# Optical Sensors based on SERS for low concentration of water contaminants

Master's Degree Thesis

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*To my family.*

*I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.*

Andrea Rovarotti

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# Glossary

**APTMS** (3-Aminopropyl)trimethoxysilane

**CCD** Charge Coupled Device

**EU** European Union

**E** Electric Field

**f.e.** for example

**GDWQ** Guidelines for drinking-water quality

**LOD** Limit Of Detection

**LOQ** Limit Of Quantification

**LSPR** localized surface plasmon resonance

**LSP** localized surface plasmon

**m** slope of the calibration curve

**MBA** 4-Mercaptobenzoic acid

**MilliQ** Purified and Deionized water

**NIR** Near Infra-Red

**NIR** near infrared

**NP(s)** NanoParticle(s)

**$NaNH_4HPO_4$** Ammonium sodium phosphate dibasic tetrahydrate

**O** Oxygen atom

**PDMS** Polydimethylsiloxane

**PLA** Polylactic acid or polylactide

**ppb** parts per billion

**ppm** parts per million

**ppt** parts per trillion

**rpm** revolutions per minute

**SAM** Self-Assembled Monolayer

**SCCM** Standard Cubic Centimeters per Minute

**SD** Standard Deviation

**SERS** Surface Enhanced Raman Spectroscopy

**SNR** Signal-to-Noise Ratio

**T** Temperature

**WFD** Water Framework Directive

**WHO** World Healt Organization

**Abstract**

The aim of this master thesis is to fabricate and characterize Surface Enhanced Raman Scattering (SERS) probes and optimize the Raman measurements for the detection of contaminants in water in a small amount of concentration.

The thesis has been realized in the framework of "RaPID" project, a project funded by NATO SPS (North Atlantic Treaty Organization, divison of Science for Peace and Security). RaPID stands for Raman Probe for chemical contaminant IDentification; the goal of this project is to realize a portable device able to detect low concentrations of contaminants in water with a relatively rapid analysis in order to understand if they're below a precise guard level imposed by law.

The SERS substrates are realized on functionalized glass that is covered with gold nanoparticles to leverage the Surface Plasmon Resonance (SPR) that allows high efficiencies for the Raman spectroscopy and identification of chemical compounds.

The target is to detect concentration of water contaminants (like Thiram) below the order of nM-µM. The SERS probe is also explored in a configuration including basic microfluidic channels for the input and the output of water.

The experiments have been carried on in the laboratories of the Department of Applied Science and Technology of Politecnico di Torino, and at the Department of Chemistry at Universitat de Barcelona.

# Chapter 1

# Introduction

## 1.1 RaPID project

This thesis has been realized in the framework of "RaPID" project, a project funded by NATO SPS (North Atlantic Treaty Organization, division of Science for Peace and Security). RaPID stands for Raman Probe for chemical contaminant IDentification; the goal of this project is to realize a portable device able to detect low concentrations of contaminants in water with a relatively rapid analysis in order to understand if they're below a precise guard level imposed by law.
Worldwide, the access to safe drinking water is a huge problem: despite being an essential ingredient for human health, an increasing number of people don't have safe drinking water everyday. For this reason, there is a critical need for easy to use, compact and sensitive techniques for water quality monitoring.
Unfortunately today, this type of control is usually expensive and time-consuming, in fact you may also require days. From here the need to have a portable device, able to give a quick response with only a little drop of water and at the same time sensitive enough to detect contamination levels within the safety limits.
Raman spectroscopy is a powerful technique to characterize chemical composition applied to many areas, including chemistry, food, material science or pharmaceuticals.
The improvement of Raman spectrometers makes possible the detection of low concentrations of chemical and pharmaceutical contaminants in water.

## 1.2 Raman Spectroscopy

Raman Spectroscopy is a vibrational spectroscopy which provides a fingerprint of a biological or chemical compound. More precisely, it probes the different vibrations occurring in a molecule excited by light: in 1921 C.V. Raman observed a light scattering shifted in wavelength ($\lambda$) with respect to the $\lambda$ of the incident light, from here this secondary radiation was named Raman effect.
The vibrations occurring in the excited molecule are highly related with the molecular structure and the frequencies of vibration depend on the mass of the atoms, on the nature and on the strength of the chemical bonds, i.e. the global conformation of the molecule. Thus, Raman Spectroscopy can be used as molecule identification.
Raman Spectroscopy is based on inelastic scattering induced by indirect transition between two vibrational levels [1]: in fact when an incident light beam interacts with the molecules, they start to vibrate and the most part of the re-emitted photons (due to scattering) have the same energy of the incoming ones (incident light beam), i.e. they undergo Rayleigh process, which is elastic scattering.

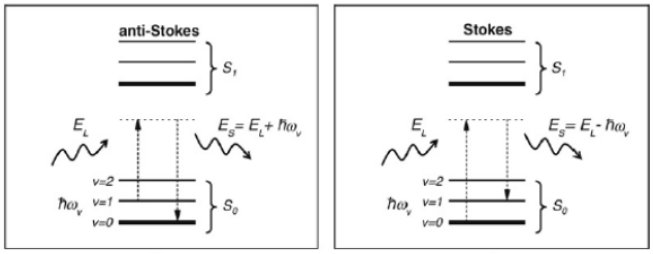


Figure 1.1: Illustration of inelastic scattering: Anti-Stokes (AS) process at left, Stokes (S) process at right

Thus Raman processes are inelastic ones, i.e. we have a difference between the energy of the incoming photons and the energy of the emitted ones. This has the consequence to have a shift in frequency, which can be positive or negative, depending if they have lost or gained energy.

In Fig. 1.1 we have a two steps process, in which the first one is the absorption of an incident photon, followed by the spontaneous emission of a scattered one. After the photon absorption, the molecule is excited to an higher energy level, which is usually thought as an intermediate virtual state. If the energy of the intermediate virtual level matches with one of the real electronic level, then a resonant scattering process is induced.
In comparison with the incident photon, the scattered one can have higher or lower energy level and the two different cases are called in different ways: Stokes and Anti-Stokes process. In case of the Stokes process, the scattered photon has an energy $E_S$ (Fig. 1.1 on right) which is lower than the energy of the incident one $E_L$ (Fig. 1.1 on right) and so $E_S < E_L$. In this case we have an excitation of the molecule from its ground state $\nu = 0$ to the first excited state $\nu = 1$ of a vibrational mode with energy $\hbar\omega_v = E_L - E_S$.
In the other case, we have an Anti-Stokes process where the excited photon is more energetic than the incident one and so $E_S > E_L$ and it corresponds to a transition from an excited vibrational state $\nu = 1$ to its ground state $\nu = 0$. The energy of the vibration is then equal to $\hbar\omega_v = E_S - E_L$.
The dipolar moment of the scattering process is written as:

$$\boldsymbol{P} = \alpha \cdot \boldsymbol{E} \tag{1.1}$$

where $\boldsymbol{E}$ is the excitation Electric Field $[V/m]$, $\alpha$ is the polarizability $[C \cdot m^2/V]$ of the vibrational mode and $\boldsymbol{P}$ is the dipolar moment $[C \cdot m]$.
An important benefit of Raman is that measurements are label-free and no special sample preparation is needed. Typical Raman cross sections are between $10^{-31}$ and $10^{-29}$ cm$^2$ per molecule. Larger cross sections can occur only under favorable resonance Raman scattering conditions, i.e. when the excitation light matches the related electronic transition energy in the molecule. Resonance Raman cross sections are typically higher by 3-4 orders of magnitude, but the sensitivity of Raman spectroscopy is rather poor. Anyway, the discovery of a very high enhancement of Raman signal when the molecule is absorbed on rough metal surface, has allowed the investigation of very low concentration of analytes and it's the topic of this thesis. [1]
In particular, the study of a nano-structured metallic surface lets us analyze the local enhancement of electric fields near metal NPs.

### 1.2.1 SERS effect

Surface Enhanced Raman Spectroscopy (SERS) is a technique that overcomes the limits of Raman Spectroscopy itself, i.e. the fact that the signal is very low. In fact, SERS enhances the signal of a targeted molecule by several orders of magnitude when in proximity of a metallic surface at the nano-scale (metallic NPs of any shape and with size varying from few tens to few hundreds of nanometers).
At the interface between any two materials where the real part of the dielectric function changes sign (as at a metal/dielectric [1] interface), there are collective electron oscillations which are called surface plasmons. The total excitation, including both the charge motion and the associated electromagnetic field, is called either a surface plasmon polariton at a planar interface, or a localized surface plasmon (LSP) for the closed surface of a small particle [5].
The LSP has two important effects: electric fields near the particle's surface are greatly enhanced and the particle's scattering has a maximum at the plasmon resonance condition.
Usually the most used metals for SERS substrate fabrication are silver and gold. This is because in the visible and in the near-infrared (NIR) frequency range, a plasmon resonance condition can be achieved. The free electron gas density of a system can have a discrete number of oscillations, which are called plasmons. [2]
In the case of a flat surface, the interaction of light with the metal at the metal/dielectric interface will create the surface plasmons (Fig. 1.2, from [1]).

The surface wave associated with the oscillations (parallel to the metal surface) is called surface plasmon that can propagate along the metal interface.
In the case of a nano-structured metallic surface a localized surface plasmon (LSP) is present since the electron cloud oscillations are confined within the nanoparticles.
The resonance condition is attained when the frequency of excitation light is equal to the proper one of the electron oscillation and the light will "highly" interact with the NPs. [1]

The local surface plasmon resonance (LSPR) condition depends upon

[1] the dielectric is air

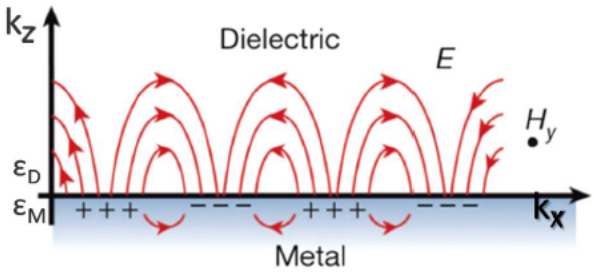


Figure 1.2: Electric Field lines related with the surface plasmons at the metal-dielectric interface. there's also the magnetic field Hy drawn in the y direction, perpendicular to the x-z plane.

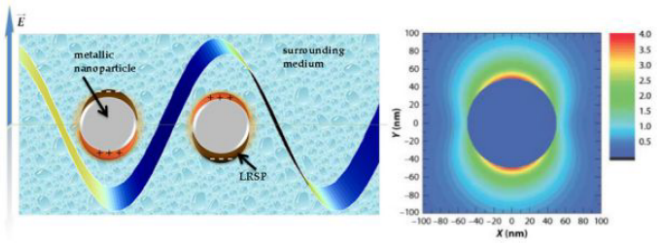


Figure 1.3: Left: schematic illustration of an incident plane wave interacting with a NP and its consequent charge displacement. Right: electric field intensity of a 100 nm silver sphere, irradiated with a 514 nm wavelength

some parameters:

- NP chemical nature
- NP size
- NP shape
- NP sorrounding media

As an example, Fig. 1.3 (from [1]) represents an incident plane wave and the NP charge displacement: it can be seen positive and negative charges are separated and the LSPR condition is achieved in the surface of the NP. On right it can be seen the Electric Field distribution in proximity of the NP

itself.
As stated before, silver and gold are the most used metals for fabrication of the SERS substrates, but in general silver reaches higher SERS response with an enhancement factor 10-100 fold greater than the one reached with gold NPs [1]. However in this work we use only gold substrates because of its properties of resistance to corrosion and oxidation [4].
A definitive and clear explanation to this phenomenon has not been given up to date, although mainly two processes contributing to the SERS have been identified: chemical and electromagnetic effect.

The chemical effect is the result of the interaction between molecule of the adsorbate and metallic surface: this interaction produces charge transfer between the molecule and metal (Fig. 1.4, from [5]).

The scattering takes place in the enhanced local optical field of the metallic nano-structures, through the excitation of localized surface plasmons. This is instead commonly known as electromagnetic effect.
In some way the main concept is that nanoparticles behave like "nano-amplifiers"(Fig. 1.5, from [5]), because they can greatly enhance the local field when an adsorbate is in proximity of a NP.

In summary, the electromagnetic field effect and the chemical one produce respectively an enhancement factor up to $10^{11}$ and of 10-100. The total enhancement factor is thus $10^{13}$.

## 1.3 Water contaminants

Water is an essential need for human beings and all the species on Earth: without it, we don't have life and nowadays is a theme of big importance since some people in the world don't have easy access to it, and not always the quality of that water is good, due to several sources of contamination.
Contamination of water happens when particles or chemical substances are introduced in water without any treatment to get rid of harmful chemicals like herbicides, heavy metals, pesticides or drugs. Contaminants are found in water mainly due to human activities.
Usually, when dealing with water pollution, we can speak about of surface water or underground water. Surface water includes oceans, lakes, rivers and the contamination has a negative impact mainly on aquatic flora and fauna.

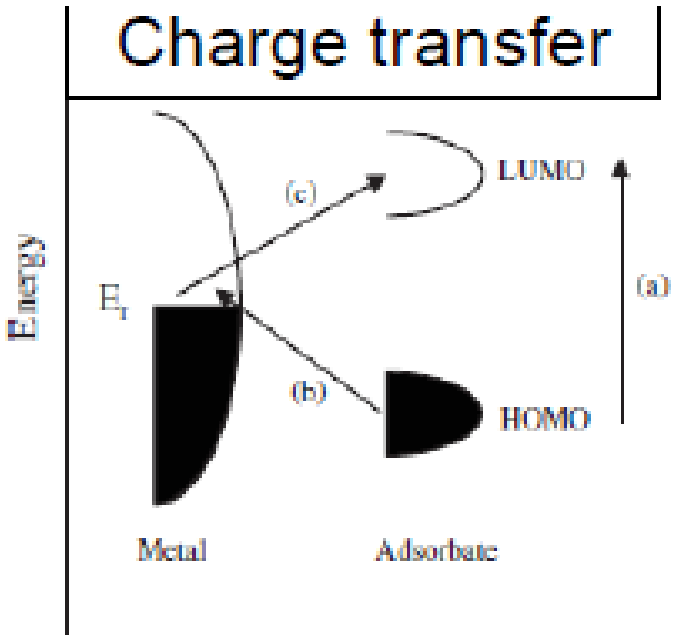


Figure 1.4: Charge transfer between the molecule of the adsorbate and the molecule of the metallic nanostructure

Underground water refers to aquifers, i.e. underground layers of rock fractures or unconsolidated materials like gravel, sand or silt. Aquifers feed rivers and provide drinkable water, so it's easy to understand how important is for human being to keep them uncontaminated. Herbicides and insecticides are the most commons.
For all these reasons, almost all governments have a strict regulation for water quality monitoring, and in general these rules are quite different in function of water: specific regularization for drinkable water, a specific one for agriculture water, etc...

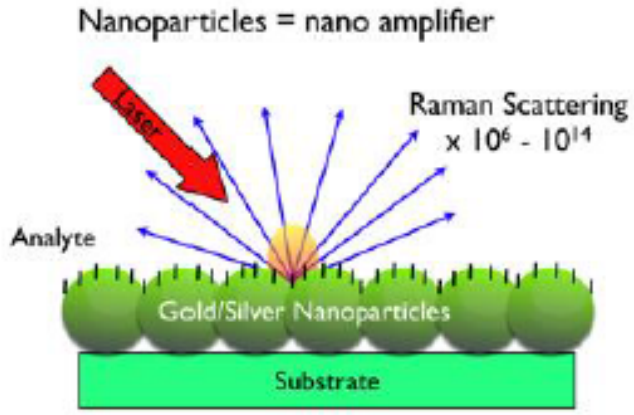


Figure 1.5: Concept of amplification of the field in proximity of the NPs

### 1.3.1 World Health Organization Guidelines

World Health Organization (WHO) "works worldwide to promote health, keep the world safe, and serve the vulnerable."[6] Its goal is to ensure health coverage, protection and well-being to "billion more people" [6].
The fourth edition of World Health Organization (WHO) Guidelines for drinking-water quality (GDWQ) during the year 2017, has formed an authoritative basis for the setting of national regulations and standards for water safety in support of public health.
Then, every government decides how to translate these guidelines into laws: the most important organisms for water laws are the Environmental Protection Agency from USA and the European Union.
Guidelines are a good starting point to ensure safety of water, with also a list of hazardous substances to be kept under control, based on scientific basis. These guidelines should be applied taking into account the context of the region or country in which they have to be translated into laws: there is not a universal approach.

According to WHO, there are three main aspects to take into account in order to ensure human health:

- Microbilogical aspects;
- Chemical aspects;

- Physical aspects.

For what concern microbiological aspects, they evaluate the presence of pathogenic microorganisms like bacteria or viruses in water, responsible of serious internal diseases or other types of infections. These contaminants can be introduced in water both directly and indirectly. The first case refers to pollutants introduced without any depuration system, whereas in the second case contaminants enter in the water through air or soil.
Chemical contaminants refer to all that substances produced by human activities reversed in the environment that cause unwanted effects on the organism; examples are non-degradable materials, heavy metals or hydrocarbons.
These are of major concerns since, when a contamination occurs, a purification process is needed and the source of contamination must be removed.
Physical aspects consist in the presence of radionuclides, i.e. unstable nuclides that emit energy (radiations) when decaying. The radiations are of alpha and beta type: they are naturally present in water, but high levels of them must be avoided.
Guidelines give an idea also about the odor, the smell and the taste of water since these three factors are really important for the human perception of safety when drinking water. It should appear colorless, without any smell or taste: any difference about this condition should be investigated.

### 1.3.2 Water Framework Directive

In the European Union (EU), the guidelines of WHO have been implemented with a directive, which again is simply a list of rules requiring member states to achieve a particular result without dictating the means of achieving that result.
On 23 October 2000, the "Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy" or, for short, the EU Water Framework Directive (WFD), was finally adopted.
This directive is quite general and it aims at the reduction of the pollution and at the protection of aquatic environments.
More specific legislation are related to the groundwater, which is the largest reservoir of freshwater in the world, accounting for over 97 % of all freshwater available on earth (excluding glaciers and ice caps). The remaining 3%

is composed mainly of surface water (lakes or rivers).
The first thing of these directives is connected to the monitoring of water quality, a key point to ensure that water is clean and free of contaminants dangerous for men. In these directives there is also how and how often you have to check the quality of water as well as how to perform this analysis.

## 1.4 PDMS

Polydimethylsiloxane (PDMS), also known as dimethylpolysiloxane or dimethicone, belongs to a group of compounds called polysiloxanes. It's the most widely used silicon-based organic polymer: PDMS is optically clear, inert, non-toxic and non-flammable.

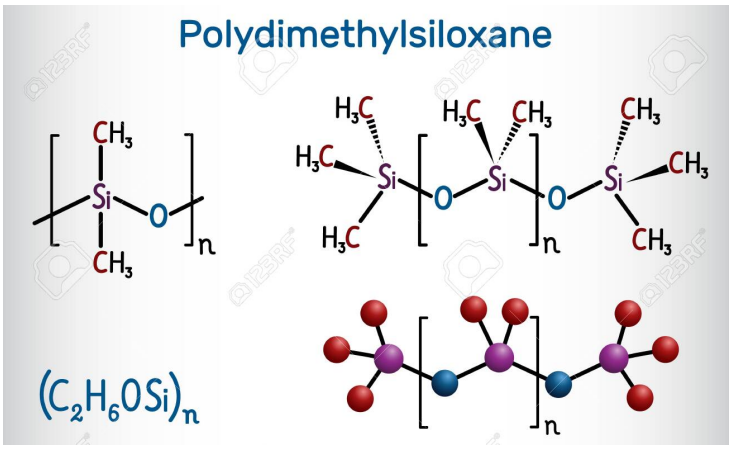


Figure 1.6: PDMS structure

As can be seen from Fig. 1.6, the polymer chain is formed by a simple monomer that repeats itself. The basis structure is formed by an oxygen atom (O) and a methyl group ($-CH_3$) bounded to a Silicon atom. There's covalent bond connecting the atoms between them, but sometimes there are dotted lines or bold lines. In the first case it means that the bonds "fall down" under the paper plane, while the bold line represents a covalent bond directed through the observer, i.e. out of the paper plane. With the normal line it's indicated a covalent bond on the same plane of the paper.
Properties of PDMS can change significantly depending on the polymeric

chain length.
PDMS is a good candidate for the fabrication and prototyping of a microfluidic chip, generally composed by a set of micro-channels etched or molded into a material (like PDMS) and connected together to obtain the desired features. The network of the micro-channels trapped into the microfluidic chip are connected to the outside by inputs and outputs pierced through the chip, as an interface between the micro and the macro world.
For the development of the project, we decided to fabricate simple microfluidic channels to deliver the water samples on a specific area of the SERS substrate. More complex microfluidic geometries were not needed, in order to allow the microfluidic to be used potentially in remote areas with a lack of complex instrumentation.

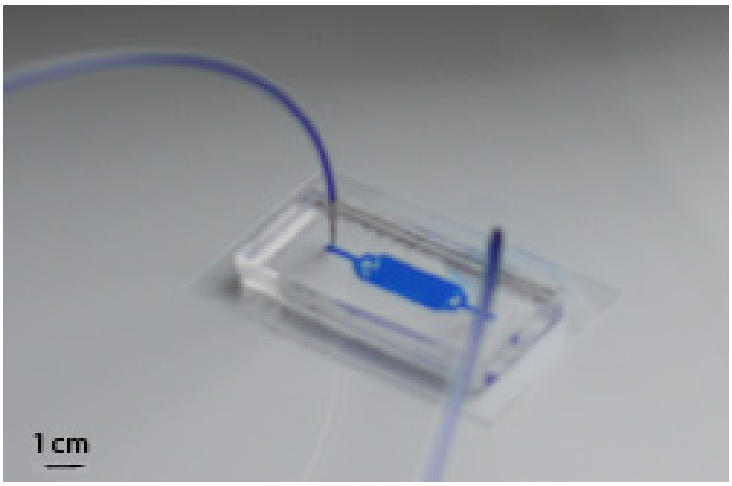


Figure 1.7: Example of a microfluidic chip made of PDMS/glass

# Chapter 2

# Substrate and Channel Fabrication

## 2.1 Glass Fabrication

The glass samples have been taken from microscope glass slides (AmScope). The slide has been cut in several pieces of around 2 $cm^2$ with a precision cutter (Fig. 2.1): revolutions per minute (rpm) of the wheel can be set before cutting.

## 2.2 Cleaning Procedure

### 2.2.1 Plasma cleaning

A plasma is a gas in which almost all the atoms or molecules has been ionized. It can be defined a sort of threshold of ionized atoms/molecules, above which charged particles will show some specific collective behavior: there's the generation of a self-consistent electric field (E), a collective response to time variations of an external E and there's conservation of charge neutrality inside macroscopic volumes, i.e. quasi-neutrality.
In general, plasma can be defined as a system containing several species, including (Fig. 2.2):

- neutral atoms and/or molecules;


Figure 2.1: Precision cutter used to fabricate the glass samples

- electrons;
- electromagnetic radiations (photons);
- ionized radicals;
- ionized atoms and/or molecules (positive/negative ions);
- neutral radicals.

Plasmas can be classified into hot and cold plasmas: the hot plasma is

characterized by an high ionization degree ($\alpha \approx 1$), which "refers to the proportion of neutral particles, such as those in a gas or aqueous solution, that are ionized to charged particles". [7] Here energy of electrons, ions and neutrals are almost equal, temperatures are really high (T $\approx 10^4 K$) and thermal equilibrium condition is almost reached.

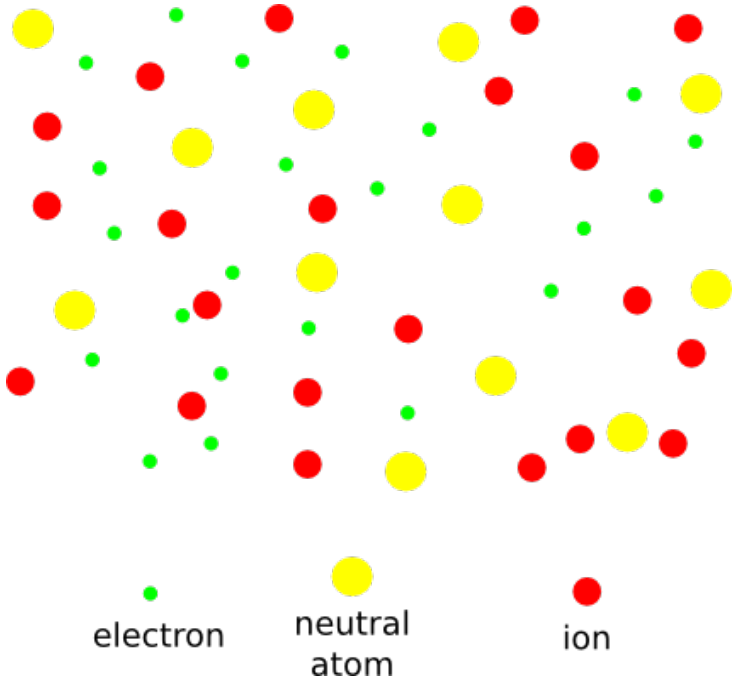


Figure 2.2: Composition of plasma, general

In cold plasmas instead a low ionization degree ($10^{-6} < \alpha < 10^{-4}$) is present, energies of electrons and of neutrals are much different ($E_{electrons} \approx 1eV$; $E_{neutrals} \approx 10meV$), the plasma is at low temperatures (T $\approx$ 300 K) and far from thermal equilibrium.
The second type of plasma is the one used to clean the glasses, simply by adding the low pressure condition.

For the experiments, a Low Pressure Table Top Plasma (Tucano) was used, shown in Fig. 2.3. This device allows to set some parameters by your choice and also to make a recipe re-usable each time you need.

This machine has two gas inlets and one gas outlet, where air flows away


Figure 2.3: Low Pressure Table Top Plasma (Tucano) used for experiments

when the plasma goes to low pressures.
It can be seen from Fig. 2.4 that the parameters are:

- Type of Gas
- Standard Cubic Centimeters per Minute (sccm)
- Direct Power
- Reflected Power

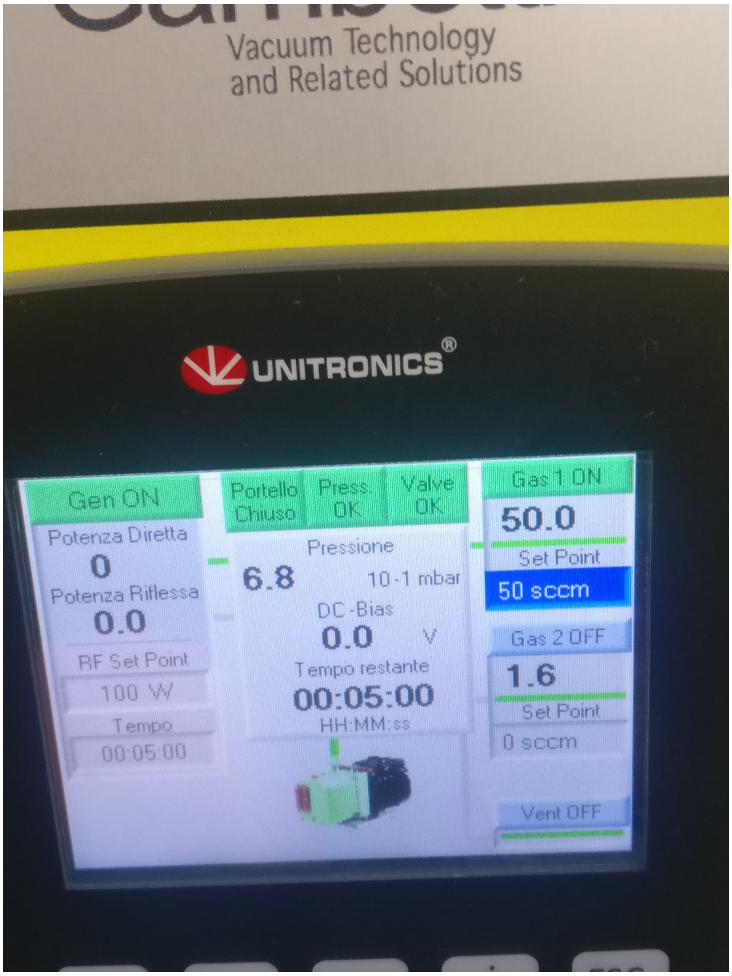


Figure 2.4: Setting parameters of plasma

- Time

For these types of cleaning, Oxygen is used (Fig. 2.5) because of the created ion species ($O_2^+, O_2^-, O_3, O, O^+, O^-$) capability to react with organic contaminants to form $H_2O$, CO and $CO_2$. These compounds have relatively high vapor pressures to be evacuated from the chamber during processing.

On top right of Fig. 2.4 there is "sccm", which is a flow measurement term $[cm^3/min]$ in standard conditions of Temperature and Pressure of the fluid (in this case the fluid is the plasma); it is a measure of mass flow rate,

Figure 2.5: Oxygen bottle

not to be confused with a measure of volumetric flow rate: one "sccm" indicates the mass flow rate of one cubic centimeter per minute of a fluid (in this case the gas of plasma).
This parameter is very important, since the chamber pressure is regulated by this mass flow rate: the two elements are proportional.
Then on top at left (Fig. 2.4) there are others two parameters, direct and reflected power. Usually for cleaning purposes, 50 W as direct power is enough, whereas the reflected one must be regulated by the operator during the treatment: in Fig. 2.3 there are two little handles above the chamber (load and

tune). These twos change internal resistances and modulate the reflected power: operator must avoid to have too much reflected power, otherwise the process would be useless. In fact, the reflected power can be thought as dissipated power.
The last parameter is the time, i.e. the time of the process: it cannot be more than 10 minutes.


Figure 2.6: Pump of the Plasma System

The instrument is turned on $\approx$ 15 minutes after the pump (Fig. 2.6), which should warm-up and stabilize. Then venting [1] is needed to put the

[1] Venting is intended as the procedure to take the instrument at ambient pressure

samples in the chamber, and at that point all the parameters mentioned before can be set as desired.

### 2.2.2 Piranha

If the plasma cleaning is not available, piranha cleaning is a good alternative. Piranha is a chemical way to remove trace amounts of organic residues from substrates.
Typically, there are two different piranha solutions used in laboratory:

- Acid piranha is a 3:1 mixture of concentrated sulfuric acid ($H_2SO_4$) with hydrogen peroxide ($H_2O_2$). It's a self-starting solution, i.e. it reacts with organics without being heated
- Basic piranha is a 3:1 mixture of ammonium hydroxide ($NH_4OH$) with hydrogen peroxide ($H_2O_2$). The base piranha must be heated up to 60 °C in order to start the reaction.

The procedure chosen in this thesis is the basic piranha, with a slightly different mixture ratio (5:1) in order to prevent dangerous situations, in fact the reaction can accelerate out of control if the piranha is "fed" with organic compounds: enormous quantities of gas and heat can be generated with sufficient "fuel" for it.
Piranha solution is useful because it will also hydroxylate most surfaces, i.e. it will add -OH groups, making them more hydrophilic (Fig. 2.7 from [15]).

A pre-cleaning procedure is made on the samples, with three baths (1 of acetone and 2 of ethanol) and subsequently stored in a falcon with MilliQ water [2].
The base piranha is formed by the subsequent reagents:

- MilliQ water
- Ammonium Hydroxide
- Hydrogen Peroxide

[2]MilliQ water is purifed and deionized water with a purification system realized by Millipore Corporation

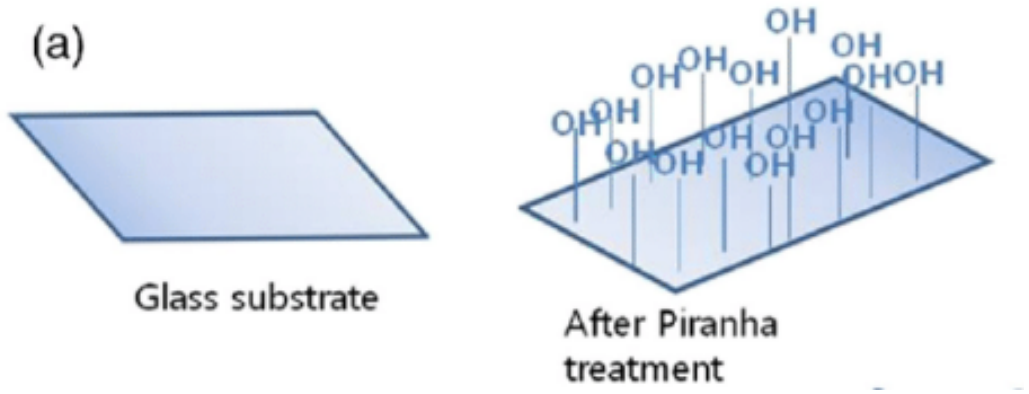


Figure 2.7: Hydroxylation of a glass surface

in a ratio 7:1:5. It is brought to 60 ° C, i.e. at the temperature at which the reaction takes off. Usually 25 mL of solution are used and taken in a glass bottle for about 30 minutes with the samples in (Fig. 2.8). Since 25 mL was the total volume of the solution, it has been used 9.62 mL of $H_2O_2$, 13.46 mL of MilliQ and 1.92 mL of $HH_4OH$.
Notice to take care of putting $H_2O_2$ as last reagent due to the high reactivity; both the acid and the basic piranhas are dangerous when hot, even if the reaction in the acid piranha is self-starting, while the basic piranha is "not active" below 60 ° C.

## 2.3 Functionalization and Seed Deposition

The functionalization technique has the objective to put a chemical agent able to bind to glass on one side and to gold seeds on the other. The idea is to use Self-Assembled Monolayer (SAM) procedure, which refers to single layers of molecules that assemble themselves in an ordered way onto a solid surface. This is achieved since the reaction is self-limited, i.e. it lasts until all the single layer is not formed, so it has the features to be very uniform and controllable.
According to the work of Karl Meissner [8], the point is to choose the aminosilane that most suite the need: in this case, (3-Aminopropyl)trimethoxysilane (APTMS) is a good candidate since it guarantees very high density of gold

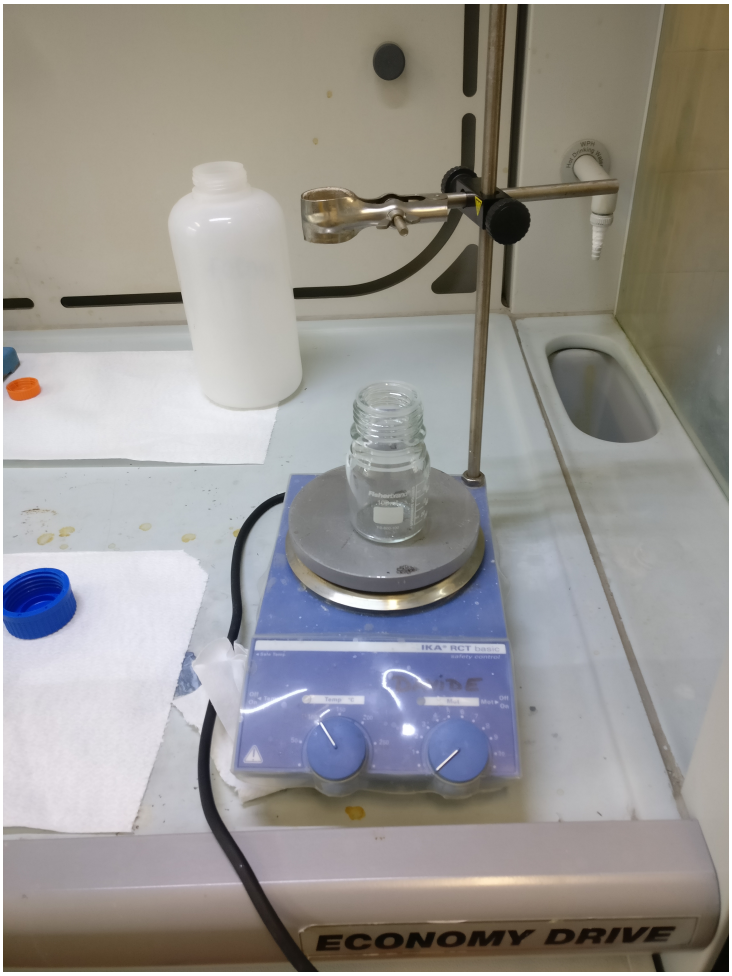


Figure 2.8: Bottle and Hot Plate used for the Base Piranha

seeds attached.

As can be seen from Fig. 2.9, it's composed by a Silicon atom, 3 oxygen atoms, 3 methyl groups and 1 amine group. The APTMS is thus chosen since the Si will bind to the glass forming a Si-O-Si bond, while the amine group will bind to gold. Notice that this technique requires a perfect cleaning procedure to avoid holes after gold deposition and also to have a uniform APTMS layer after deposition.
APTMS solution is thus prepared following the work of Messner [8]: 2.5%

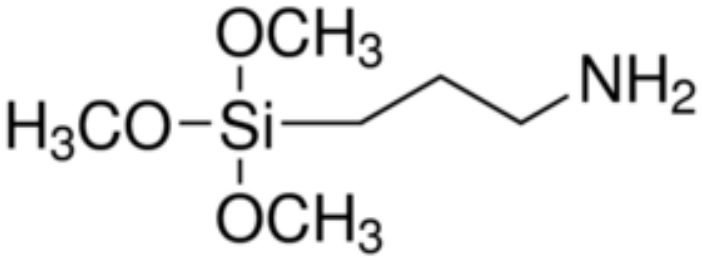


Figure 2.9: (3-Aminopropyl)trimethoxysilane (APTMS) structure

in weight of APTMS and 97.5 % in weight of ethanol for the total solution. 4g of solution for each sample are used, 2.5 % of the total solution to obtain 0.1 g of APTMS needed for this experiment. 10 glasses usually are done, so the solution is of (4x10)g = 40g composed by (0.1x10)g = 1g of APTMS and 39g of ethanol.
Notice that APTMS is put on the falcon under chemical hood (medium) to avoid that APTMS goes in contact with humidity, because this could degrade the chemical.
After that, the solution is divided one for each falcon, where subsequently the glasses are introduced.
When the cleaning procedure is ended, glasses are further cleaned in two bath of MilliQ: these are fundamental to make -OH groups to which Au nanoparticles will bind. After that, they're dried under $N_2$ flow, paying attention not to put glasses in contact with whatever surface. When dried, glasses are put in solution with the 10 falcons containing APTMS and ethanol and at rest for about 2h.
After 2 hours, another cleaning procedure is required to remove the excess APTMS from glass surface: a bath in ethanol for 5 minutes, and a further one, again in ethanol. Then another drying step is required, but this time is faster since the ethanol evaporates more easily.
After the functionalization, there's the step of seed deposition: stabilized suspension of gold nanoparticles ( 10 nm diameter) in citrate buffer from Sigma Aldrich. The solution must be kept in the fridge and shows a weak red, the typical color of the NPs of this dimension. Due to the high costs, only 3 mL for each falcon has been used for the samples. In fact the most important thing is that the glass samples are totally covered by the AuNP solution, with 3 mL this requirement is satisfied.

Typically the samples are immersed in the solution overnight.
The following day, 3 baths are needed (1 in ethanol and 2 with MilliQ) and samples should be no more transparent but showing a weak pink, meaning that seeds have successfully attached to APTMS.

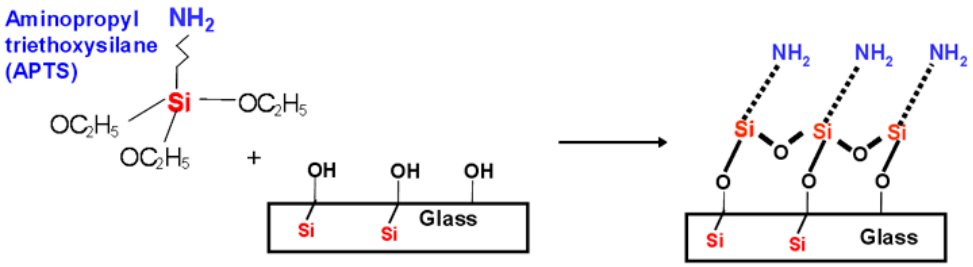


Figure 2.10: Schematic of APTMS absorption on glass

Fig. 2.10 from [9] shows the two steps, i.e. the functionalization (left), where the -OH groups are on top of the surface after the cleaning procedure and the MilliQ baths. On the right of the same figure, it can be seen the situation after 2h in the APTMS solution: the amine groups then will be on the top and they will attach to the Au NPs during the seed deposition step.

## 2.4 Au spherical nanoparticles (NPs) growth

After the night, the Au NPs growth procedure can start. First thing to do is to prepare a solution 0.28 mM of $HAuCl_4 \cdot 3H_2O$. A stock solution of $HAuCl_4 \cdot 3H_2O$ 0.01 M (10 mM), was used and diluted with the correct quantity of water.
The goal is to have 5 mL for each falcon:

$$0.28mM : 10mM = x : 5mL \tag{2.1}$$

From here x = 0.14 mL, which is the quantity of the solution of $HAuCl_4 \cdot 3H_2O$ 0.01 M to be taken. In order to obtain 0.28 mM of this solution, this must be diluted with MilliQ water. The correct quantity is 5 mL - 0.14 mL = 4.86 mL.

In this way, diluting 0.14 mL of $HAuCl_4 \cdot 3H_2O$ 0.01 M with 4.86 mL of purified water (MilliQ), it has been obtained $HAuCl_4 \cdot 3H_2O$ 0.28 mM.
After that, 2 baths in ethanol and one in MilliQ are done, then 1.75 % of the solution (which is 0.0875 mL = 87 $\mu L$) must be added with hydrogen peroxide $H_2O_2$ and then vigorous magnetic stirring for 3.5 min.
The correct thing to do is to put $HAuCl_4 \cdot 3H_2O$ and water, mixing for 30 seconds the solution under vigorous magnetic stirring, and only after that, adding the glass and the hydrogen peroxide: in this way, possible scratching of the glass (in the first 30 seconds) are prevented.
This procedure is usually done for 3.5,4,4.5,5,5.5 min with two samples and two falcons at the same time under stirring.
Then the samples undergo 2 baths of ethanol, 1 bath of MilliQ and then they're stored in a clean falcon.
With this procedure spherical nanoparticles should grow, it means that they keep the original shape of the seeds, but increasing in volume. This is an isotropic growth, i.e. the growth is the same in all directions.
The reaction taking place during this procedure is a reduction of Gold(III) of the $HAuCl_4 \cdot 3H_2O$ solution to Gold (O), which then will deposit on seed surface. The reduction happens thanks to the reducing agent $H_2O_2$; $HAuCl_4 \cdot 3H_2O$ acts as oxidizing agent, whereas gold seeds act as catalyst.
Gold seeds will speed up the reaction (this is the meaning of being catalysts) and the new formed Gold(O) will deposit on seeds.
It's important to remember that hydrogen peroxide must be added when the glass sample is already in the solution, since $H_2O_2$ is the responsible of the reduction reaction.
After this treatment, samples are washed with ethanol, MilliQ and dried under chemical hood (medium).
As reaction time goes on, nanospheres will grow bigger and this is more or less demonstrated, even if this factor is influenced also by the gold seeds present at the beginning of the procedure.
A first idea is given by the color of the sample itself, which remains weak pink if the nanostructure is not grown correctly. Other proofs can be done, like UV-Vis Spectroscopy [10].

## 2.5 PDMS Casting

PDMS casting is the next step, when the functionalized substrate (the sensing part of the device) is ready. The contaminated water should be analyzed, and a basic microfluidic channel is needed for the purpose, achieved thanks to the use of polydimethylsiloxane.
PDMS casting is made with the use of Sylgard 184 from Sigma Aldrich and a cross-linker or curing agent (accelerant) bought from "Farnell". The first thing done is to print the mold in Polylactic acid or polylactide (PLA) as in Fig. 2.11 since from Finland there are samples of Silicon of 100 mm as diameter. Each hole has a different diameter:

- 1.5 mm;
- 2 mm;
- 3 mm;
- 4 mm;
- 5 mm.

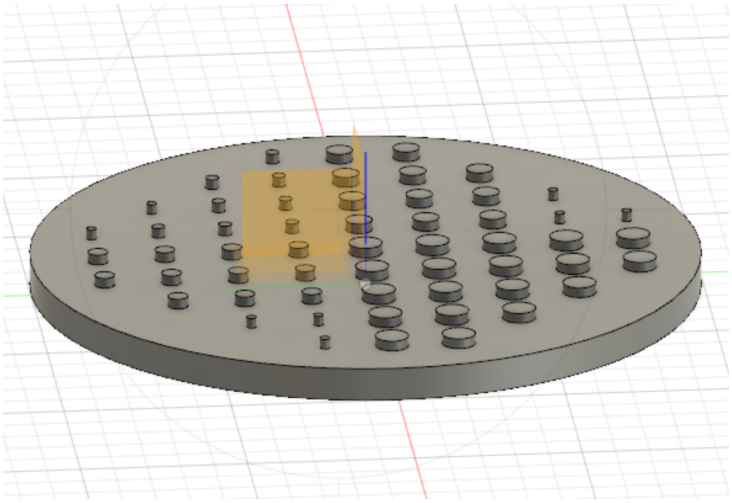

Figure 2.11: 3D printed mold

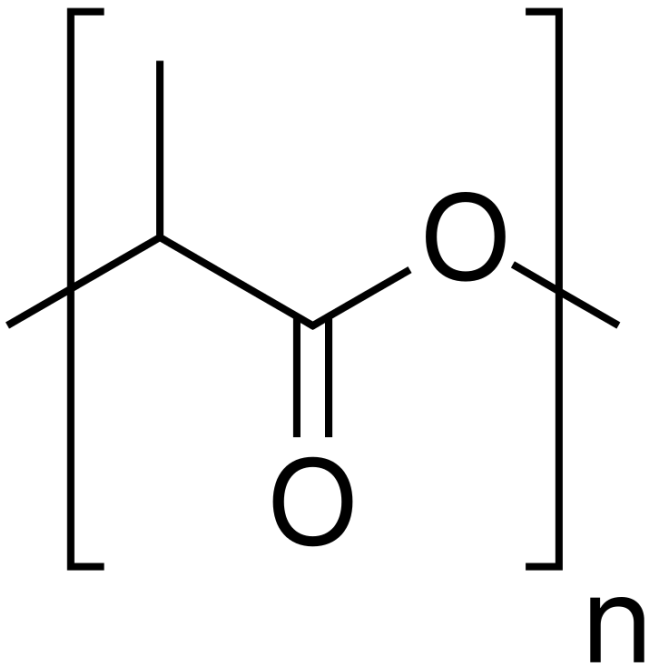


Figure 2.12: PLA structure

PLA is a thermoplastic polyester, thus it has the features of being a material moldable at certain elevated temperatures, it solidifies upon cooling and it contains the ester functional group [3] in its main chain, as shown in Fig. 2.12. Then the solution made of Sylgard 184 and the cross-linker is prepared: 19g of Sylgard 184 are mixed with 1.9g of the cross-linker in a 10:1 ratio.
A vigorous mix of the solution with a metal spoon in a styrene cup is done, paying attention not to make the solution attaching to the styrene cup or to the spoon.
After that there's the de-bubbling, because at normal atmosphere there are air bubbles forming in the solution and it's obviously unwanted: the de-bubbling is made with a vacuum pump which can reach 15 mbar (Fig. 2.13). The styrene cup is inserted in a vacuum chamber and the machine is turned-

[3]Ester is a chemical compound in which at least one –OH (hydroxyl) group is replaced by an –O–alkyl (alkoxy) group

on to make air flow out and consequently push down the pressure. It's visible at naked eye that the bubbles come at the surface of the solution, which must not go out of the cup. This is prevented by regulating the air through a sort of "tap" by which the user can control the air flowing in and out of the structure.
It's not a precise way to control the pressure inside the chamber, but precision is not needed: the only need is to prevent bubbles go out of the styrene cup and this is greatly achieved.

Figure 2.13: Debubbling of the mix in the styrene cup

After that, when no more bubbles are present at the top of the solution, the pump is stopped and atmospheric pressure is again reached. The styrene cup now can be taken and poured onto the PLA cast (Fig. 2.11 and Fig. 2.14), be careful not to cover the surface of the little cylinders. It's better also to incline the PLA structure in order to make the viscous fluid flows better in the empty spaces.

PDMS solidifies in about 2h and then the de-molding step can be done. Unfortunately this method doesn't work, PLA was more lucid and PDMS seemed to be disappeared: maybe the PDMS penetrates into the PLA since PLA is not too resistant. So a method based on lacquer spray is tried, which

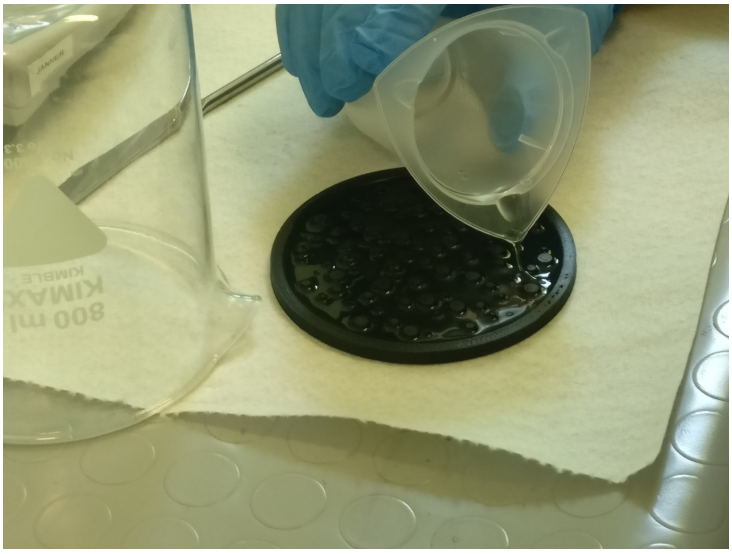

Figure 2.14: Pouring of PDMS into the PLA structure

is an acrylic paint (Fig. 2.15) that can prevent this phenomenon to happen.

The trials were made onto little samples in order not to use too much PLA. The procedure was to spray one time, then cook the sample on a hot plate at 85-90 ° C for 5 minutes: subsequently spray again the lacquer and then cooking again on the hot plate at same conditions. For the last spray, tilt of the spray angle was an issue in order to cover all the parts that maybe were not covered in the previous 2 steps: the last cooking is made and so the solution of Sylgard 184 and cross-linker can be prepared again.

Here the procedure is the same as described before, with the vacuum chamber and the casting.
Three little samples are studied, one of them cooking on a hot plate (with an Aluminum foil to improve contact) for 1h at 85-90 ° C (Fig. 2.17), whereas the others 2 are left to harden, with a baker on them, at room T. Only the one on the hot plate hardened, whereas the others 2 do not present the same problem as before of penetrating into the PLA (Fig. 2.16), but they show high difficulty to solidify.
Another problem founded here is the fact that, due to this cooking at $\approx$ 90 °C for 5 minutes (before the pouring of PDMS), the surface of PLA was


Figure 2.15: Acrylic paint

deformed on the edges and this was another issue to be solved, because it's desirable to have a perfectly flat base to work with.

Little changes on the procedure are done, in terms of hot plate and also type of the used paint. Different little samples with different height (2 mm, 4 mm, 6 mm) are prepared and tested with the same acrylic paint in comparison with another, which is specified to be suited for plastic (Fig. 2.18).

The procedure is always the same, but with little changes: when spray the lacquers onto the PLA samples, it's absolutely avoided to cook on the

Figure 2.16: Two of the three little samples used just as try

hot plate.
Then the PDMS is prepared and poured on the PLA samples: 3 samples are covered with the acrylic paint and 3 with the new spray; all the 3 different heights are analyzed (2 mm, 4 mm, 6 mm).
At this point the hot plate is used, but at 40 ° C in order not to have PLA deformations and preventing unwanted reactions between the paint and PLA or even PDMS.
Cooking for 2h at 40 ° C and then de-molding: unfortunately, the PDMS with the paint suited for plastic (the white one) was difficult to remove and full of scratches, so the first lacquer (at lower T with respect to the first trials) is better, because the PDMS is easily removed and the samples are not too much scratched.
The point is that they're a bit contaminated and so there's the need to clean them: a bath in acetone (5 min), IPA bath (also 5 min) and then put them in water. After that they're put in the petri to dry.

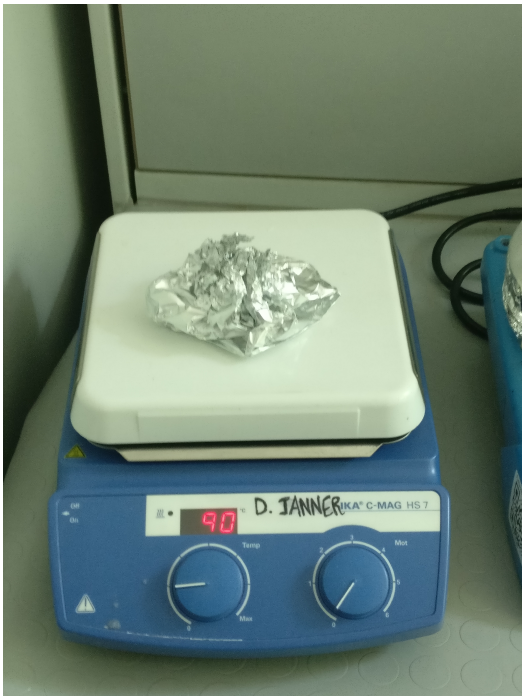


Figure 2.17: Sample closed in an Aluminum foil to improve the contact at 90 C

### 2.5.1 Basic Microfluidic Channel

The trials to obtain a little microfluidic channel were made at Universitat of Barcelona, where there were the possibilities to produce Silicon masks to use as master mold for PDMS; since the process is not very cheap, the first trials are made trying to avoid this technique.
First of all it has been started with PARAFILM, making a little hole of 5 mm diameter (Fig. 2.19) and the matter was how to stick it on the glass. It was used an hot plate at 85 ° C for 15 minutes with a piece of TEFLON above it in order to stick it, but it was too much time and also too much weight since PARAFILM attached to the Teflon and also to the glass, but easily removed, so this technique is abandoned.

Notice also that 60 ° C is the PARAFILM melting temperature $T_m$, so this is another issue: it's said that with the hot plate at 60 ° C with a pressure of 200 psi for only 1 minute, maybe the desired result can be reached,


Figure 2.18: Spray Paint suited for plastic

but it doesn't worth the risk.
In Barcelona was analyzed the problem of the objective that, during Raman measurements, can touch the device, i.e. the glass sample that will be contaminated.
In order to test if this problem of the objective touching the sensor can be solved, a little droplet (100 $\mu L$) of a solution of nanoparticles (1.7 micrometers of diameter, 10 $\mu L$ of an already prepared solution of suspended nanoparticles) in 10 mL of water and put it on the hole and another problem of PARAFILM arises: water goes underneath it and it's also absorbed or, in

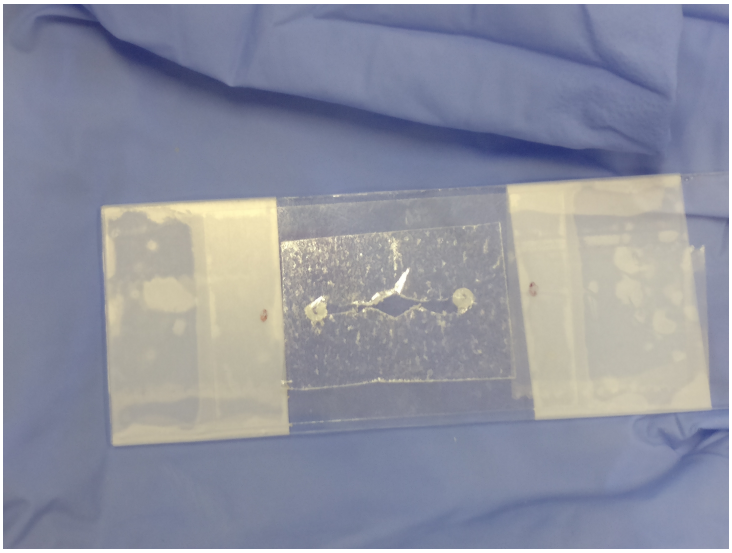

Figure 2.19: First trial with PARAFILM

the worst cases, the droplets don't fill the cavity of the channel and remain still above the glass (Fig. 2.20), so this is another reason to forget about this technique.

A cover glass is an alternative to the PARAFILM, two holes are cut for inlet and outlet. The cover glass is thick 100-120 $\mu m$ so must be handled with care, and cut with a manual driller (Fig. 2.21, usually accompanied by an oil in order to grease, Fig. 2.22).

20 rpm are used paying attention not to scratch the sample by putting another piece of glass below it as structure holder. In order to make the simple microfluidic channel, a tape very thin (25 micrometers) is used with a central pool in between the inlet and the outlet. Again lots of trial are made due to the fragility of the cover glass, and also a more compact structure is done with smaller pool, because the sample glasses are not too big (1 $cm^2$ or max 2 $cm^2$).

The second trial was done using the laser cutter. It's an EPILOG laser cutter (Fig. 2.23), working at the 30 % of the power, in the InfraRED. It's a $CO_2$ laser. It was used to make the holes in a more precise way and of the correct diameter. They are distant 14 mm and have a diameter of 1 mm, in

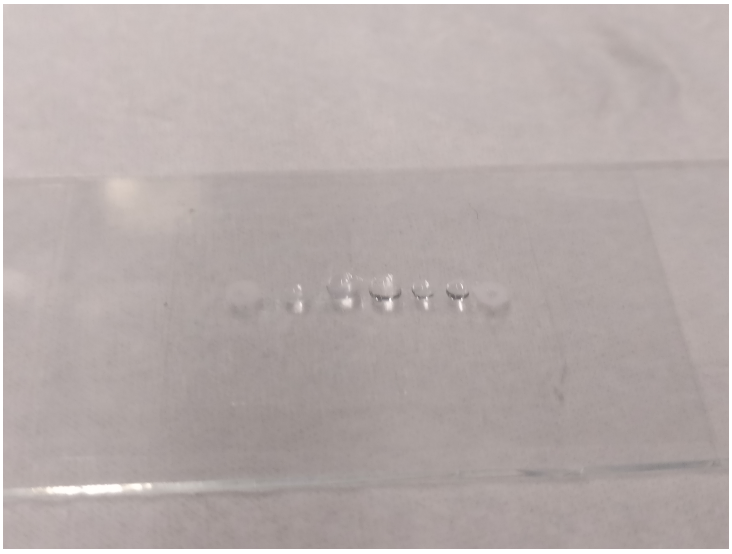

Figure 2.20: The cavity of the channel is not suited for the purpose

principle the same diameter achievable by the manual driller, but in theory more precise since there aren't lateral movements when working with that.

It's said in theory because in reality they suffer of the same problem since the glasses are not perfectly straight (differently from the support), and so it can happen that they can be accidentally moved when they're in the machine.
The working principle of the laser cutter is to overheat some regions, the final pattern to be cut. Since the glasses are very thin (ranging from 100 to 120 $\mu$m), it's not a good idea to improve too much the laser power, otherwise there's the possibility of scratching; the advice is to be patient, and repeat the process the times it needs to obtain the desired holes.
In this case repetitions were 19 for each glass: 6 glasses in the machine (Fig. 2.24), and usually one process lasts less than 2 minutes, so it's understand is not a slow process.

When the drills are done it was tried to put the tape on the glass, but unfortunately the method of cover glass doesn't work, since the tape is too thin and also the drills again are not perfectly done, so the little drop of water remains over the cover and does not fill it, unless the cut is perfect: in

Figure 2.21: Manual driller used at Unviersitat of Barcelona

one case we achieve it and it works, so it's only a matter of avoiding lateral movements, both in the manual driller work and in the laser cutter.
At the end also this method was abandoned. As already said, in Barcelona there is the possibility to have Silicon masks like the ones in Fig. 2.25, the first and the last one maybe can be used for this thesis purpose. The PDMS is prepared using 30g of Sylgard 184, and 3 g of curing agent in a 10:1 ratio. Then these twos are mixed and put in a vacuum pump to make the so called "de-bubbling" and wait until the bubbles are completely removed. At the end pouring of PDMS on the already prepared Silicon mask and putting it


Figure 2.22: Oil used with the manual driller

in the oven in a Temperature range of 50-60 ° C overnight.

After the night, the poured PDMS is put again in the vacuum pump to remove the residual bubbles. A little glass is cleaned with de-ionized and purified water and dried with a Nitrogen gun. The glass is then placed on an hot plate at ≈ 50 ° C. PDMS is "engraved" on one side and on the other is not, so the part with the pattern must be put in contact with the glass (Fig. 2.26). Since this procedure is made on the hot plate and since the PDMS sticks very quickly on the glass (but fortunately not too strong), press onto

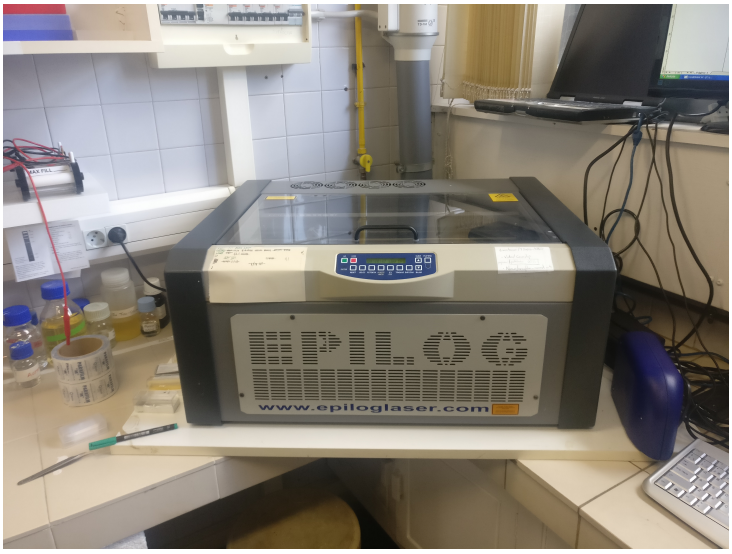


Figure 2.23: Epilog Laser Cutter used in Barcelona

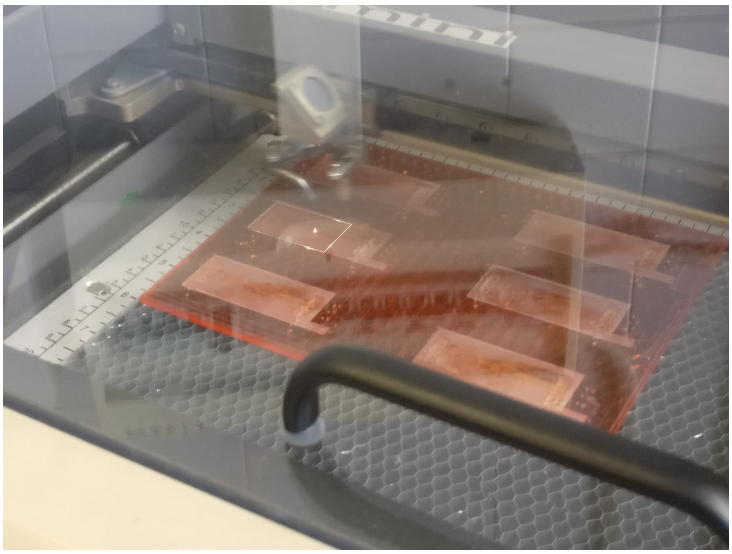

Figure 2.24: Laser Cutter while working

the PDMS sample is required, to make sure no air bubbles are inside it.

At this point the photo-curable glue is put in between the PDMS and the

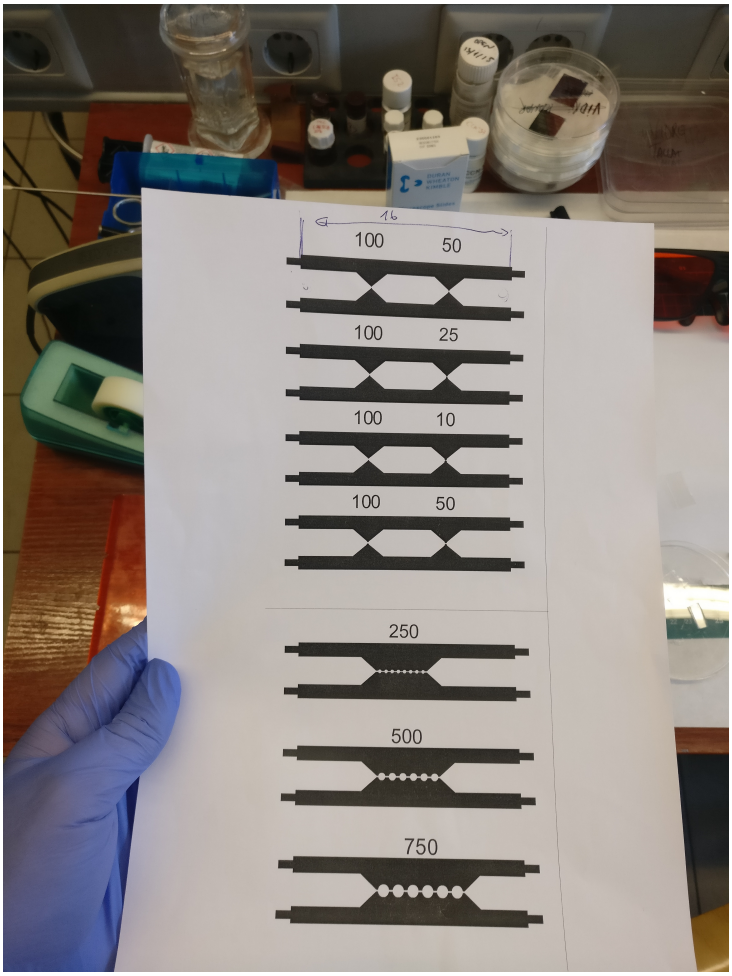


Figure 2.25: Schematic of Silicon Masks

glass, and it fills the "engraved" parts of the PDMS (Fig. 2.27). Then a UV cure is done for 7 seconds at the 70 % of the intensity: with this procedure, the glue will be solidified and replicates the pattern.

Then there's the critical part: the PDMS must be gently removed and then put a clean glass over the bottom glass, making sure it will be attached to it. To do that, another cure at the 70 % of the intensity is done for again 7 seconds.
The last step is to fill the sides of the glass with the glue: this is done to


Figure 2.26: PDMS on the hot plate

prevent breaking of the device. After the last cure, the channel is ready to be filled with contaminated water (Fig. 2.28).

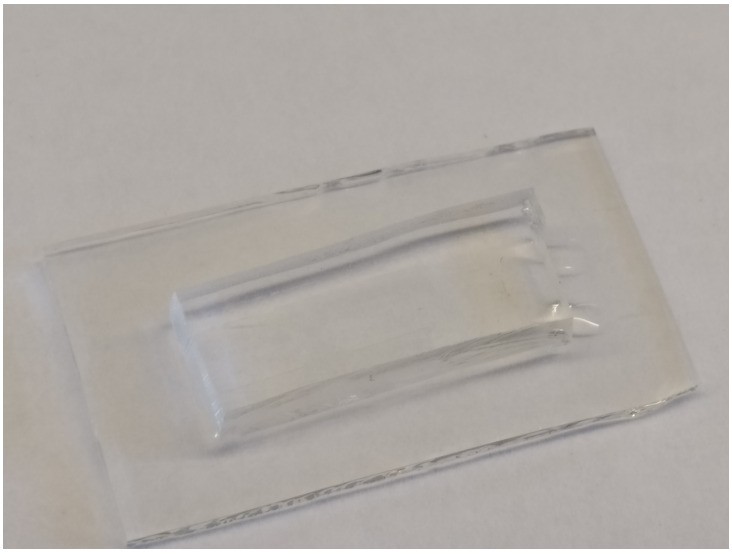

Figure 2.27: Glue is inserted from one side and it fills the "engraved" parts by capillarity

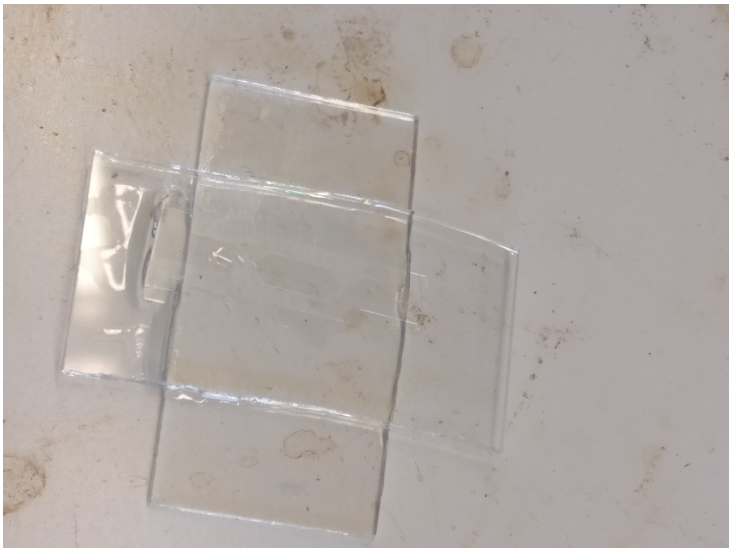

Figure 2.28: Final basic channel ready to be filled

# Chapter 3

# Raman Analysis

## 3.1 Raman Instrumentation

The analyses presented in this chapter are made with the inVia micro-Raman microscope by Renishaw (Fig. 3.1).


Figure 3.1: InVia micro-Raman Microscope by Renishaw

The objectives used in the analysis are the 5X, 20X and the 50X, available in the micro-Raman Microscope (Fig. 3.2).
The micro-Raman instrument consists of:


Figure 3.2: Raman Objectives used for analysis: 5X, 20X and 50X

- A monochromatic light source (a laser);
- The microscope, responsible of shining light on the sample and collecting the scattered one;
- Notch filters to filter out all the light (Rayleigh scattering) except for the tiny fraction that has been Raman scattered;
- A diffraction grating able to split the scattered light into components wavelengths, i.e. a spectrum;
- A CCD camera, a light-sensitive device able to detect Raman scattered light;
- A computer able to control the instrument, the motors and analyze and store the data.

A little drop of contaminated water is put on the glass sample ($\approx 20\mu L$) and let it dried as shown in Fig. 3.3. As can be seen, two microscope slides keep the sample (which is inside the petri) covered by dust that can accidentally drop on the glass itself, in fact it was not possible to work in a clean room for these measurements.
The other reason to use this dry procedure is to make water drying not too

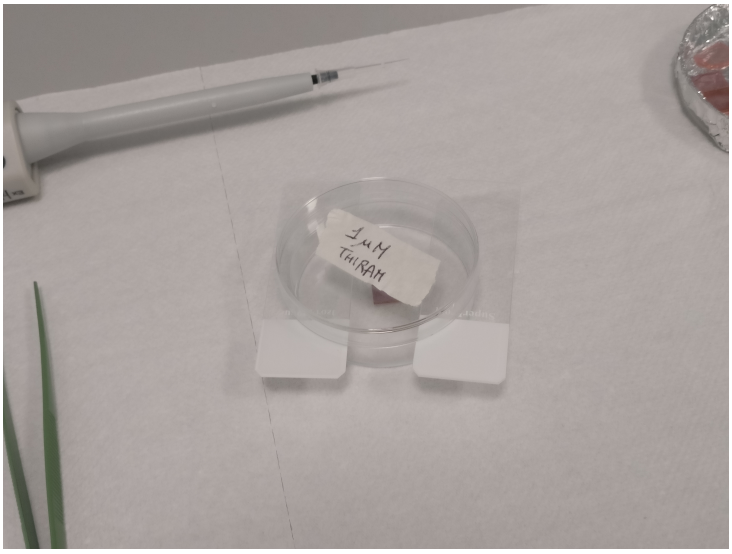


Figure 3.3: Sample dry procedure

fast: in this way, the probability for the contaminant (overall when in low concentration) to attach to the substrate is enhanced. In fact it was also explored at first pouring of contaminated ethanol, which in reality evaporates (and so dries) very fast: this can lead to low probability for the contaminant to attach to the functionalized substrate.
In this fashion, it is important to keep in mind the so called "coffee ring effect": it indicates the pattern left by a particle-laden liquid drop after evaporation, similar to what happens with the coffee left in the cup after drinking it. In this case it's important because in the proximity of the end points of the droplet, we know that there will be an higher probability for the contaminant to attach to the glass substrate, because of this effect.

## 3.2 Analysis technique

In order to make measurements, user must be aware of few preliminary things that should be done each time he turns on the instrument. It's not really an issue, but it's an advice to turn on at first the instrument, then the computer with the Wire Software and only after that, the lasers that will be used in the measurement procedure. Usually 15-20 minutes are waited before

starting, in order for the lasers to warm up and stabilize and also because the CCD camera will take approximately that time to cool to its operating temperature.
After that, there's the calibration step to be done: this is very important, because Raman instrument will acquire the spectrum of Silicon and will take it as a standard reference for all the next acquirement; without this step, unwanted shifted Raman spectra may be acquired.
User places the Silicon reference standard sample on stage (Fig. 3.4) and sets the instrument to optical mode by rotating the corresponding wheels (Fig. 3.5 and 3.6).

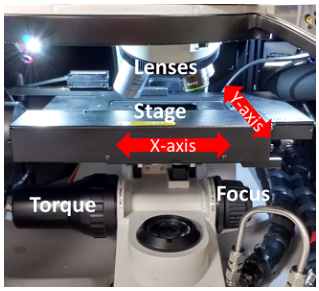


Figure 3.4: Composition of the Raman Microscope

The objective 5X is now moved into position above the Silicon reference sample and, with the help of the torque and of the focus (Fig. 3.4), the reference is put on focus. Then the objective turret is rotated to the 20X position and focus on the sample. Same procedure for the last objective, the 50X, paying attention to do all these steps SLOWLY so as not to crash the objective onto the sample, otherwise the objective will be damaged.
On the Sample Review window (Fig. 3.7) user should select the correct laser: with this instrument, three types of lasers can be selected. They're:

- Green laser, wavelength 532 nm;
- Red laser, wavelength 633 nm;
- NIR laser, wavelength 785 nm.

When the proper laser is selected, laser shutter can be opened (Fig. 3.7, on left) with the pinhole IN or OUT. Laser will illuminate the sample with a little hole (point) in the first case, whereas with the other setting the sample


Figure 3.5: Wheel to be rotated in order to set the instrument in Laser (4) mode or Optical (1) mode

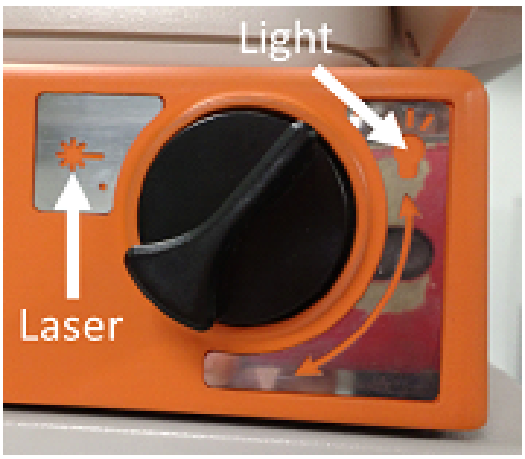


Figure 3.6: Further Protection for the user: without also this setting, Raman laser does not work

is illuminated by a stripe: it can be chosen for the different applications needed.
For the calibration purpose is not important, because it is a standard process done by the instrument, but it matters just to put also the laser on focus, since the optical focus plane is slightly different from the laser focus plane

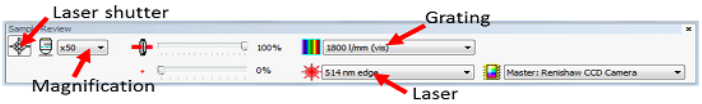


Figure 3.7: Sample Review window of the Wire Software present in the computer of the micro-Raman instrument

(just few $\mu m$).
In the sample review window is also important to check that the selected objective is the one that is effectively pointing at the sample (looking at Fig. 3.7), and also that the correct diffraction grating is selected. Three different diffraction gratings can be selected:

- 2400 l/mm;
- 1800 l/mm;
- 1200 l/mm.

where l/mm stands for "lines per millimeter" and they're the diffraction grating lines per unit of length (mm in this case). The first and the second ones are suited for green laser, the second also for the red one, whereas the third grating line is suited only for the 785 nm laser.
At this point there's the command "Quick Calibration" and the instrument will return back a spectrum, which should be something like Fig. 3.8, with an high peak at 520 cm $^{-1}$:

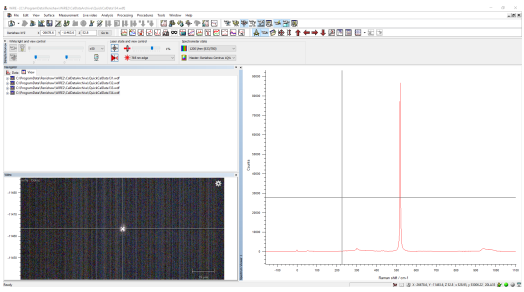

Figure 3.8: Silicon Spectrum, pinhole IN

In this figure, it can also be seen on left the pinhole IN, it means that measurement was done with the laser pointing at the reference Silicon sample

with a little hole.
If the Silicon spectrum is not similar to something like Fig. 3.8, a new calibration should be done and maybe also the calibration offset should be corrected. In fact it can happen that the higher peak is not perfectly pointed at 520 cm $^{-1}$ like it should be, so setting a positive/negative offset should correct the calibration. If the peak is centered at values greater than 520 cm $^{-1}$ (f.e. 523 cm $^{-1}$), user should put a positive offset (in this case equal to 3 cm $^{-1}$) and repeat the calibration. A negative offset (in this case equal to -3 cm $^{-1}$) is used in the opposite case.
Once the calibration step is terminated, instrument is ready to make measurements on the desired samples.

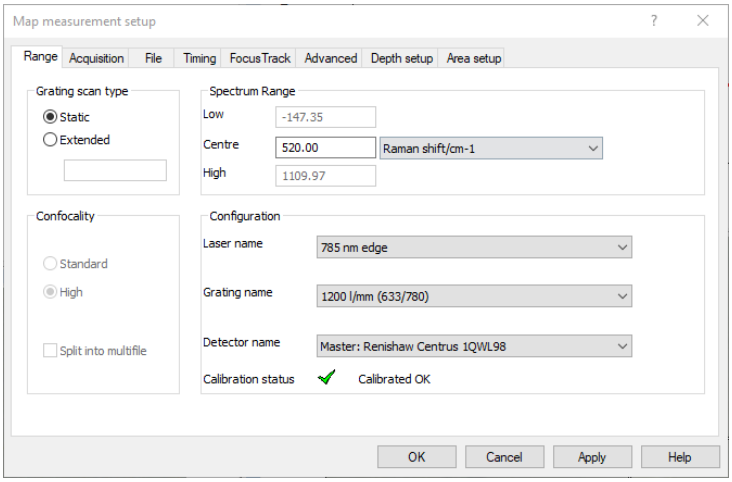


Figure 3.9: Range Tab in the Spectral Acquisition set-up

When the sample under analysis is put on stage, the measurement can start. With this instrument there are several types of measurements available for users as follows:

- Spectral Acquisition - standard method;
- Depth Series Acquisition - measurements in z direction;
- Map Image Acquisition - measurements in (x,y) directions.

The second and the third are simply spectral acquisitions developed in z axis or in the (x,y) one.

In Fig. 3.9 is represented the window that the user sees when a spectral acquisition measurement is going to be done.
In the Range Tab, several settings are available:

- **Grating Scan Type**:
  - Static Scan: the center point of the Spectrum Range can be set (here 520 $cm^{-1}$ for the Silicon Reference Sample) and the system will automatically generate the upper and the lower limit in function of the desired point. This scan can have an exposure time of less than one second per accumulation.
  - Extended Scan: user here can set the upper and the lower limit of the Spectrum Range and the instrument will scan continuously over that range. The scan is limited to exposure times of 10 seconds or greater.
- **Spectrum Range**: it depends on the sample, usually in this work starts from $\approx 600$ $cm^{-1}$ and ends at $\approx 1800$ $cm^{-1}$. Raman Shift should appear in the box to the right.
- **Confocality**: it sets the collected sample volumes:
  - Standard: it uses a larger volume to increase signal strength;
  - High: it reduces the volume and consequently the signal strength in order to increase the depth resolution.
- **Configuration**: in general it is just a check that the selected laser and grating correspond to the one really needed (CCD camera can be just one for this instrument).

In Fig. 3.10, there is the Spectral Acquisition Tab:

- **Title and Description**: this will give a name and eventually a short description of the scan to be done. This will not be saved when exporting the file, but it is just an information saved by the instrument in order to distinguish it between lots of measurements.
- **Exposure Time**: this is the time of exposure at the detector (CCD camera), longer exposure times will improve SNR. It is important to

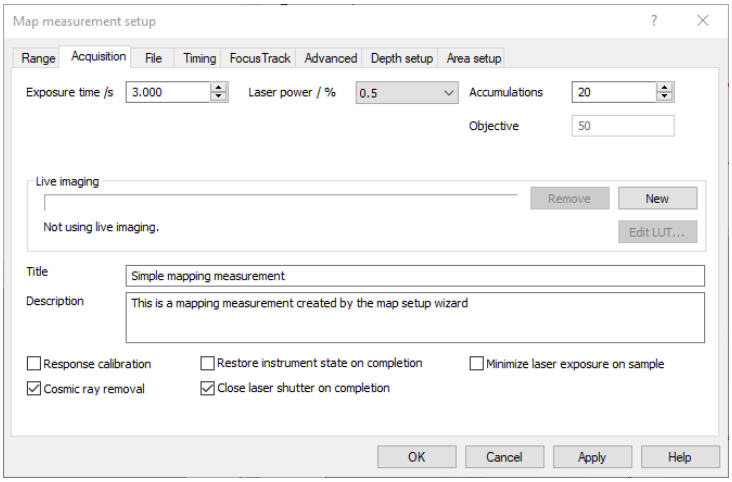


Figure 3.10: Spectral Acquisition Tab

remember that this can not be set below 10 seconds when an extended measurement is performed.

- **Accumulations**: this is the number of scan repetitions made by the instrument, they will be added together and an high number will improve SNR as well. If the cosmic rays removal is selected, then two additional scans will be processed in addition to the number of accumulations selected (in the case of Fig. 3.10, 20 accumulations + 2)

- **Objective**: this is the objective selected in the Sample Review window and must be changed every time the user changes the objective manually on the microscope.

- **Laser Power**: this indicates the percentage of the power being used on the sample, higher power translates into higher SNR. The sample can be susceptible to being damaged by the laser as in this work case, so usually 0.5% or 1 % is selected.

- **Cosmic Rays Removal**: this box is selected to have 2 additional scans to remove random cosmic peaks. This removal can also be done in post-processing.

- **Close Shutter on Completion**: selecting this box, the laser at the end of the acquisition will be closed and the laser exposure on the

sample will be limited.

In this micro-Raman apparatus, the power on sample generally will be halved. This means that f.e. red and green lasers have little power: 17 mW and 50 mW respectively, at the end on the sample will arrive only the half. The 785 nm laser is the most powerful: 300 mW, on the sample will arrive $\approx$ 150 mW. If in this work is indicated a measure at 1 % of the power, it means that on the sample will arrive 1.5 mW of power.

## 3.3 Results

The calibration process is usually composed by several steps [11]:

- Plan the experiments;
- Make the measurements;
- Plot the results;
- Carry out statistical analysis on the data to obtain calibration function;

The first step can seem trivial but it is not: the number of measurements to be done must be decided and also in what concentration range, ideally the calibration range should be established so that the majority of sample concentrations fall towards the center of that range.
The best way to prepare the concentration of the so called "calibration standards" must also be decided, because usually the standards should be independent, i.e. they shouldn't be prepared from a common stock solution, otherwise any error of the stock will be propagated through the other standards leading to a bias in the calibration [11].
In this thesis work the concentration standards were prepared as discussed in Appendix A, i.e. diluting a more concentrated solution with purified and de-ionized water in order to obtain the next concentration standard: this was done because of the low availability of contaminants, due to their high costs.
The second step is as well as very important because the instrument must be calibrated as already explained in the previous section, and also the measurements should be performed in the same conditions. The drop of water on the substrate is in fact always equal to 20 $\mu L$ and the drying procedure

is always the same (Fig. 3.3).
In the third step the plots are shown, where the contamination of Thiram was studied in comparison with three different substrates, one made of spherical NPs from Barcelona (Fig. 3.11 and Fig. 3.12), a substrate made of NanoStars (Fig. 3.13 and Fig. 3.14) and one of Nanospheres made in Turin (Fig. 3.15 and Fig. 3.16):

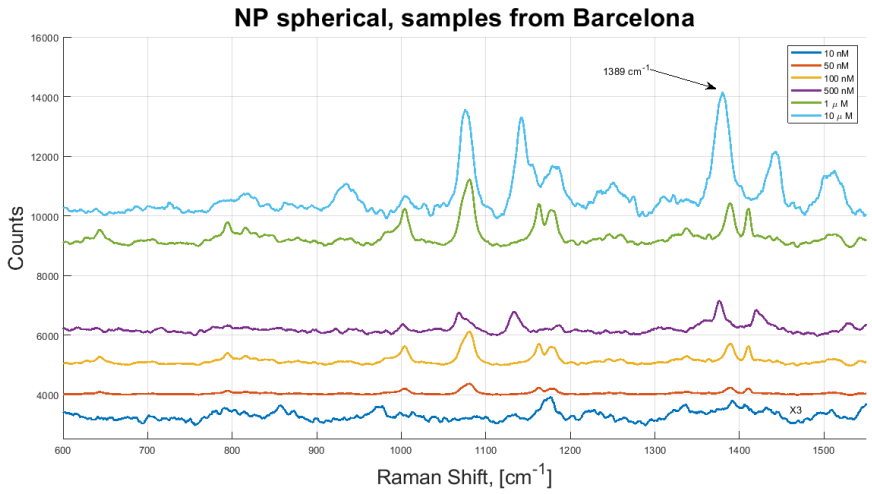


Figure 3.11: NP spherical (samples from Barcelona), Raman Spectrum at different concentrations

In analytical chemistry, the detection limit, i.e. the lower Limit Of Detection (LOD) is the minimum quantity of a substance that can be distinguished from a blank value (absence of that substance) [12] with a stated "confidence" level (generally 99%) [1].
The Limit Of Quantification (LOQ) is the lowest concentration of an analyte in a sample that can be detected with acceptable precision and accuracy, with a stated confidence level.
It must be underlined the fact that sufficient analyte concentration must be present in order to produce an analytical signal that can be distinguished from the "analytical noise", the signal produced in the absence of the analyte (blank) [13].
However, some methods are proposed [14] for the estimation of LOD and

[1]Confidence level is a quantification of the probability that a certain deterministic parameter falls into a precise interval

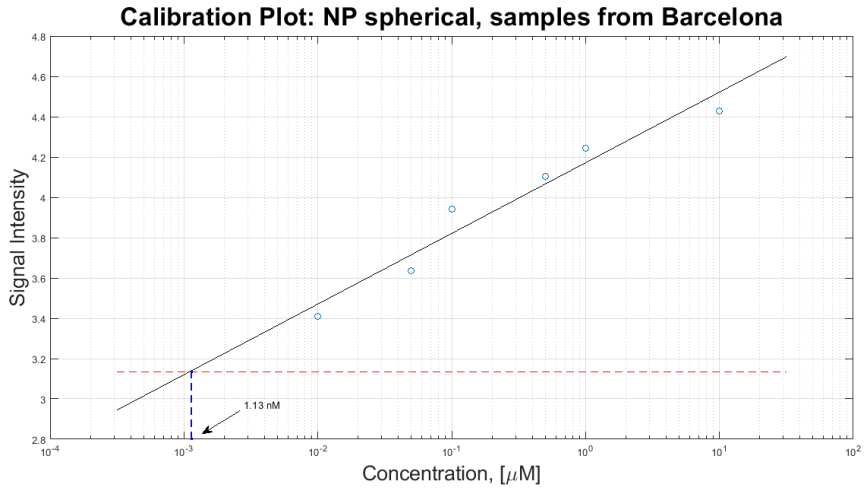


Figure 3.12: Calibration Plot of NP spherical (samples from Barcelona)

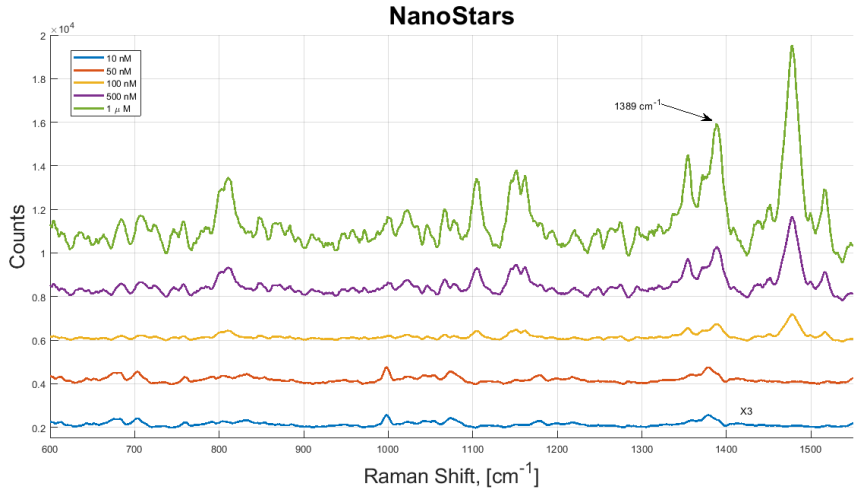


Figure 3.13: NanoStars, Raman Spectrum at different concentrations

LOQ and used in this work:

$$LOD = \frac{F1 \cdot SD}{m} \tag{3.1}$$

$$LOQ = \frac{F2 \cdot SD}{m} \tag{3.2}$$

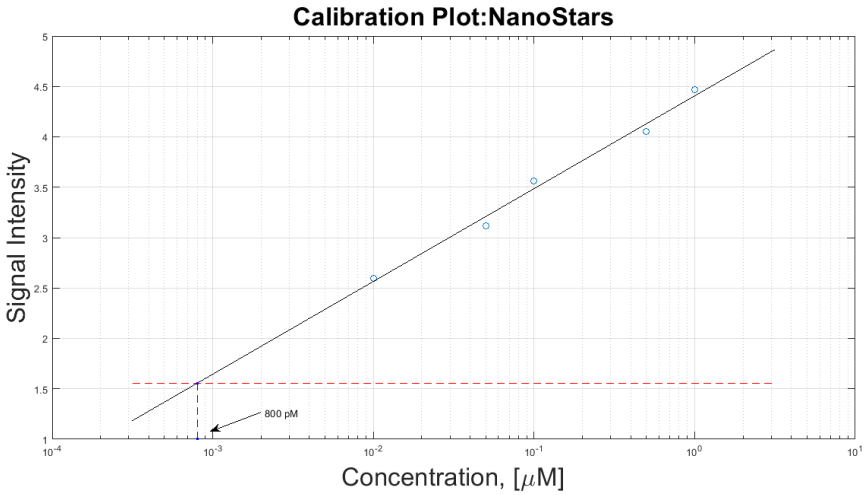


Figure 3.14: Calibration Plot of NanoStars

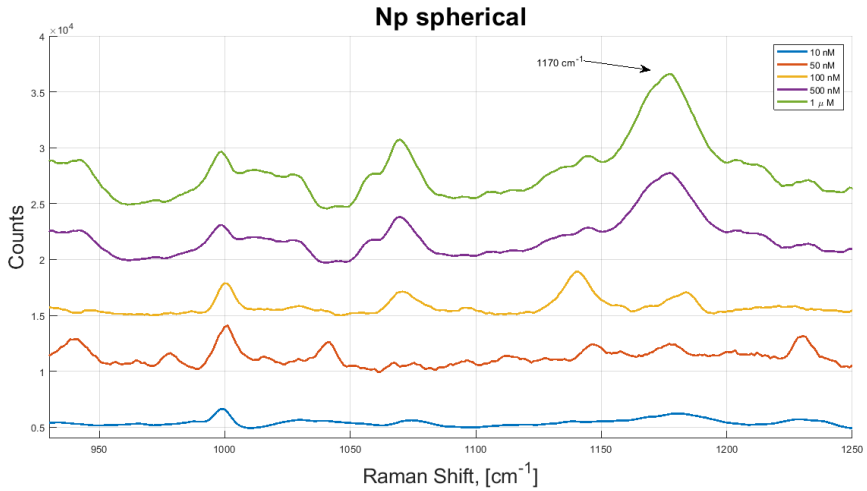


Figure 3.15: Np spherical, Raman Spectrum at different concentrations

where F1 is a factor of 3.3 for LOD, F2 = 10 for LOQ. SD is the standard deviation of the blank, here calculated as an estimate of the SD of the ordinate intercept. Finally, $m$ is the slope of the calibration curve and it is also the last parameter indicated in the figures.
The peak at 1389 cm$^{-1}$ is taken as a reference for the calibration plots, because is the CN stretching mode and symmetric $CH_3$ deformation mode of

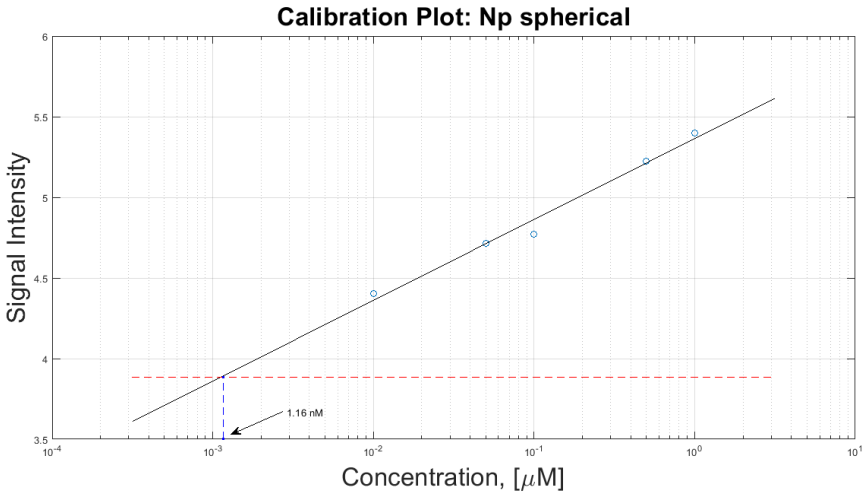


Figure 3.16: Calibration Plot of NP spherical

Thiram and it's the strongest peak of the contaminant[16].
In the nanosphere case (Fig.3.15) the peak at 1170 $cm^{-1}$ is taken as a reference, which is the rocking mode of $CH_3$ and the strectching mode of CN [11]. Quite good results are reached for the Nanostars (Fig. 3.14), which shows a LOD of 800 pM ($\approx$ 200 ppt) and this is due to the lightning rod effect in which there are greater electromagnetic fields at the corners and edges of the nanoparticles. In fact an-isotropic particles like nanostars show corners and edges that spherical ones don't have.

For the Nato Project, it has been requested to detect also organics like 4-Mercaptobenzoic acid (MBA) 1 ppb ($\approx$ 7 nM), Ammonium sodium phosphate dibasic tetrahydrate ($NaNH_4HPO_4$) 300 ppb ($\approx$ 1 $\mu M$) and Calcium nitrate ($Ca(NO_3)_2$) 300 ppb ($\approx$ 1$\mu M$) and this was achieved, as shown in the following pictures.

From Fig. 3.17 can be easily seen the typical peak present at 1000 $cm^{-1}$ of this substance, then in Fig. 3.18 is visible the peak present just slightly after 1000 $cm^{-1}$, whereas in the MBA case (Fig. 3.19) the peaks present are around 1080 $cm^{-1}$ and 1570 $cm^{-1}$, which assure the presence of the MBA.
The titles of the figures are self-explaining: f.e. the one of Fig. 3.19 tells us that user was looking for 7 nM of MBA, he has done a measurement of 5 seconds, with 30 accumulations using the 50X objective at the 0.5 % of the power. When not indicated as in this picture, it means that the laser was

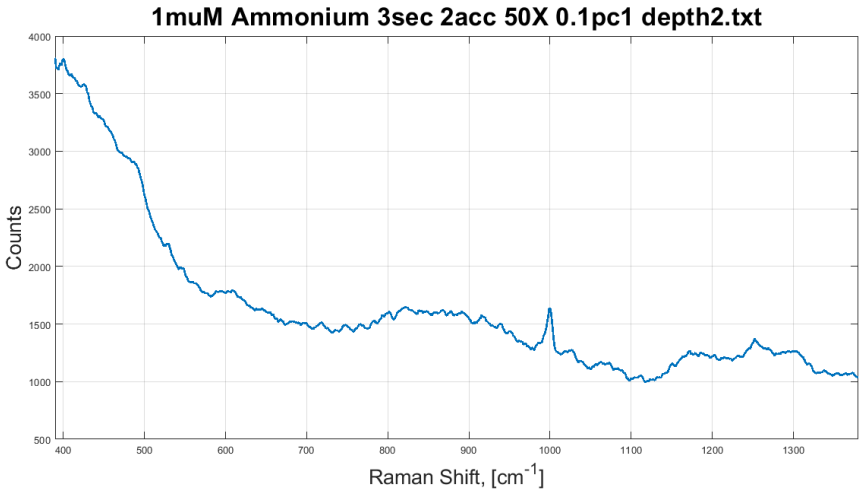


Figure 3.17: Ammonium sodium phosphate dibasic tetrahydrate Raman Spectrum

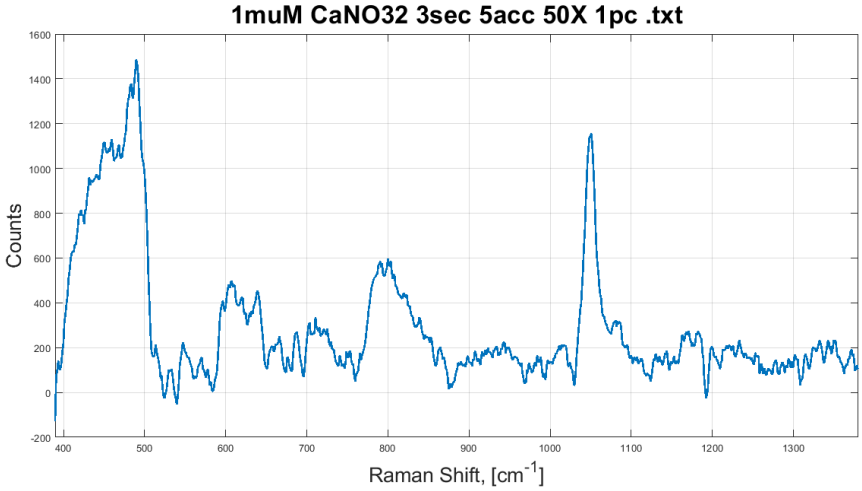


Figure 3.18: Calcium nitrate Raman Spectrum

used with the stripe and not with the pinhole; it's generally the most used feature since it gives the best SNR.

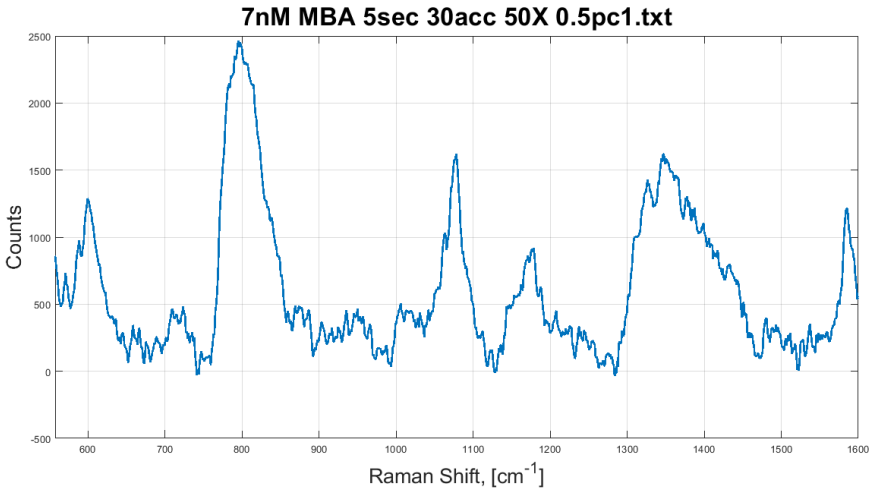


Figure 3.19: MBA Raman Spectrum

# Chapter 4

# Conclusions

During this work, in the framework of RaPID project, several substrates have been tested, showing the possibility to have a fast, easy to use and cost-effective optical sensor for monitoring water quality. Tests have been performed on three types of substrates: gold nanospheres, gold nanorods and gold nanostars. Raman Spectroscopy has been chosen as a spectroscopic technique to analyze them when contaminated. Nanorods do not present good results since it is not easy to remove MBA with a plasma cleaning, whereas nanospheres and nanostars show good LOD.
Calcium Nitrate, Ammonium sodium phosphate dibasic tetrahydrate and MBA have also been tested to detect a precise concentration.
The analysis technique involves Map Image acquisition on a little area ($\approx 100\mu m$ x $100\mu m$) and then Depth Series acquisitions on the points that show the best SNR. For each Depth Series acquisition, a Single Scan is acquired in order to further improve SNR by increasing accumulations (usually decreasing the power) or using the stripe instead of the pinhole.
The laser used in this thesis work is the 785 nm: the use of a near infrared excitation source can eliminate sample fluorescence from most organic molecules. The wavelength of 785 nm has been found to be optimum for these applications, as it largely avoids fluorescence but still returns a Raman signal sufficient to enable detection by a CCD at a reasonable SNR.
Raman Fiber Probes are then versatile accessories for lab applications, and they're available also for the 785 nm laser: this gives to Raman Spectroscopy a good appeal for an easy to use and portable device able to detect low concentration of water contaminants.

# Appendix A

# Thiram solution

The contaminant solutions are made with Thiram diluted in water.
103 mg of Thiram are put in 10,328 g of acetone: since Thiram is not dissolved in water at high concentrations, acetone has been used as a starting point. In this fashion, knowing acetone density as 784 kg/m$^3$, i.e. 784 g/L, i.e. 0.784 g/cm$^3$ and Thiram density as 1.29 g/cm$^3$, the concentration of the just prepared solution can be understood. The total volume of the solution V is:

$$V = \frac{0.103g}{1.29g/cm^3} + \frac{10.328g}{0.784g/cm^3} = 13.25331cm^3 = 13.25331mL \qquad (A.1)$$

knowing the relationships 1 dm$^3$=1 L and 1 cm$^3$ = 1 mL.
In order to understand how much is the concentration, it's known 103 mg of the solute are diluted in a total volume of 13.25331 mL and the Thiram has a molecular weight of 240.4329 g/mol, so the total concentration C is:

$$C = \frac{1}{0,784g/mL} \cdot \frac{103 \cdot 10^{-3}g}{240.4329g/mol} \cdot \frac{1}{13.25331 \cdot 10^{-3}L} \cdot 10^3 = 41.303mM \quad (A.2)$$

In reality this is the molality, since we have mol/g, but anyway is a measure of concentration of moles: 50 $\mu$L of the solution are taken, which is $103mg/13.25331mL \cdot 0.05mL = 0.38852mg$ and they're dissolved with water in order to achieve a concentration of 50 $\mu M$.
Thanks to the excel file [1], it's needed 0.0324 L of purified water, in which 0.38852 mg are dissolved, in order to achieve a concentration of 50 $\mu$M. So from here lots of samples are prepared with different concentrations: the

[1] Molar converter.xls, Appendix B

minimum to be achieved is 100 pM.
6 mL of the solution 50 $\mu$M are taken and diluted with 24 mL of purified water (MilliQ) in a 5:1 ratio, in fact the total of the solution is 30 mL: 1 part is of the 50 $\mu$M solution, 4 parts are of water. In this way the achieved concentration is 1/5 of the starting one (which was 50 $\mu$M), i.e. 10 $\mu$M. Then the next step is to achieve 10 $\mu$M, which is 1/10 of the just obtained concentration. In this fashion 4 mL of 10 $\mu$M solution are taken and diluted with 36 mL of MilliQ, obtaining a total solution of 40 mL composed by 1 part of the 10 $\mu$M solution and 9 parts of purified water.
The next steps are sum-up on the Table *A*:

| starting solution [mL] | water [mL] | achieved concentration [M] |
|---|---|---|
| 50 $\mu$L of 41.303 mM | 32.4 mL | 50 $\mu$M |
| 6 mL of 50 $\mu$M | 24 mL | 10 $\mu$M |
| 4 mL of 10 $\mu$M | 36 mL | 1 $\mu$M |
| 15 mL of 1 $\mu$M | 15 mL | 500 nM |
| 6 mL of 500 nM | 24 mL | 100 nM |
| 10 mL of 100 nM | 10 mL | 50 nM |
| 6 mL of 50 nM | 24 mL | 10 nM |
| 10 mL of 10 nM | 10 mL | 5 nM |
| 6 mL of 5 nM | 24 mL | 1 nM |
| 10 mL of 1 nM | 10 mL | 500 pM |
| 6 mL of 500 pM | 24 mL | 100 pM |

# Appendix B

# Molar Converter

For the conversion of molar to ppb (or vice versa) it has been used an excel file where the inputs to insert are:

- Desired Concentration C ($\mu M$);
- Molecular Weight of the solute MW (g/mol);
- Known Density of the solution D (g/mL), usually is =1 because the solute is dissolved in water;
- volume of the solution V (L);

and the outputs will be the ppm and the ppb. The calculation is:

$$ppm = D \cdot \frac{V \cdot C \cdot MW}{V \cdot 10^3} = D \cdot C \cdot MW/10^3 \tag{B.1}$$

$$ppb = 10^3 \cdot ppm \tag{B.2}$$

For the opposite case, when the ppb is the first input (instead of C), the concentration C ($\mu M$) can be calculated as well:

$$C = \left[\frac{1}{D} \cdot \left(\frac{ppm}{10^3}/MW\right)/V\right] \cdot 10^6 \tag{B.3}$$

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