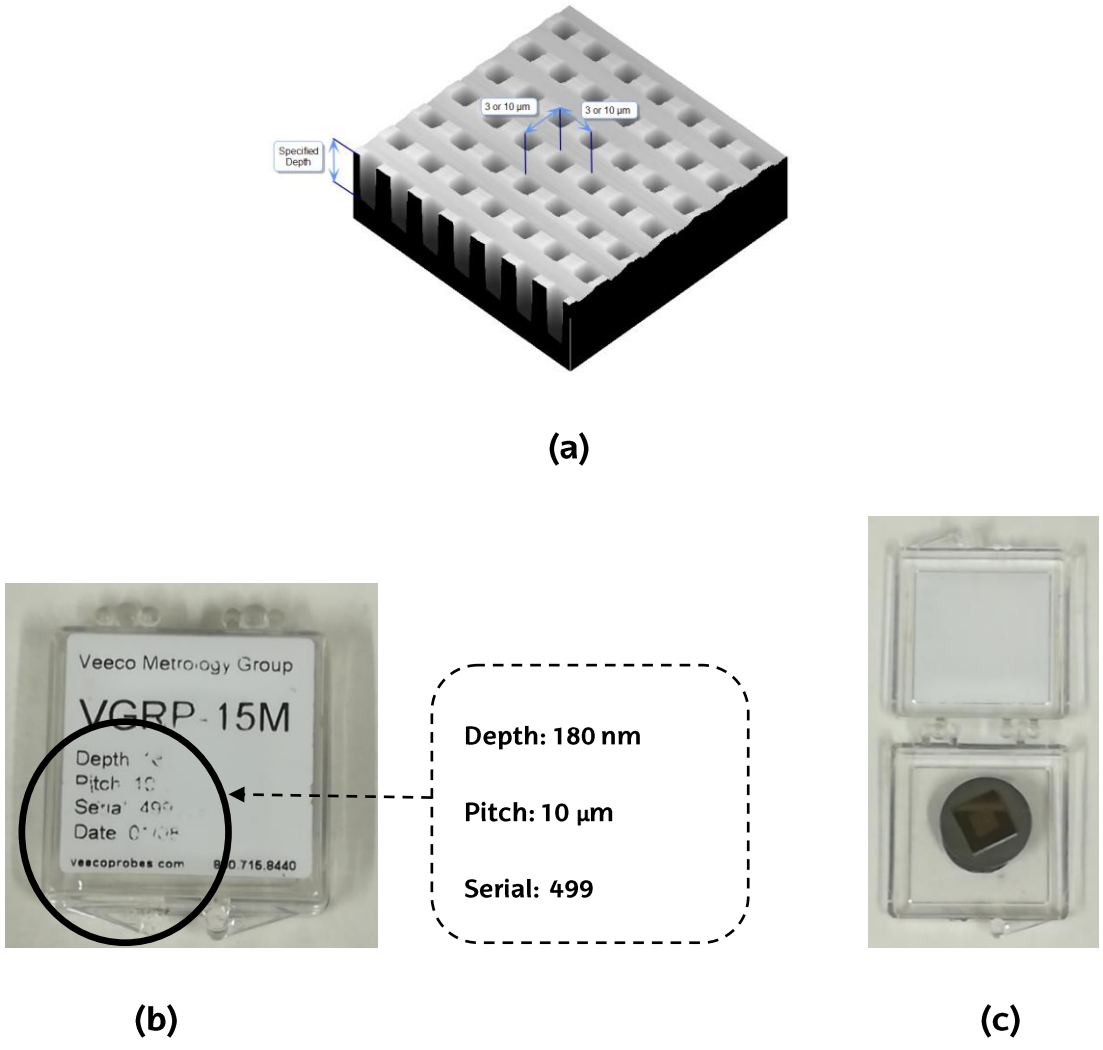


Figure 14: calibration reference (a) virtual diagram (b) characteristics (c) real reference [13]

A calibration reference consists of a silicon substrate having a regular series of pits, at a depth specified on the container (typically 180 or 200 nm), which is plated with platinum. Pits are spaced apart on 10 µm.

The reference has to be scanned with "Contact mode". The image obtained should be like Figure 15 shows:

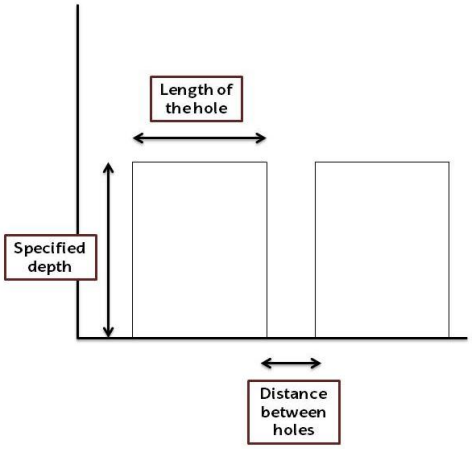


Figure 15: expected image of the calibration reference

T3. Preparing the samples for AFM and sample thickness measurements

T3.1 Sample preparation

The material chosen for nanomechanical analysis was polyhydroxialkanoate, PHA. The goals are to obtain samples of this material, to measure the thickness and to carry out a characterization of them.

The dissolutions that will be prepared are (Table 2):

Table 2: PHA dissolution at different concentrations

Concentration	PHA mass	Chloroform volume
3% wv	0.6 g	20 mL
5% wv	1 g	20 mL
7.5 % wv	1.5 g	20 mL
10 % wv	2 g	20 mL

These dissolutions will be mixed in four vials, and then they will be deposited in some metallic bases, which will be the platform to examine them in the AFM.

The first thing to do is to take some metallic bases and clean them so that PHA can be deposited on them. To do so, the metallic plates are drown in chloroform during a couple of days. Once the polymer they had from previous samples was dissolved, the plates are cleaned with soap and water until the bases are completely clean and polymer free.

Then, the dissolutions are prepared. They are shaken during a day. The next step is then to deposit the polymer dissolutions over the metallic bases. In order to achieve this, the spin coating method will be used.

Samples are required for two purposes:

1. To measure the film thickness.

2. To analyze completely in the AFM.

For each purpose, four samples will be prepared at each of the following velocities:

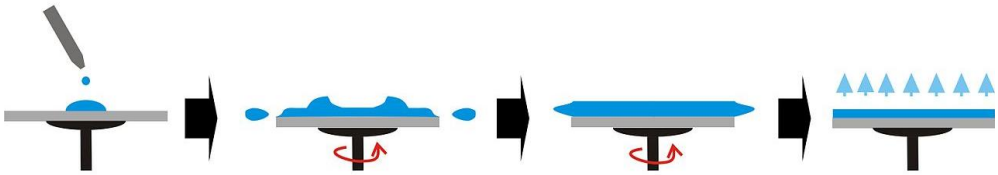
1. At 14 rps **2.** At 25 rps

3. At 40 rps **4.** At 60 rps

Therefore, 32 samples are to be done.

For samples used for purpose 1, it would be ideal if the polymer could deposit only on half of the base, so that the other half would be free and the step between both parts could be measured with the AFM. To do so, half of the metallic plate is covered with Teflon. Then, the spin coating method is applied: the polymer dissolution is coated in the base, the plate is rotated and the surface is dried. The spin coater of the laboratory is shown in Figure 16.

(a)



(b)

Figure 16: (a) spin coater of the laboratory (b) spin coating method [14]

T3.2 Sample thickness measurements

To measure the thickness of the samples, half of the polymer film from the sample should be taken out, so that the AFM can measure the step that is created. To do so, the next steps are:

1. The line where the step will be located will be marked with a sharp pen. The reason for this is to see the pen ink in the AFM and easily locate the spot where the scanning has to be carried out. Figure 17 shows how the samples are marked.

2. With a chloroform wet special cloth for laboratory uses that does not leave any tiny hair, half of the polymer is manually removed from the sample.

3. The AFM is prepared with the sample. The separation line is centred and vertically set,

Figure 17: sample marked with a red pen to locate the step

4. The separation spot between the part with polymer film and the part without it is searched. This part is scanned with the tip. The way of locating this spot is searching for the pen mark and visualizing a grey vertical straight line that can only be a rough difference in the sample and not any dirt or casual morphology that could mislead us because it is also shown in screen as grey irregular lines.

5. The area is scanned. A clean spot is scanned, that is, that no other grey lines like the mentioned before are close to the separation line.

6. The parameters that will be used in this scanning will be:

Mode: Scan Asyst in Air

When looking for the sample spot: Scan Size = 0

When everything is ready to scan, the desired parameters are set.

7. Once the scanning is finished, the image is visualized with the NanoScope Analysis software.

T4. Probe tip election criteria

Bruker provides a tip catalogue. The catalogue briefly explains the elements of the AFM, the different modes and extensively presents the probe tips available for each mode.

The probe model is indicated, with some basic characteristics; the material of the tip and tip side is stated as well. Also, a reference to the catalogue page where a deeper explanation will be carried out is indicated. Finally, the modes where this type of probes can be used is marked.

Most common materials in probe tips are: silicon, silicon nitride, magnetic cobalt-chromium, super sharp silicon -golden coated-, electrical platinum, doped diamond, wear resistant carbon...

www.brukerAFMprobes.com is Bruker online purchase resource for probe tips. On it, several specific properties can be compared, as well as detailed cantilever and tip characteristics and specifications are shown. There is also a filter search in case any specific characteristic in our probe tip is needed. The filter also allows us to search by mode, by sample, by AFM or by application.

In case a clear criteria is not up to choose between the probe tips, Bruker offers us some graphics, like the one shown in Figure 18 **[9]** as a method to decide whether the tip suits our experiment or not.

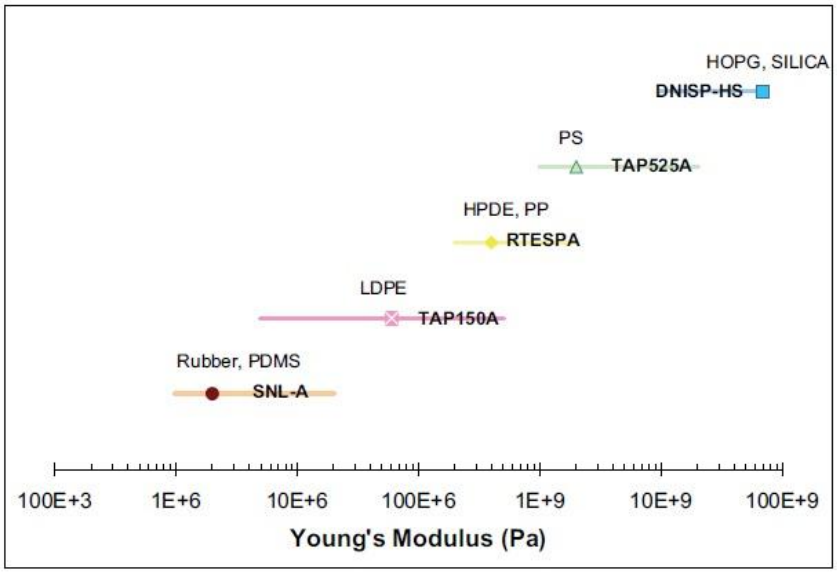


Figure 18: probe tip selection criteria graphic [9]

Figure 18 shows the Modulus range of each probe and which one should be chosen when working in PeakForce QNM mode. The aim is to obtain a tip that penetrates enough in the sample as well as keeps enough sensitivity throughout the whole scanning process.

T5. Adjustment of the AFM

One of the main skills the user should acquire is adjusting the AFM. The adjustment refers to all the calibration and parameter settings that should be made before starting to scan the sample.

The steps to adjust the AFM are: **[15]**

1. Inserting the tip in the cantilever
2. Loading the experiment
3. Loading the sample
4. Aligning the laser
5. Adjusting the photodetector
6. Engaging

The first step is to insert the tip in the cantilever. This should be carried out carefully and with gloves and clamps, due to the tiny surface of operation (the tips are only a few nanometres long) that could provoke a breakage of the probe tip. In order to avoid this, already broken tips available in the laboratory will be used to practice the operation, so that the delicate process gets mastered.

The cantilever with the tip in the end is in the boxes of purchase of Bruker. The whole cantilever plus tip element should be introduced in the holder. The operation is simple: the cantilever is clipped with the clamps. The probe holder is on the table, upside down with the groove facing up. Gentle upward pressure is applied against the plunger to lift the spring clip up.

Then, the cantilever is slid in the holder taking advantage of the elevated free space. Finally, the pressure is relieved from the holder and the clip traps the cantilever. Figure 19 shows how this operation is carried out.

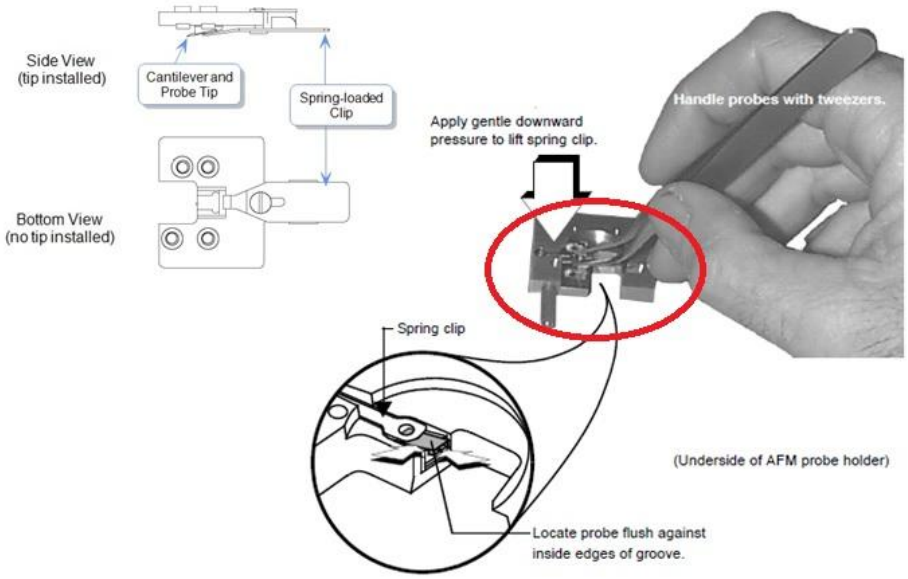


Figure 19: cantilever insertion procedure [15]

After the probe holder is loaded with a probe, the probe holder will be placed inside the SPM head and clamped into position using the clamping screw at rear of head, as shown in Figure 20.

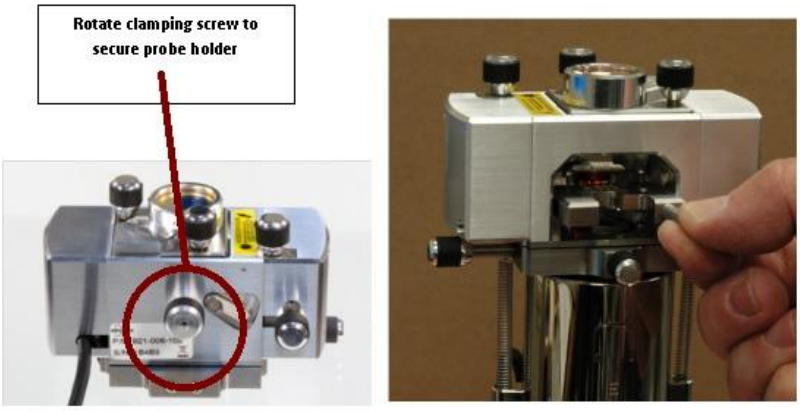


Figure 20: holder insertion in the SPM head procedure [15]

The second step is loading the experiment. It is as simple as double clicking in the computer software of NanoScope AFM. Then, a window like the shown in Figure 21, will appear on screen.

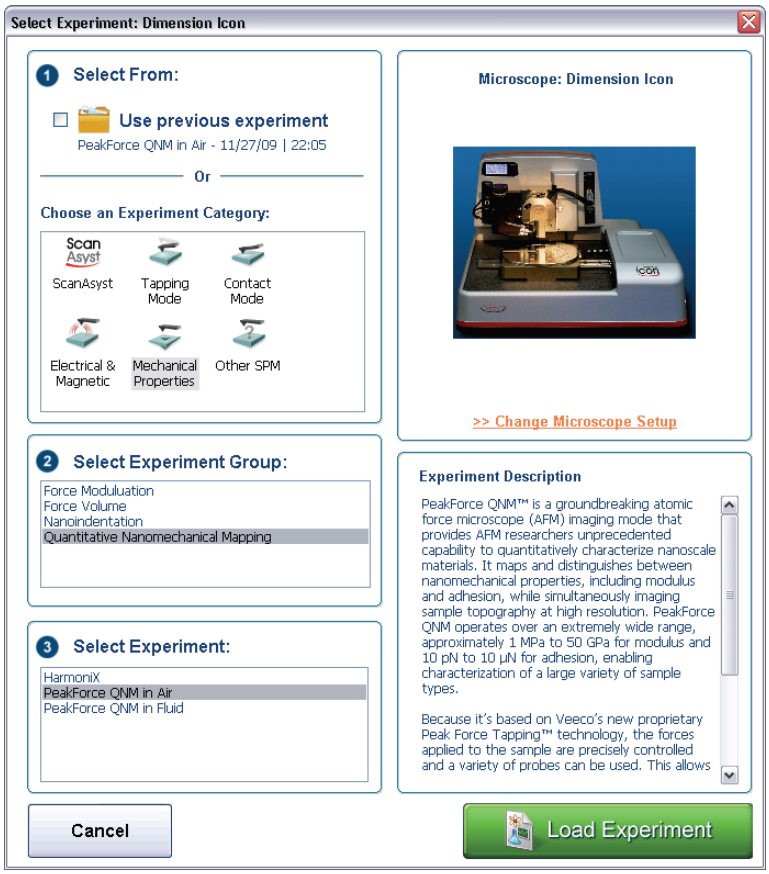


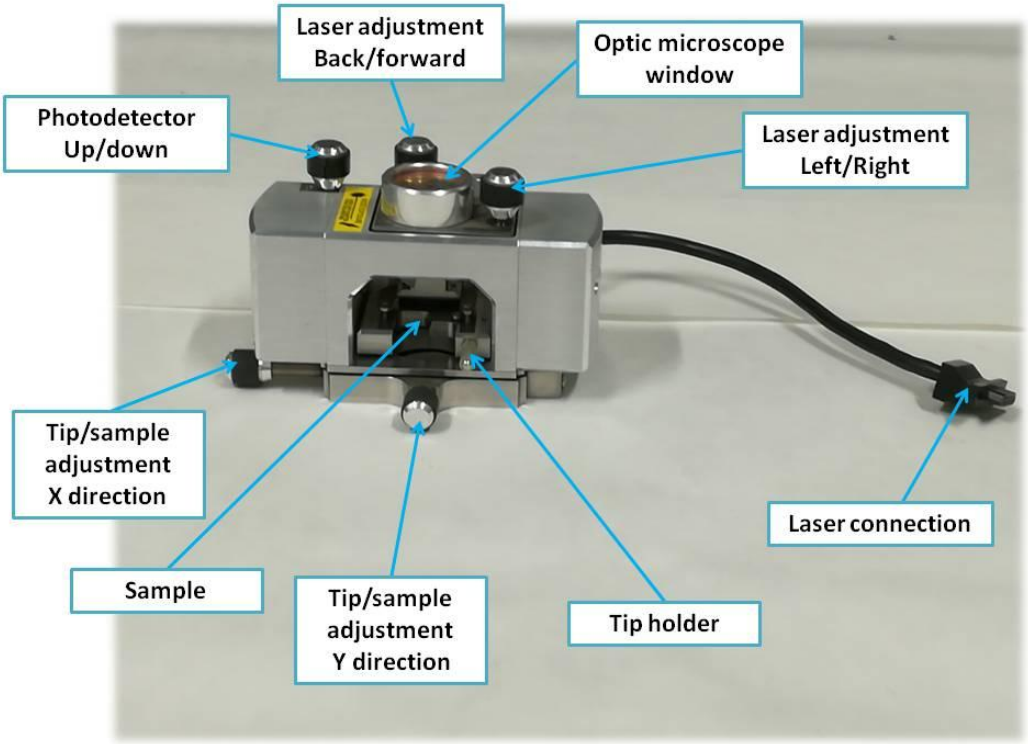
Figure 21: experiment selection window

There, the mode of operation has to be selected.

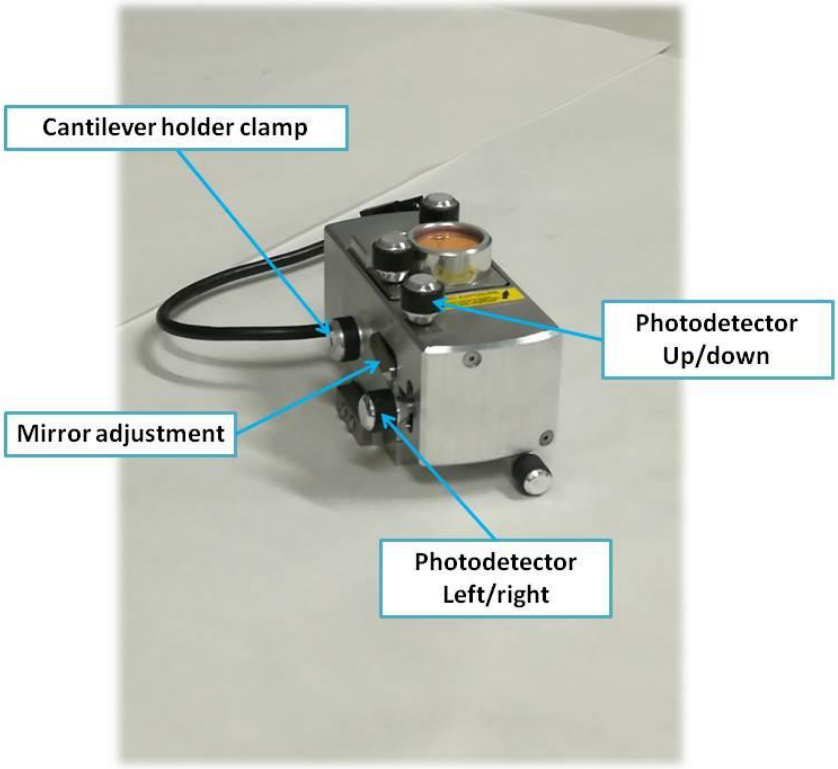
Thirdly, the sample should be loaded and the holder should be introduced. The sample is placed on top of a magnetic sample holder. Being the sample a polymer with mica on a metallic plate, the magnetic effect sticks the sample to the magnetic sample holder.

Then, the sample holder is set in position in the microscope. The SPN head is also set in position. It should be taken into account not breaking the tip in all the process and holding the head throughout all the process, being such an unique and key element. The head is attached to the microscope by two springs.

The SPN head has a lot of little elements, adjustment wheels and roulettes.. All of them are explained in Figure 22.



(a)



(b)

Figure 22: elements and function of the head

The fourth step is aligning the laser. In order to do that, first, the light MI-152 of the optic microscopy should be activated (Figure 23). This light is installed in the laboratory and will be necessary to operate the laser alignment.

Figure 23: MI-152 light

Then, the "UP" button in the microscope has to be pressed, to put the sample as close to the tip as visual operation can handle, without getting it too close and breaking the tip. In case the sample and the tip get too close, the "DOWN" button should be pressed. These buttons are shown in Figure 24.

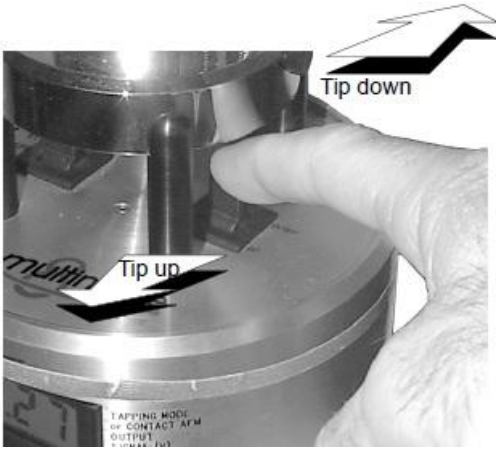


Figure 24: tip and sample approach buttons [15]

Then, the right button of the mouse will be pressed in the screen of the computer and, by pressing "Undock", the operation can be seen full screen.

Next, these steps will be followed:

1. A small piece of white paper is placed covering the photodetector.

2. The intensity of the light is lowered.

3. The different wheels of the SPN head that move the laser are moved, until the following sequences are obtained:

- "laser appears in the white paper, then disappears, then appears again" in Direction 1

- "laser appears, then disappears" in Direction 2

The reason of these steps is that, when the laser is reflecting on the cantilever, it reaches the photodetector. If it does not, nothing will be seen in the paper. The explanation is shown in Figure 25.

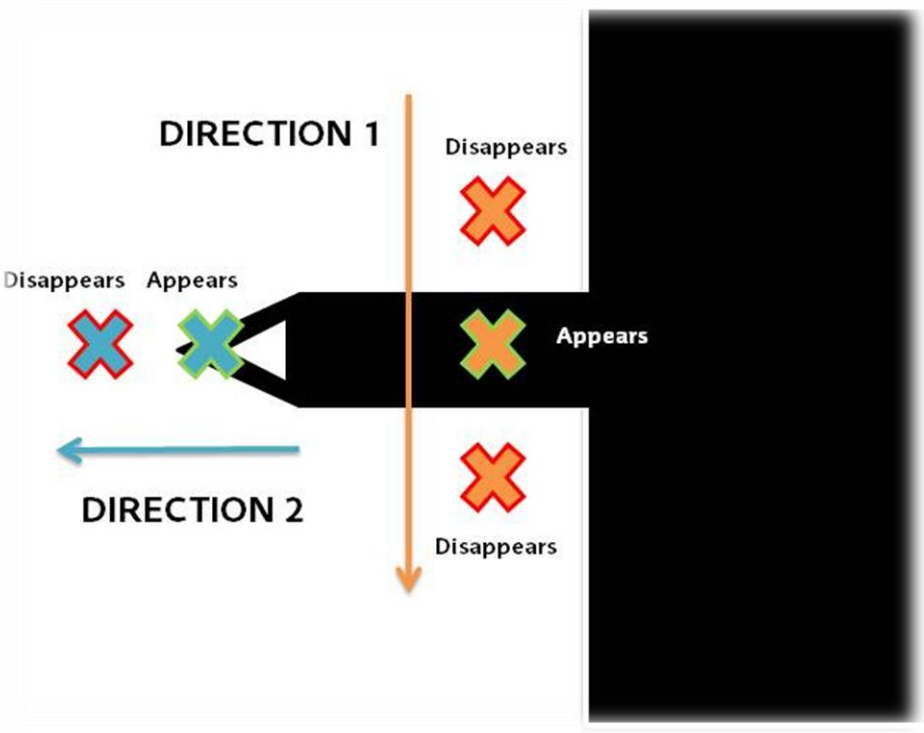


Figure 25: laser alignment

If these steps are not followed, it would be very hard to directly localize the laser in the computer screen, whereas after successfully doing the "paper

method", it is ensured that the laser is very close to the tip and slightly turns in the wheels will show the laser red dot on screen.

Therefore, the laser should be shown in the video window on screen and then moved exactly to the point where the tip is visualized. It should be also considered moving the image shown on screen by moving the elements shown in Figure 26 down in the microscope.

Finally, the photodetector must be adjusted, by moving in the SPN head the little lever that moves the mirror. The goal is to maximize the number appearing in SUM in the microscope electronic panel.

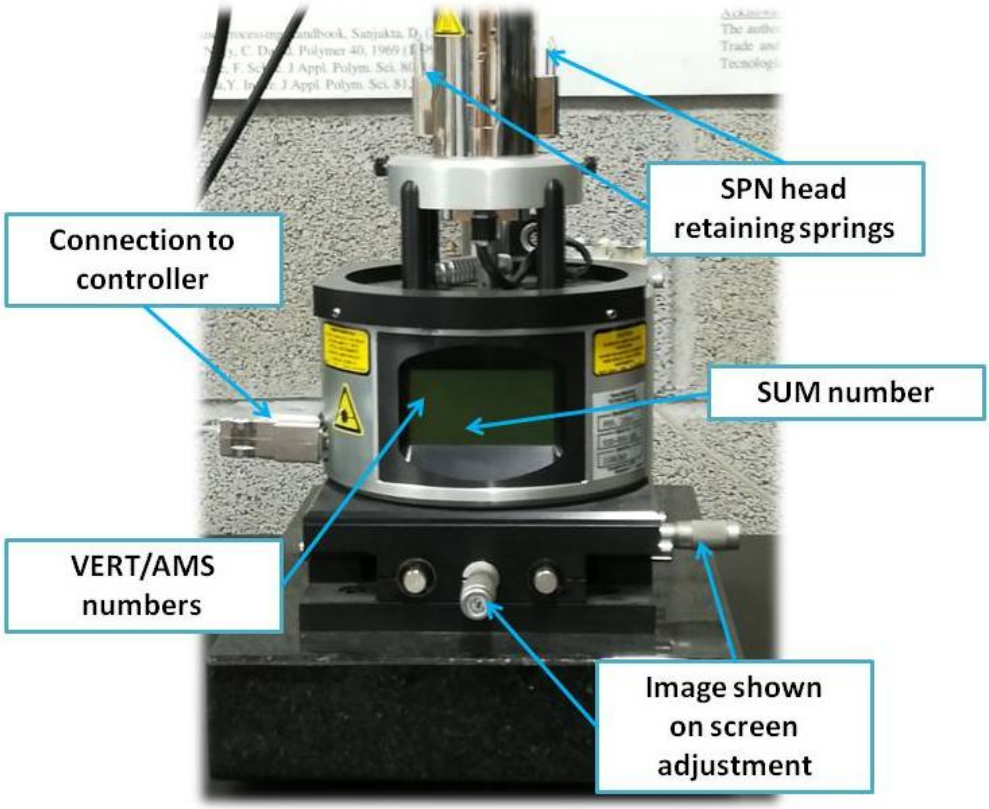


Figure 26: elements of AFM and function

Once this is done, the Engage button is clicked and the tip and sample will get closer until the first interaction is noticed and then, the scan will start. If the engaging takes too long, it should be considered to put manually closer tip and sample with the previously mentioned UP and DOWN buttons in the microscope, shown in Figure 24.

T6. Characterization of the sample

After organizing the laboratory, checking connections and calibration, preparing the samples, choosing the tip probe and adjusting the AFM, the final part would be the actual scanning of the samples using the PeakForce Tapping mode.

Nevertheless, the PeakForce mode settings, procedure and parameters will be explained below in order to get an insight of what the characterization of the sample stage takes.

Firstly, a probe that can cause enough deformation of the sample and still retain high force sensitivity has to be chosen. Therefore, cantilever stiffness should be selected based on the sample stiffness. Bruker's recommendations are shown in Table 3.

Table 3: probe selection recommendations

Sample Modulus (E)	Probe	Nominal Spring Constant (k)
1 MPa < E < 20 MPa	ScanAsyst-Air	0.5 N/m
5 MPa < E < 500 MPa	Tap150A, P/N MPP-1220-10	5 N/m
200 MPa < E < 2000 MPa	Tap300A, (RTESPA), P/N MPP-12220-10	40 N/m
1 GPa < E < 20 GPa	Tap525A, P/N MPP-13120-10	200 N/m
10 GPa < E < 100 GPa	DNISP-HS	350 N/m

To reduce optical interference, probes should be coated on their back side.

Next step is to set the mode selector switch on the MultiMode base to AFM&LFM. This is the appropriate mode for using PeakForce Tapping. The Peak Force Tapping mode, the core technology behind PeakForce QNM and ScanAsyst modes, performs a very fast force curve at every pixel in the image. The peak interaction force of each of these force curves is then used as the imaging feedback signal, that is, the setpoint. Peak Force Tapping mode modulates the Z-piezo at ~2 kHz with a default Peak Force Amplitude of 150 nm (0-peak).

Then, the software for experiments should be opened in the computer; click the SELECT EXPERIMENT icon. Here, two options are valid for working with PeakForce Tapping mode, as shown in Figure 27.

1. Scan Asyst, and then Scan Asyst in Air or in Fluid

2. Mechanical Properties, and then PeakForce QNM in Air

Figure 27: experiment selection windows

The focus will be set in PeakForce QNM mode, with the aim to measure mechanical properties as well as getting the scan phase image

So, when the experiment is chosen, the steps explained before in T5 have to be followed. Once they are done, initial scan parameters have to be set. These are a suitable Scan Size (normally around 500nm), Scan Angle (0º) and set ScanAsyst Auto Control to ON. This will avoid manual control of integral, derivative and feedback gain.

Then, the calibration of the mode has to be carried out. This setup is absolutely necessary to start using the PeakForce Tapping mode, recently installed in the laboratory. The following parameters are those needed to calibrate PeakForce QNM:

- **Deflection Sensitivity**

- **Spring Constant**

- **Tip Radius**

- **Poisson's Ratio**

- **PeakForce Setpoint**

There are two methods to calibrate these parameters:

- **The Relative Method:** uses a sample of know modulus to obtain the ratio of spring constant to the square root of tip end radius.

- **The Absolute Method:** instead of using the reference sample, the tip end radius is measured by scanning a tip calibration artifact and analyzing the resulting image. This procedure has the benefit that there is no concern over the accuracy of the modulus of the reference sample or whether it ages over time or becomes contaminated.

Deflection Sensitivity

Given that PeakForce QNM mode ramps the Z piezo and acquires force curves, measuring deflection sensitivity requires fewer steps than the normal ramp procedure. The steps are:

1. Set the Scan Size to 0 nm

2. Engage the probe onto a clean sapphire (required for cantilevers with k› 200 N/m) or silicon surface.

3. Activate RAMP mode by clicking the RAMP icon in the Workflow Toolbar. This causes the system to stop scanning, and the probe to position above the center of the previous image.

4. Enter the following parameter setting in the designated panels of the Ramp Parameter List (Table 4)

Table 4: parameters in Ramp Parameter List

Ramp panel	
Parameter	**Setting**
Ramp output	Z
Ramp size	100 nm - 1 µm
Scan Rate	1.00 Hz
Number of samples	512

Mode panel	
Parameter	**Setting**
Trigger mode	Relative
Trig threshold	0.2V

Channel 1 panel	
Parameter	**Setting**
Data type	Deflection Error
X Data Type	Z
Display Mode	Deflection Error vs. Z

5. Click the RAMP SINGLE icon (Figure 28) on the NanoScope toolbar.

Figure 28: ramp single icon

6. Move two cursors onto the Deflection vs. Z plot.

7. Arrange the cursors so that they surround the contact portion of the graph, as shown in Figure 29.

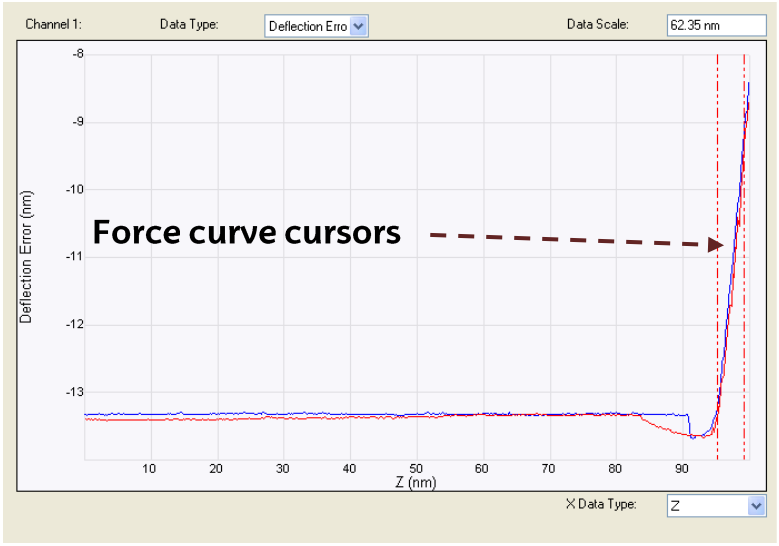


Figure 29: force curve cursors setting

8. Click the UPDATE SENSITIVITY icon (Figure 30) in the NanoScope toolbar. The software will automatically calculate the deflection sensitivity and open the Set Real-time Channel Sensitivities window. Click OK in the dialogue box and it will automatically be entered into the Deflection Sensitivity parameter.

Figure 30: update sensitivity icon

Spring Constant

1. Click the THERMAL TUNE icon.

2. Select a frequency range that includes the resonant frequency of the cantilever. Stiff cantilevers may require the 5-2000 kHz range.

3. Click ACQUIRE DATA in the Thermal Tune panel (Figure 31).

4. Click LORENTIZAN (AIR) button. Then adjust the MEDIAN FILTER WIDTH, to remove individual spikes. This replaces a data point with the median of the surrounding n (n=3, 5, 7) data points. Adjust the PSD Bin Width to reduce the noise.

5. Drag markers in from the lets and right plot edges to bracket the bandwidth over which the fit is to be performed.

6. Click FIT DATA. The curve fit, in red, is displayed along with the acquired data. Adjust the marker positions and fit the data again to obtain the best fit at the thermal peak.

7. Enter the cantilever Temperature.

8. Click CALCULATE SPRING K. If you agree with the calculated value, press OK.

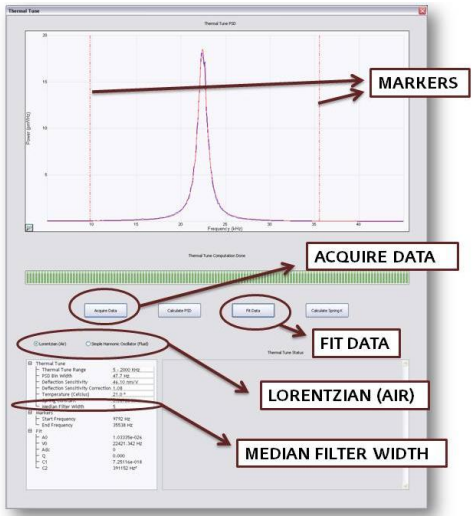


Figure 31: spring constant setting window

Tip Radius

Tip radius may be measured using a tip characterizer sample and the Tip Qualification function in NanoScope software.

1. Scan the characterizer sample. Set the Scan Size to 1.5 μm.

2. Set the Samples/Line and Lines to 512. Set the Aspect Ratio to 2.0. Set the Scan Rate to 0.5 Hz or less. Set ScanAsyst Noise Threshold to 1.0nm.

3. CAPTURE the characterizer image.

4. Open the Height channel of the saved image in the NanoScope Analysis package (Figure 32).

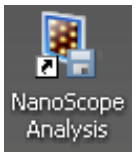


Figure 32: NanoScope software icon

5. Flatten the image by clicking the PLANE FIT icon (Figure 33). Select XY as the Plane Fit Mode. Select 1ST as the Plane Fit Order. Click EXECUTE.

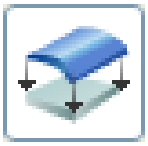


Figure 33: plane fit icon

6. Click the TIP QUALIFICATION icon. (Figure 34).Enter the measured average Deformation into the Height 1 from Apex field.

Figure 34: tip qualification icon

7. Click ESTIMATE TIP. Click QUALIFY TIP. It will show the Estimated Tip Radius.

Poisson's Ratio

Poisson's ratios of the sample is used to calculate the sample modulus, E_s from the measured reduced modulus E*.

The reduced modulus is related to the sample modulus by the following equation (Equation 1):

$$\mathbf{E}^* = \frac{\mathbf{1} - \boldsymbol{\nu}_t^2}{\boldsymbol{E}_t} + \frac{\mathbf{1} - \boldsymbol{\nu}_s^2}{\boldsymbol{E}_s}$$

Equation 1: reduced modulus

where $\boldsymbol{\nu}_t$ and $\boldsymbol{E}_t$ are the Poisson's ratio and Young's modulus of the tip and $\boldsymbol{\nu}_s$ and $\boldsymbol{E}_s$ are the Poisson's ratio and Young's modulus of the sample. The assumption of the tip modulus $\boldsymbol{E}_t$ being much larger than the sample modulus $\boldsymbol{E}_s$, is made, and can be approximated as infinite, and calculate the sample modulus using the sample Poisson's ratio.

Poisson's ratio generally ranges between 0.2 and 0.5 giving a difference between the reduced modulus and the sample modulus between 4% and 25%. Because the sample's Poisson's ratio is not generally known, many publications report only the reduced modulus. Recommended values for the sample's Poisson ratio $\boldsymbol{\nu}_s$ are shown in Table 5.

Table 5: recommended values of Poisson ratio

E_s	ν_s
E_s<100 MPa	0,5
0,1<E_s<1 GPa	0,4
1GPa<E_s<10GPa	0,3

Then, the obtained parameters should be included in the Cantilever Parameters dialogue box, as shown in Figure 35.

Figure 35: Cantilever Parameter dialogue box

Peak Force Setpoint

A Peak Force Setpoint that is too high can either damage the sample or wear the tip. It is generally desirable to reduce it to as small a value as is possible. However, in order to achieve accurate Elastic modulus measurement, sufficient sample deformation is needed. If the deformation is less than 2nm, increase the Setpoint.

12. RESULTS

T1. Equipment, inventory and optimization

The first task consisted in getting used to laboratory, reading bibliography and manuals about them, inventorying the equipment and accessories and carrying out an optimization of the workspace to ensure the success of the project. The results of this stage are presented in the following lines.

Table 6 shows the inventory that was carried out in the laboratory. Table 7 shows the accessories that were at use too. The model and characteristics have been highlighted. It should be recalled that the controller was modified when introducing the new PeakForce Tapping Mode.

All equipment which was not useful for the AFM was removed from the room. A huge wardrobe in the left side of the room kept nothing but useless bibliography, old equipment and non-AFM stuff. Therefore, the full furniture was removed.

Also, the controller and the PC tower were located on top of the table. This was not the optimal location, because it could lead to poor ventilation of the equipment, overheating and failure of the controller, the most important element of the AFM. Therefore, the storage was moved under the table, so that more working space was available, and the controller was located on top of it, ensuring a proper air flux to ventilate it.

Figure 36 shows the organization of the laboratory before and after the optimization. Some of the elements in the laboratory were removed in the graphic to get a better insight of the changes.

Table 6: inventory, elements

Equipment	Model	Characteristics
Atomic Force Microscopy (AFM)	Bruker Multimode 8-HR	Includes Laser Class 2M,1 mW maximum at 690 nm (IEC and US CDRH)
Scanner	Bruker AS-130, model J	Scan size 125x125 μm z = 5 μm
Microscope base	Bruker 920-006-101	With LCD Display Panel
Controller	Bruker NanoScope, model NSV	Previously, the controller model was III, but it was upgraded to model V.
Computer	Bruker, 481-009-600	NanoScope Software Version installed is 9.15 Operating system: Windows XP
Temperature control module	Bruker 840-002-337	-
Vibration Isolation Table	TMC, model CleanBench Vibration Isolation Table	600x600 mm, fed with a N2 line installed in the laboratory

Table 7: inventory, accessories

Accessory	Number of units	Characteristics
Clamp kit	5	-
Adhesive stickers	+100	Model: STKYDOT
Scissors	-	-
Metallic disks	17	15 mm diameter, SD-102
	66	12 mm diameter, SD-101
	10	6 mm diameter, SD-104
Mica sample disks	+100	-
Silicon pattern sample	1	Model: VGRP-15M, surface topography reference
Holders	1	Force Modulation Probe Holder, MFMA
	1	Torsion Resonance Mode Probe Holder, MMTR-TUNA-CH-2
	1	Fluid Cell Holder, MMTMEC

Bruker acquired Veeco's Scanning Probe Microscopy area in 2010. That is why in the equipment the Veeco logo is sometimes included.

BEFORE AFTER

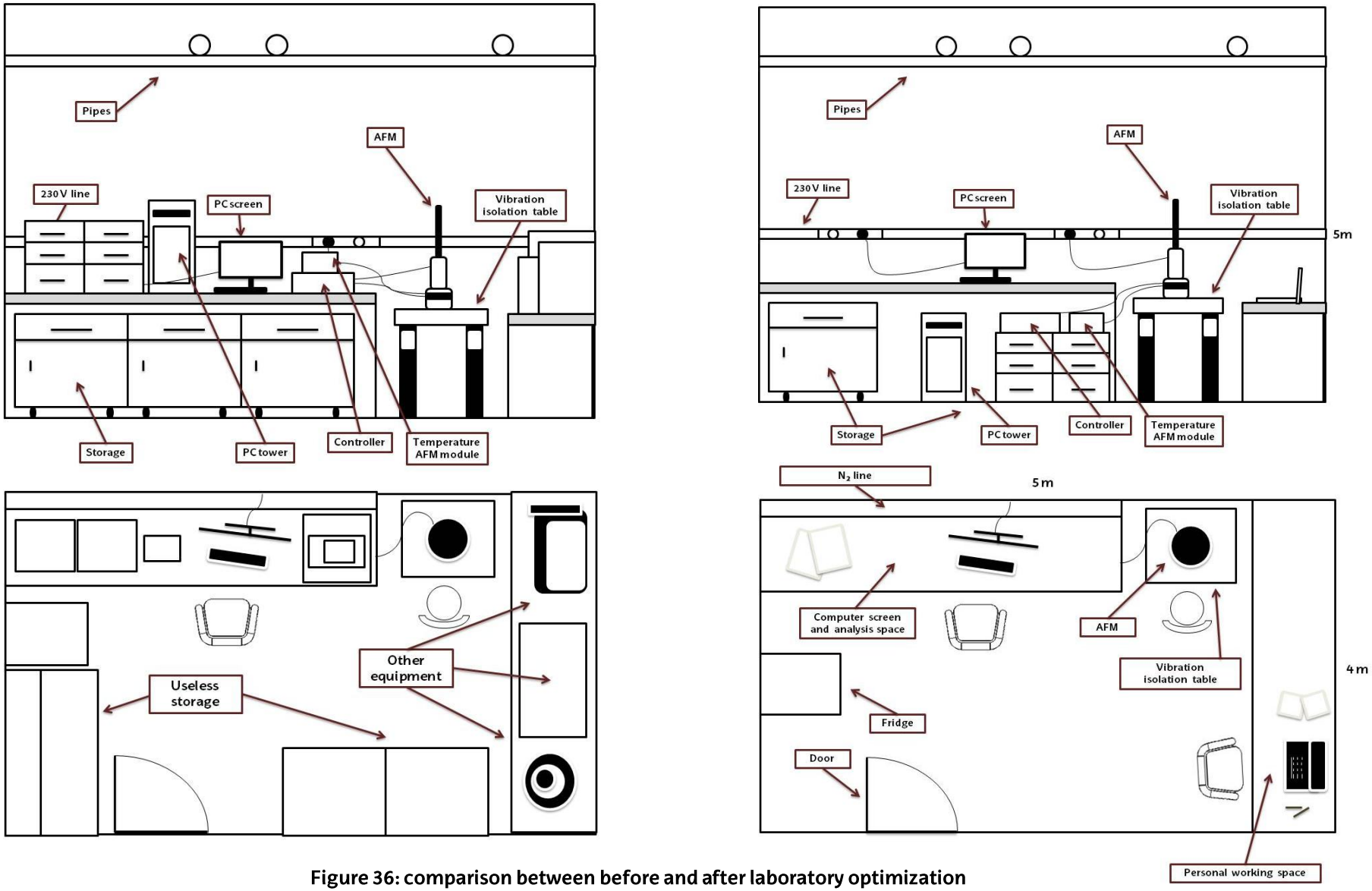


Figure 36: comparison between before and after laboratory optimization

T2. Checking connections and calibrating the unit

In the stage of checking the voltage of the controller, the results showed that the voltage was right in all spots; however, in the spot showed in the bottom left, the measure was slightly lower than the actual set value. Little disturbances in the polymer or in the way the measure was made could lead to this, so the project continued.

T3. Preparing the samples for AFM and sample thickness measurements

When the spin coating is finished, the result of checking if the film has been formed is negative. The reason for this is that Teflon is an hydrophobic material and covering half of the plate with this material is not a valid solution. Therefore, another way to solve this issue has to be found.

The solution adopted is the following: complete films in the samples will be made, for both purposes 1 and 2 mentioned earlier. For the samples whose thickness wants to be measured, half of the film will be taken off with chloroform.

However, when working with the samples that spin at 25 rps and even faster, an issue is raised up: the spin coater drives the metallic plates away. So, a solution has to be found. The solution adopted is using mica.

Therefore, some mica available in the laboratory is cleaved until thin ultraflat surfaces of the diameter of the metallic plates are obtained.

The method that will be followed takes these steps:

1. Vacuum is created in the spin coater to hold the mica thin surface

2. The mica is spun to the desired velocity

3. While it is spinning, two drops of the dissolution are carefully added.

4. Waiting some seconds is required.

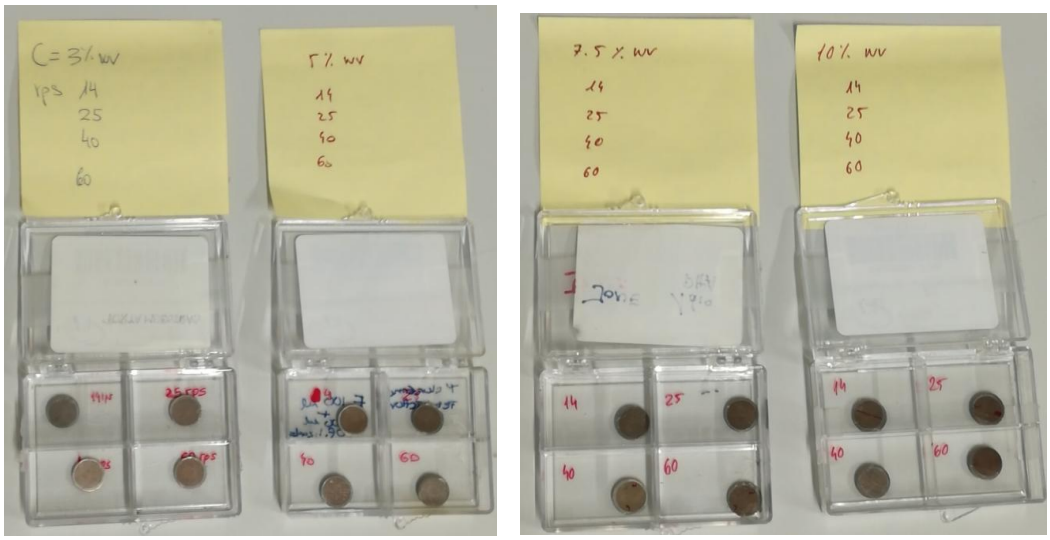
5. The spin coater is stopped.

6. The mica is adhered, on top of which the polymer surface has been created, on the metallic plate. To do so, some adhesives like the ones shown in Figure 37 are used.

Figure 37: adhesives

By this method, the desired result is achieved. The mica is not taken off the spin coater when spinning it at high velocities. Also, the polymer film is created. Furthermore, the mica surface is flat and perfect so that it does not interfere in the AFM analysis.

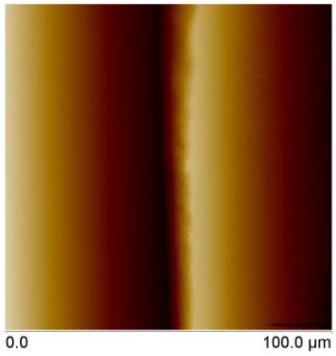
The samples obtained are shown in the following Figure 38:



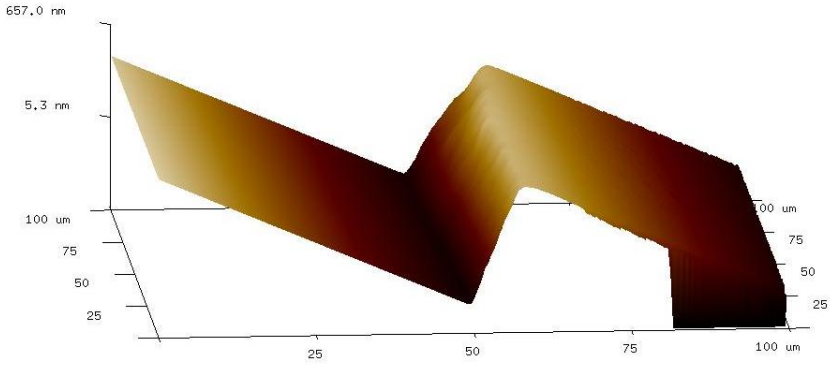
(a) **(b)**

Figure 38: obtained samples

The first sample to scan is the one of 10% wv dissolution and 14 rps. The images obtained are shown in Figure 39.



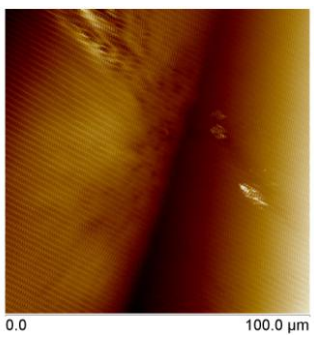
(a)



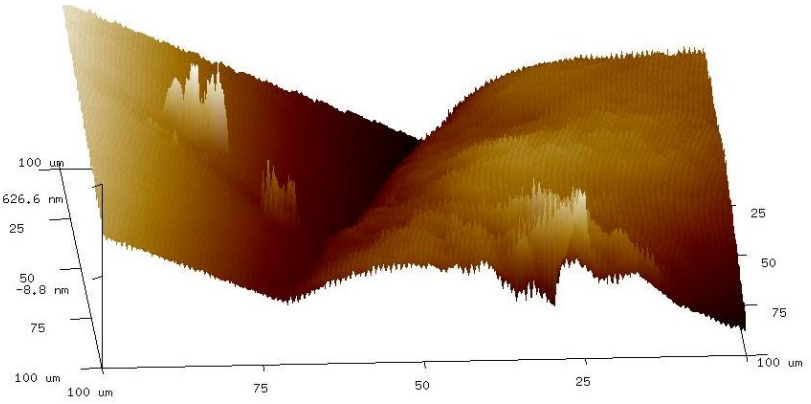
(b)

Figure 39: PHA images in AFM, at 10% wv and 14 rps

The sample of the same dissolution but at 25 rps is also scanned. The images obtained are shown in Figure 40:



(a)



(b)

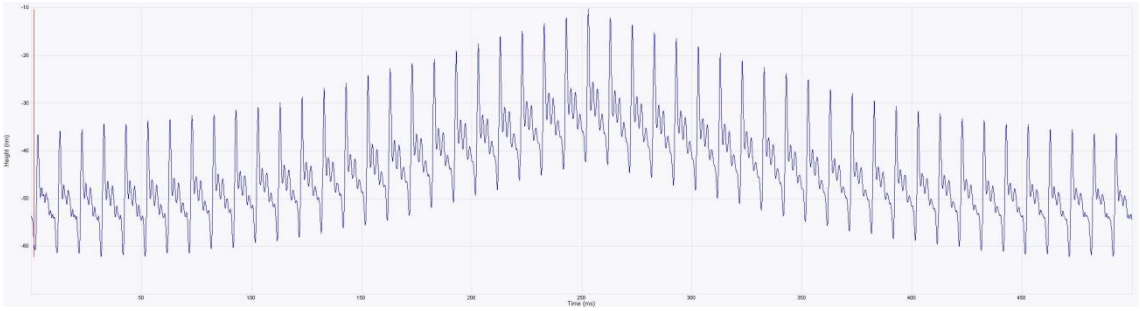
Figure 40: PHA images in AFM, at 10% wv and 25 rps

Two problems are seen in the results:

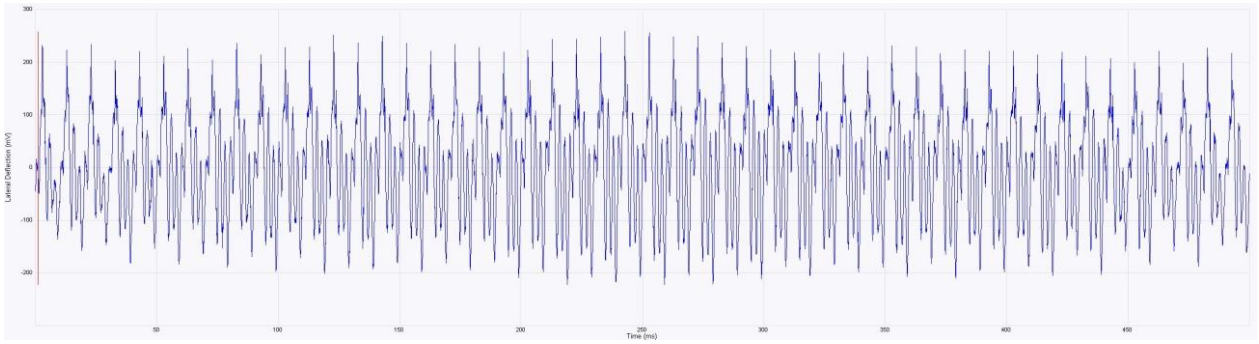
1. The sample looks like a ramp, like if it was rotated. (see Figure 39 and Figure 40). The step is seen and both surfaces (polymer and mica) are flat but the state of the 3D image makes its analysis and measurements difficult. This could be because the holder is not correctly attached and balanced or because the parameters have not been correctly set. However, this is not a highly problematic issue.

2. The image is obtained with a lot of noise (see Figure 41). This is a highly concerning issue, since the problem could be in the controller.

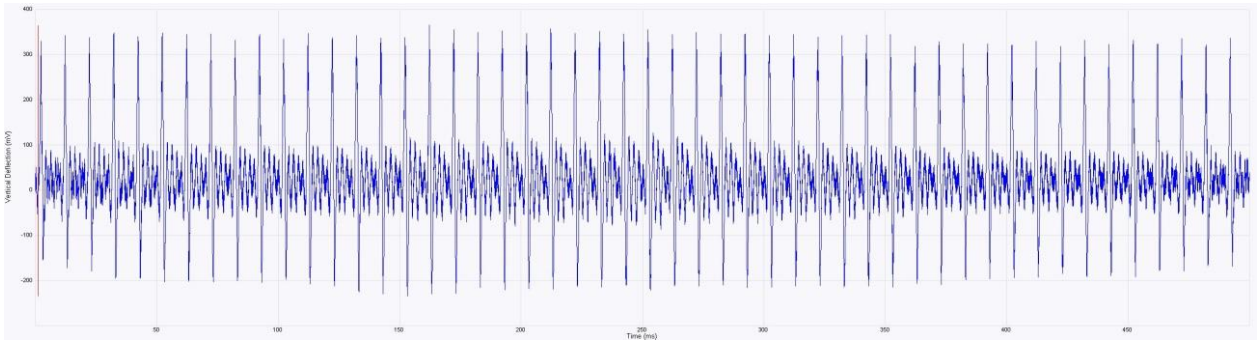
To check how the controller is scanning and driving the tests, the Peak Force curve is analyzed, and the vertical, lateral and z deflection of the cantilever throughout the process. The following graphics are obtained. (Figure 41)



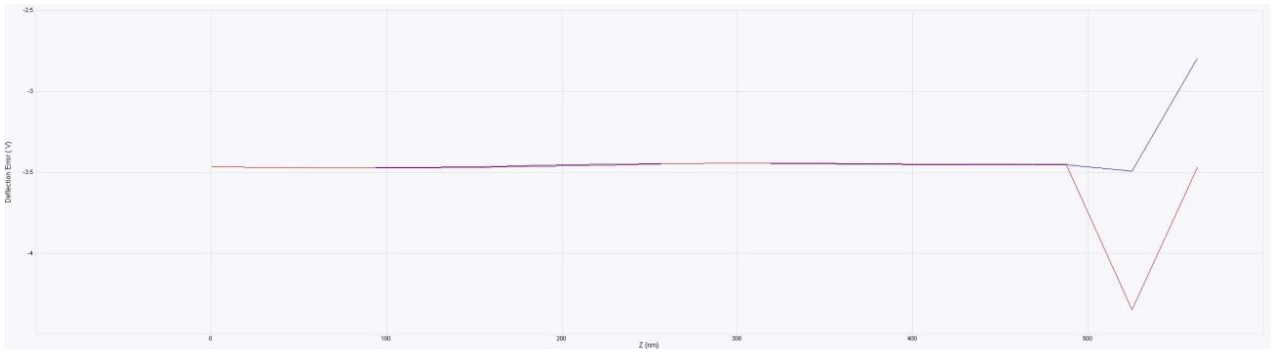
(a)



(b)



(c)



(d)

Figure 41: cantilever graphics while scanning (a) vertical deflection (b) lateral deflection (c) z deflection (d) force curve

There is a huge noisy background in the experiment. However, the Peak Force curve graphic look like what it was expected from the theoretical approach.

Also, when engaging the tip to start the scan, some trouble with the piezoelectric voltages is faced. The AFM sounds loud and the voltages are unexpectedly high or low at some stages, with a red light indicator in the screen referring to the scanner.

With all these results, a proper image of the samples cannot be achieved. Also, the test cannot be safely done, without breaking tips in the process, due to the high voltages that make unsteady volume variations of the scanner and excessive approach of the tip to the sample. So, the experiment is stopped and the technical support from Bruker is called, to set which is the issue with the controller and the AFM.

Bruker will send a technician to try to make a diagnosis. Meanwhile, the samples cannot be tested. Since two steps have only been measured, the desired graphics (PHA composition vs. sample thickness and Spin Coater velocity vs. sample thickness) cannot be done.

T4. Probe tip election criteria

The laboratory has purchased several probes. The available tips are shown in Table 8:

As the inventory of Table 8 shows ,six different types of probes are available. They are kept in seven bags. Six of them are labeled as each operation mode and new tips are kept there. The seventh bag keeps the already used tips and the broken ones. This tips will be reused to practice their introduction in the cantilever as part of the experience of the user.

In our experiment, PHA will be used, which has a Young's Modulus of approximately 3,5 GPa. Attending to Figure 18, SNL-A or TAP 150 A tips should be used. Attending to our inventory, the used tip will be PeakForce QNM TAP.

Table 8: inventory of tips in the laboratory

OPERATION MODE	MODEL	QUANTITY
PeakForce QNM	(TAP 525A) MPP-13120-10 Tapping	**1**
	(TAP 150) MPP-12120-10 Tapping	**1**
	(RTESPA) MPP-11120-10 Tapping	**1**
	ScanAsyst- Air	**2**
Scan Asyst	ScanAsyst- Air	**3**
	ScanAsyst- Fluid	**1**
	ScanAsyst- Fluid+	**1**
Torsion	MPP-21100-10	**1**
Tapping Mode	MPP-11100-10	**5**
Super sharp	MSNL-10	**3**
Contact Mode	NP-10	**2**

T5. Adjustment of the AFM

After some training with already broken tips, the user gained experience and practised several times in the laser alignment method. Therefore, this stage was successfully satisfied.

T6. Characterization of the sample

Due to the bad state of the controller, proved when scanning the thickness of the samples, this task could not be carried out.

12. GANTT DIAGRAM

A Gantt Diagram (Figure 42) is a useful tools to plan and visually get an insight of projects. Dates, tasks, length of the project are shown in the diagram to organize and check how the project development Is carried out.

In our project, several landmarks can be distinguished:

- Preparation of the project: reading bibliography about the AFM, equipment organization and inventory, installation at the laboratory...

- Sample preparation: as explained before with the spin coating technique and materials in the laboratory.

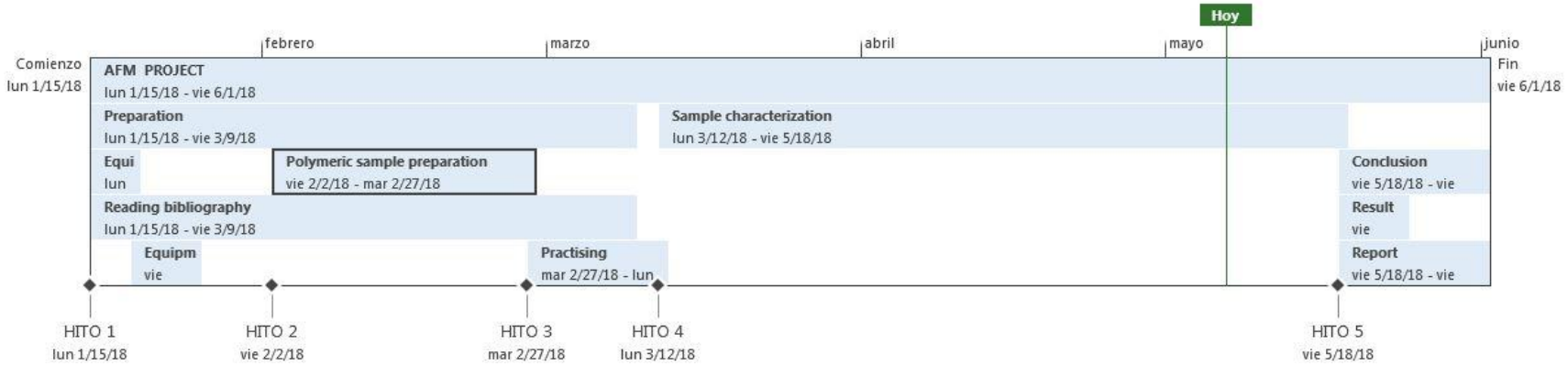
- Getting expertise in laboratory and AFM basic tasks: such as inserting tip in the cantilever or removing half of the sample film.

- Characterization of the sample

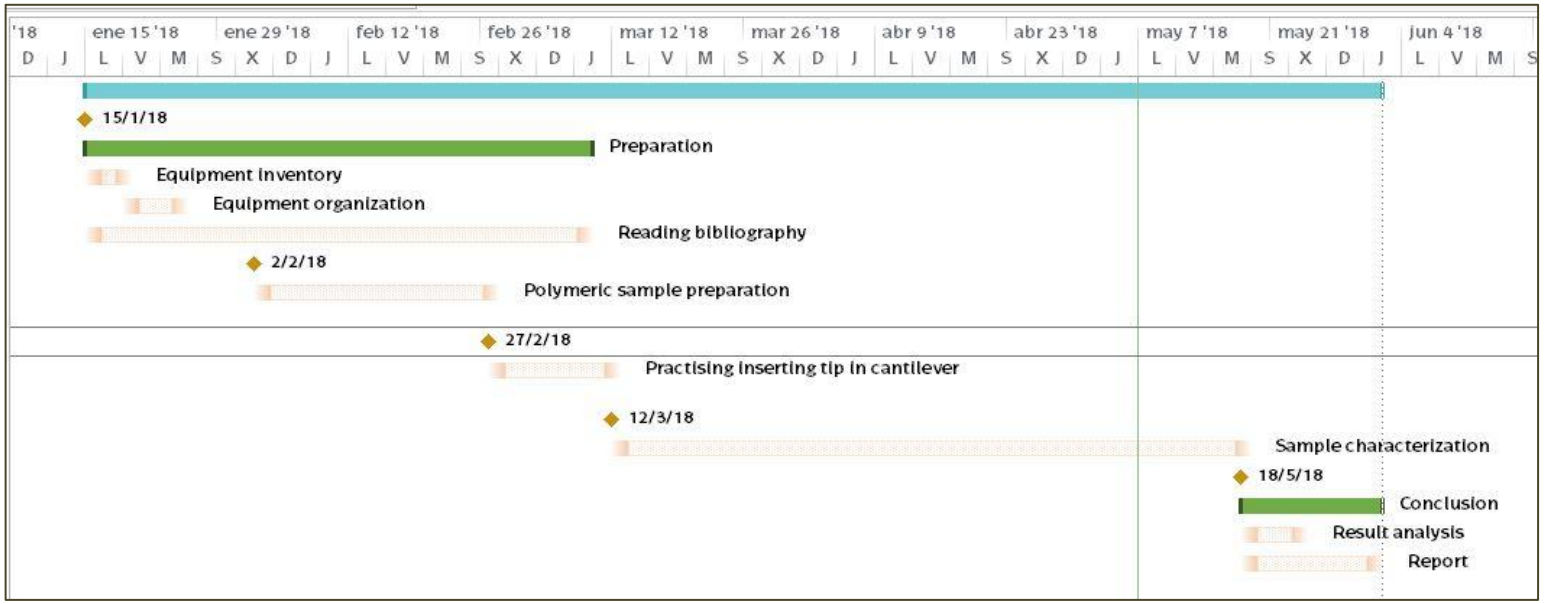
- Conclusions of the project: analysis and reports.

Nombre de tarea	Duración	Comienzo	Fin
AFM PROJECT	**100 días**	**lun 1/15/18**	**vie 6/1/18**
HITO 1	**0 días**	**lun 1/15/18**	**lun 1/15/18**
Preparation	**40 días**	**lun 1/15/18**	**vie 3/9/18**
Equipment inventory	5 días	lun 1/15/18	vie 1/19/18
Equipment organization	5 días	vie 1/19/18	jue 1/25/18
Reading bibliography	40 días	lun 1/15/18	vie 3/9/18
HITO 2	**0 días**	**vie 2/2/18**	**vie 2/2/18**
Polymeric sample preparation	18 días	vie 2/2/18	mar 2/27/18
HITO 3	**0 días**	**mar 2/27/18**	**mar 2/27/18**
Practising inserting tip in cantilever	10 días	mar 2/27/18	lun 3/12/18
HITO 4	**0 días**	**lun 3/12/18**	**lun 3/12/18**
Sample characterization	50 días	lun 3/12/18	vie 5/18/18
HITO 5	**0 días**	**vie 5/18/18**	**vie 5/18/18**
Conclusion	**11 días**	**vie 5/18/18**	**vie 6/1/18**
Result analysis	5 días	vie 5/18/18	jue 5/24/18
Report	11 días	vie 5/18/18	vie 6/1/18

(a)



(b)



(c)

Figure 42: Gantt diagram

13. BUDGET

Table 9: budget

Inhouse labour hours

	Hour unit cost	Hours	Cost (€)
Student	15	185	2775
Tutor	45	50	2250

Depreciation

	Quantity	Usage life (years)	Prize (€)	Depreciation (€/h)	Usage time (h)	Cost (€)
AFM	1	20	2000000	13,89	100	1388,89
Balance	1	10	420	0,01	2,5	0,01
Micropipette	2	10	260	0,004	5,5	0,02

Destructive material

	Quantity	Appearence	Prize (€)	Prize per unit	Cost (€)
Glass big vials	4			3,00	12
Glass small vials	32			0,20	6,4
Probe tips	8	10-pack	250	25,000	200
PHA	100	500 g pot	1000	2	200
Mica	32	100 kg pack	10	0,1	3,2

Outhouse labour hours

	Hour unit cost	Hours	Cost (€)
Technician	50	30	1500

Total cost (€)	8335,52 €

The AFM is a valuable equipment with a high depreciation cost. This is the reason for good maintenance advises throughout the entire set up and run-through project.

Also, the technician is a key element in the budget of the project. It is a non-expected cost. Anyway, it had to be paid due to inconvenient issues appearing in the project.

About the products, PHA and tips are the most expensive ones. PHA is obtained in form of pellets, whereas tips can be purchased in 10-tip packs.

The laboratory aims to buy tips in large quantities in order to get volume purchase discounts. Unfortunately, large pack sizes have been identified as a frequent cause of inconvenience to the customer and corruption of probe quality over time:

• Large pack sizes have a "hidden cost" that customers share with friends and colleagues. The process of repackaging and distributing probes from larger pack sizes is time consuming and subjects the probes to potential handling damage.

• Large packs must be opened and closed repeatedly throughout their lifetime, continuously exposing the entire package to static and environmental contamination, thus degrading probe quality over time. **[8]**

In an effort to allow customers to continue to enjoy volume discounts while enabling them to preserve the initial quality of the probes, Bruker AFM Probes offers most probes in only 10-tip packs (Figure 43).

	Price per 10-Pack, When Purchased in Quantities Indicated						
Model	**1**	**2 - 6**	**7 - 15**	**16 - 24**	**25 - 37**	**38 +**	**Wafer**
DNP-10	$250.00	$212.50	$187.50	$162.50	$137.50	$125.00	$4,750.00
DNP-S10	$265.00	$225.25	$198.75	$172.25	$145.75	$132.50	$5,035.00
NP-10	$250.00	$212.50	$187.50	$162.50	$137.50	$125.00	$4,750.00
NP-S10	$265.00	$225.25	$198.75	$172.25	$145.75	$132.50	$5,035.00

Figure 43: tip packs prizes [8]

14. CONCLUSIONS

In the development of this project, several conclusions have been brought up:

1. The project has not been completed since the equipment was broken. Characterization of the samples was impossible due to controller failure.

2. When organizing the workspace, ventilation of the equipment, personal workspace enabling, noise avoidance and inventory of the equipment of the laboratory are useful tools in order to achieve **a successful run-through** of any project. In the AFM, these are even more relevant due to the fragility of some parts and precision required in the measures.

3. In the preparation of polymer samples, **the spin coating method is very useful** to obtain thick films to analyze in the AFM. However, the **use of mica** is the key to obtain flat surfaces that allow a better characterization in the AFM.

4. The **measures to adopt** so that the equipment is kept in good state are:

4.1. Avoiding switching off the computer while the controller is on.

4.2. Fixing the bolts to avoid noise.

4.3. When trying to maximize the SUM number, moving the mirror and not the laser to do so.

5. The Atomic Force Microscopy, AFM, may be very useful tool in polymer characterization at nanometric scale. Just regarding bibliographic information and the images obtained in the measurements of the thickness of the polymer film, the **advantages of this microscopy are clearly seen**. The images are taken much faster and easier than working with other modes, and the information the software gives is more complete.

15. FUTURE PROJECTS

As it has been explained in the previous lines, this project has not been concluded, due to technical issues and lack of time. However, once the AFM operates correctly again, it could be concluded and developed.

Thus, **this project is to be followed**. Indeed, if the controller worked, the samples should be analyzed. The aim is to obtain mechanical properties and images of their structure at a nanometric scale. Another purpose is to measure the same polymers once they have been heated at different temperatures, or even at a fixed temperature using the module the AFM offers.

Polymer characterization by Atomic Force Microscopy is part of a bigger project carried out by the research group ZIBIO. The bigger project consists in the development of materials for uses in biomedical engineering.

One of the materials in research are the polyhydroxyalkanoates (PHAs). It is believed that these materials are useful for tissue regeneration and biomedical purposes, because of their biodegradability, good biocompatibility and mechanical properties.

Most part of the characterization of these materials has been already made by the polarized light optic microscopy. However, this microscopy method holds two drawbacks.

1. Limitations in lateral resolution

2. Two-dimensional images

Though, the key is to know deeper about the crystallization of these materials, because their properties get largely affected by the way this process is carried out. PHAs tend to do small crystals whom morphology and kinetic have to be known as a previous step to design the final product.

The Atomic Force Microscopy (AFM) is, thus, a better method to get a greater insight in these materials. It provides information about crystallization at a smaller scale, as well as tridimensional images. In the AFM we can do in situ crystallizations as well.

In the AFM, the PeakForce Tapping method holds the best characteristics to fulfill the purposes of the project, since it operates at lower frequencies, ensuring better stability of the "in situ" process and enabling to measure mechanical properties while scanning the sample.

Therefore, after this project (setting up the PeakForce Tapping mode and carrying out an initial run-through some polymeric samples), a greater research could be done; it could be possible to reach our final aim of obtaining a better characterization of PHA, to hold data in order to apply these materials into biomedical uses.

17. BIBLIOGRAPHY

[1] Díaz, J. *Aplicaciones de la Microscopía de Fuerza Atómica (AFM) cómo método de caracterización de polímeros* (2004)

[2] Meyer, G. and Amer, N.M. Appl. Phys. Lett. 53, No. 12, 1045-47 (1988)

[3] Kaemmer, S. *Introduction to Bruker's ScanAsyst and PeakForce Tapping AFM Technology*, Application Note 133, *Last seen:* www.bruker.com

[4] Berquand, A. *Quantitative Imaging of Living Biological Samples by PeakForce QNM Atomic Force Microscopy*, Application Note 135, *Last seen:* www.bruker.com

[5] Kallesøe, C., Larsen, M. B. B. S., Bøggild, P., & Mølhave, K. *3D mechanical measurements with an atomic force microscope on 1D structures.*(2012)

[6] *Bioscope Catalyst with ScanAsyst*, Experiment Guides, Bruker (2009)

[7] Prater, C. & G. Maivald, P. & Kjoller, K. & G. Heaton, M. *TappingMode Imaging Applications and Technology* (1995)

[8] *Bruker AFM Probes*, Bruker Manuals, *Last seen:* www.brukerAFMprobes.com (2013)

[9] Pittenger, B. *Quantitative Mechanical Property Mapping at the Nanoscale with PeakForce QNM*, Application Note 128, *Last seen:* www.bruker.com

[10] Pavel, T, Kaufmann, J., Volz U., *On the use of peak-force tapping atomic force microscopy for quantification of the local elastic modulus in hardened cement paste* (2012)

[11] Díaz Martínez, V. *La industria de los PHA: cuando el plástico es natural,* BioTech Spain, *Last seen:* www.biotechspain.com/generate_pdf.cfm (2010)

[12] Ali, I. & Jamil, N. Front. Biol. 11: 19. *Polyhydroxyalkanoates: Current applications in the medical field* (2016)

[13] *Installation and Maintenance Guide*, Bruker, *Last seen:* www.nanophys.kth.se/

[14] Jiang, P and McFarland, M *Large-Scale Fabrication of Wafer-Size Colloidal Crystals, Macroporous Polymers and Nanocomposites by Spin-Coating* (2004)

[15] Kelley, V. *Veeco PeakForce QNM User Guide*, 004-1036-000, Bruker Manuals, Bruker (2010)