A low-cost Raman microscope for detecting microplastics in the ocean

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Abstract

Microplastics are a significant source of pollution in lakes and oceans worldwide. To address this issue, we propose constructing a low-cost Raman microscope specifically designed for use on a boat during scientific sailing expeditions. Raman spectroscopy, which analyzes light scattered by a laser beam on a sample, can uniquely identify plastics based on their optical fingerprints. When coupled with a microscope, this technique allows us to study plastics down to <1 μm in diameter. Our project involves two main components: (i) reviewing existing low-cost Raman microscope systems in the literature, and (ii) building a functional Raman microscope suitable for future Sailowtech cruises. The successful completion of this project will contribute to quantifying environmental plastic pollution in water sources.

1 Introduction

1.1 Motivation: Plastics in the ocean

The increasing use of plastics since the 19th century was a decisive turning point on our planet. Production has skyrocketed, along the diversity of plastic applications. This has led to a huge concern : the all over presence of microplastics in our environment, particularly in our oceans. Microplastics, defined as tiny plastic fragments, fibers, or particles less than 5 millimeters in size [3], pose a significant threat. They can originate from the breakdown of larger plastic debris, or even be manufactured specifically for various uses, such as microbeads in cosmetics. The importance of detecting microplastics is real. As these plastic fragments become smaller, their numbers explode, exponentially increasing their potential impact on a vast array of marine species. While the dangers posed by larger plastic debris are evident, the smaller, more discrete microplastics present a really big knowledge gap. Data on the risks of these miniscule particles, particularly those less than 20 micrometers in size, remains scarce [1]. This lack of information is even more concerning because we don't have any idea of the extent of the problem. The potential consequences of microplastic contamination extend far beyond the health of marine organisms. These tiny plastic fragments enters the food chain when ingested by plankton, fish, and even whales. This contamination can then bioaccumulate, finally reaching our dinner plates through seafood consumption [1]. The potential health risks for humans associated with microplastic ingestion are still under investigation. However, research suggests they may cause irreversible damage to the digestive and immune systems [9]. The pervasive nature of microplastics is another cause for alarm. These contaminants have been found in everything from the deepest ocean trenches to our drinking water, highlighting their enormous presence. In ocean sediments, microplastics disrupt the delicate balance of the ecosystem. They can alter the permeability and thermal diffusivity of the sediment, impacting the development and growth of organisms that rely on these factors for survival. Studies have even shown that microplastics can disrupt the hatching process of turtles, affecting the sex ratio of their eggs [6, 7]. Given these significant threats, the development of effective methods for detecting and quantifying microplastics in the environment is rather obvious. By understanding the extent of microplastic pollution and its impact on ecosystems, we can begin to develop targeted strategies to mitigate plastic pollution.

1.2 Aim: A low-cost Raman microscope

1.2.1 Raman microscope to detect microplastics

Raman microscopy is a crucial tool for analyzing the smallest microplastic[1]s, providing data on their numbers, size, shape, and polymer types. Compared to Fourier transform infrared (FTIR) spectroscopy[18], Raman spectroscopy offers advantages such as narrow characteristic peaks, easier substance identification, insensitivity to water, and simpler sample preparation, making it more user-friendly.[2]

1.2.2 Why low-cost

Investigating this question is crucial because current Raman microscopy solutions are prohibitively expensive and inaccessible for many academic and research institutions. The high cost, starting at $ 12,000 for commercial Raman spectrometers[3], limits their availability to well-funded laboratories and restricts educational opportunities in less affluent settings. Moreover, existing systems are often bulky and non-portable[4][5], further reducing their practicality for diverse applications. By exploring the feasibility of designing a low-cost Raman microscope, we can potentially democratize access to this powerful analytical technique, enabling broader use in educational settings and expanding its application to important areas such as microplastic detection in water. This research has the potential to significantly impact scientific education and environmental monitoring by making advanced spectroscopy techniques more affordable and accessible.

1.2.3 Research question

Every aspect of this project tends to answer to following question. Is it possible to design and built a low-cost Raman microscope? And if it is possible, to what cost ? How precise can we get with a low budget ?

1.3 MAKE project

This semester project is part of an EPFL MAKE project called Sailowtech [6]. Sailowtech intends to contribute to the development of innovative and frugal scientific instruments to study the marine ecosystem through field measurements. The equipment developed is designed to be simple and efficient innovation using a minimum of means. These ocean measuring instruments are meant to be boarded on scientific and educational sailing expeditions in order to minimize their environmental impact, make oceanic research more accessible and contribute to the global understanding of the oceanic ecosystem. Most of the scientific prototypes are tested on Lake Geneva and then on the open sea.

2 Working principle of a Raman microscope

A Raman microscope is an instrument that combines a chemical analysis technique called Raman spectroscopy with a traditional light microscope, in order to be able to analyze smaller samples [7]. Raman microscopy (also called micro-Raman spectroscopy or μ-Raman) was first proposed by Puppels et al. in 1990 [2].

2.1 Light-matter interaction and Raman scattering

Three different phenomena can happen when photons of light hit matter [1]:
- light might pass through the material or be reflected without interaction,
- photons might be absorbed while exciting molecules (which might cause fluorescence),
- light might be scattered (light dispersion process).

Lets now focus on this third phenomena. Scattering happens when photons of the excitation source excite electrons of a molecule of matter which jump into a labile, so-called virtual energy state (the energy of the photons is absorbed by the molecule). These electrons are unstable in this virtual energy state so they tend to fall back immediately to a lower level of energy, releasing photons in the process [5].

The molecule can undergo two types of scattering [8, 1, 9] (see fig.1):
- Rayleigh or Mie scattering (depending on the particle size): it is an elastic scattering, meaning that it happens without a change of photon energy (the energy of the scattered photon is the same as the exciting photon's energy): this type of energy is non informational to the chemical makeup of the molecule being interrogated but it is the most likely type of scattering,
- Raman scattering: it is an inelastic scattering, meaning that there is a change of photon energy: this scattering is highly informational to the chemical makeup of the molecule being interrogated.

This last type of scattering (inelastic scattering of photons) theorized in 1923 by Adolf Smekal [7] and then it was first observed in 1928 by C.V. Raman and K.S. Krishnan and was thus named the Raman effect [1, 3].

Raman scattering can be sudivided into two categories [8, 1] (see fig.1):
- Stokes Raman scattering: the photon scattered has a lower energy than the excitation photon and the molecule falls back to a vibrational state with higher energy than before (an electron absorbs energy), frequency of the scattered photon is lower than that of incident photon
- Anti-Stokes Raman scattering: the photon scattered has a higher energy than the excitation photon and the molecule falls back to a vibrational state with lower energy than before (an electron emits energy), frequency of the scattered photon is higher than of the incident photon.

Raman scattering (Stokes and anti-Stokes) is very unlikely [1, 8]. For strong scattering molecules, the intensity of the light being Stokes scattered is only about 10^{-6} of the intensity of the incident light. Anti-Stokes scattering is even more rare and leads to weaker Raman bands. In fact, for anti-Stokes scattering to happens, the molecule must already by in an excited vibrational state when the light arrives on it (see fig.1). But at room temperature, only few molecules are in excited vibrational states. This is why Stokes scattering is the most common type of Raman scattering that is measured by Raman instruments today [8].

Despite Raman effect being weak, it can still be detected quite well thanks to technology advancements in solid state lasers, gratins and CCD detectors [8].

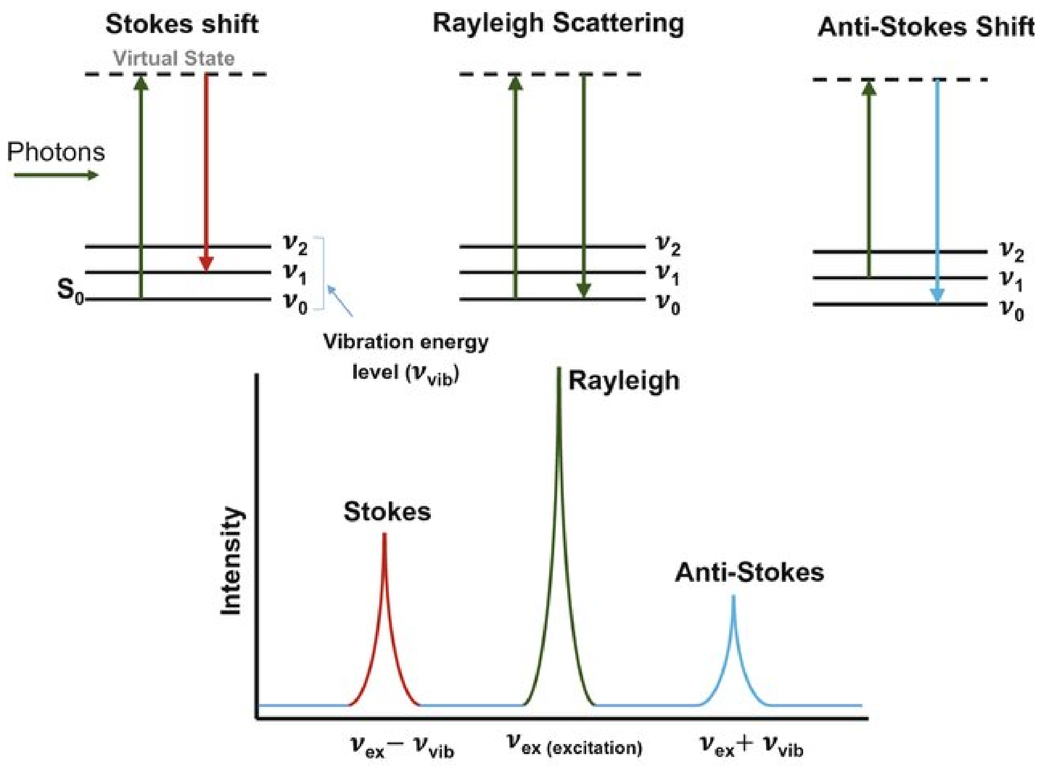

Figure 1: Rayleigh scattering versus Stokes Raman and anti-Stokes Raman scattering [10]

2.2 Raman spectroscopy

Raman spectroscopy is a technique that uses Raman scattering to characterize and identify chemical substances. In fact, the energy absorbed or released by a molecule when it does Raman scattering depends on the type of bonds and the specific atoms of the molecule that the light photons interact with [8]. And by doing inelastic scattering, the molecule changes the frequency of the light. Detecting this frequency shift, called Raman shift, between the original beam of light and the Raman scattered light allows to recover the frequencies absorbed or released by the molecule. This is how Raman scattering allows to identify molecules, acting like a fingerprint.

Therefore, the aim of Raman spectroscopy is to detect the frequency shift. The principle of Raman spectroscopy is the following [1, 7]. A sample is radiated with a monochromatic light source (that has a known frequency). Some of the laser light is absorbed by the sample to excite molecular vibrations, causing Raman scattering. The scattered light is collected and headed towards a detector which captures its frequency. From that, the Raman scattering of the different bonds and bond types can be recovered. It is usually represented in a spectrum as a function of the Raman shift against the intensity [8] (see fig.8).

This spectrum, also referred as the fingerprint vibrational information, can be used for reliable identification of chemical compounds as it provides information about the structure and properties of the studied molecules from their vibrational transitions [3, 8]. The major advantages of this identification technique are that it is nondestructive and that it allows to study aqueous samples as the interference of water is minimal (water molecules do not exhibit strong Raman scattering features)

[4, 3]. With calibration, the Raman signal can also give quantitative information as the intensity of the Raman signal are functions of excitation power, scattering cross-section of the sample and the material's concentration in the sample [8, 3]. The Raman spectrum obtained is the algebraic sum of the individual components' contributions [3]. Additionally, when coupling a probe to a Raman system, it is now possible to measure these bonds and their changes in real time [8].

2.3 Raman microscope

The principle of a Raman microscope is to combine a Raman spectroscope with a microscope to be able to analyse very tiny objects. To do so, an objective lens is added and the sample is irradiated through this objective. The scattered light is also collected through the objective. The sample is placed on a microscope stage which allows to select the region of interest for analysis and to do the focus of the laser light onto the sample. An objective lens is also added to direct the laser light to the sample and to focus it.

3 State-of-the-art

3.1 State-of-the-art of microplastic detection using Raman microscopy

Raman spectroscopy and Raman microscopy have limitations, particularly in analyzing certain chemical compounds with weak Raman signals. This challenge is less significant for microplastics[7], which generally exhibit strong Raman activity and produce intense spectra. Both Raman microscopy (RM) and µ-FTIR spectroscopy provide detailed data on polymer types through their spectroscopic fingerprints, with RM detecting particle sizes as small as 1 µm or below and µ-FTIR detecting sizes around 10–20 µm. While Raman spectroscopy focuses on the backbone of chemical compounds, IR spectroscopy is more about functional groups.[1]

Table 1 Advantages and disadvantages of Raman microscopy for the analysis of microplastics

Advantages	Disadvantages
Determination of material → Reliable identification of microplastics → Plastics are quite Raman active → Identification of other Raman active substances, such as additives, pigments	Possible interferences of adhering or included substances (e.g., additives, pigments, humic acids) → Potential misidentification/false negatives
Information on particle number, size, shape, (color)	No information on particle mass; only via estimation
Low detection limit (particle size) → Toxicological relevance?	Long measurement time
Low or no sample preparation necessary No interference of water	Extensive sample preparation for environmental samples possible/necessary
Nondestructive	Burning of particles is possible

Figure 2: Advantages and disadvantages of Raman microscopy for the analysis of microplastics [1]

Table 1 outlines the advantages and disadvantages of RM in analyzing microplastics (MP). RM is highly effective for identifying polymer types in MP as small as 1 µm. Larger items can also be analyzed as long as they fit within the instrument. By comparing the particle spectrum with standard spectra from databases, the polymer type can be reliably identified.[1]

RM, combined with imaging, also provides data on particle number, size, shape, and color. The quantification of small MPs is crucial, as plastics fragment into increasingly smaller particles in the environment, exponentially increasing their number. Generally, minimal or no sample preparation is needed for Raman measurements, and unlike IR spectroscopy, water interference is negligible due to its weak Raman signal.[1]

However, environmental samples can have interfering compounds that complicate analysis. Substances like dyes, pigments, and humic acids can produce their own Raman spectra, absorb laser light, or cause fluorescence, which can dominate the spectrum and hinder identification. These interferences can sometimes be managed with extended measurement times or prior sample preparation steps, such as alkaline, acidic, oxidative, or enzymatic treatments to remove impediments.

Environmental MPs may also show changes in characteristic bands due to aging processes, affecting band intensity or introducing new bands. Despite these challenges, RM's nondestructive nature allows repeated measurements to improve spectral quality. However, caution is needed to avoid burning small or dark-colored MPs, which can happen with high laser power.

Raman microscopy remains a relatively slow method with long measurement times. Yet, its ability to integrate imaging and spectral data makes it invaluable in detecting and analyzing microplastics. Techniques like confocal micro-Raman, surface-enhanced Raman scattering (SERS), and resonance Raman are being explored to enhance signal intensity and overcome limitations like weak Raman signals and low signal-to-noise ratios.[2]

Researchers like Schymanski et al. and Lenz et al. have demonstrated the capability of micro-Raman spectroscopy to detect tiny MP particles, underscoring its high application value and prospects in this field.[20]

3.2 State-of-the-art of low-cost Raman microscopes

There are few example of low-cost Raman microscopes in the literature. However, multiple examples of low-cost Raman spectroscopes and of low-cost microscopes already exist. The idea of this project is then to see if it's possible to combine these elements to design a low-cost Raman microscope.

3.2.1 Low-cost Raman spectroscopes

Traditionally, raman spectrometer are really expensive. But there have been lots of different efforts to provide low cost solutions for raman spectrometery. To lower the cost, different idea can be followed. First one might be repurposing parts: Instead of needing expensive, custom-made components, some low-cost designs may rely on lasers and detectors already found in schools. Also a simpler design and using 3d printed open source parts to avoid using expensive optic equipement.

One example is OpenRAMAN, a completely free, open-source Raman spectrometer you can build yourself using readily available parts. This is great for educational purposes or researchers who want to customize their instrument. There are also commercially available options like the StellarNet Raman System, known for its affordability and user-friendliness. These advancements shows that Low cost Raman can be done and may still be enhanced.

3.2.2 Low-cost microscope

Several open-source, low-cost microscopes have been developed to make microscopy more accessible. One notable example is the OpenFlexure Microscope, which is a 3D-printed microscope that offers high-resolution imaging capabilities. It uses widely available and inexpensive materials, combined with open-source software, to create a fully functional microscope that can be customized and modified according to user needs. Another example is the Public Lab's Community Microscope is designed for environmental monitoring and uses a webcam-based system for capturing images. This low-cost solution promotes community science by allowing individuals to conduct their own investigations and share findings openly. These open-source microscopes not only reduce the cost barrier but also encourage collaboration and innovation in scientific research and education across the globe.

3.2.3 Low-cost Raman microscopes

2 low-cost Raman microscopes: [2] and [11]

Another insteresting Raman microscope (not low cost but worth mentioning): development of underwater in-situ microplastics detection system based on micro-Raman spectroscopy [12]

4 Constraints

For this Raman spectrometer, there are lots of differents constraint made either by the environnment it is going to be used in or by user's needs. The Sailowtech association would like to have a working specrtometer for this summer Expedition. The Arvor [13] expedition is going to take place in The Channel from 18 July to 11 august in partnership with the Pacific Foundation.

The constraints made by the environnment are the following ones :
-The device should be water resistant and protected against humidity.
-The device should work with a car battery and uses low power.
-The device must resist to vibration and pitch and roll of the boat.
The constraints made by the users are the following one :
-The price should be at the lowest possible.
-The device should be small enough and transportable.
-The user interface should be easy to use and don't need anything to work.
-The device should be precise enough to analyse microplastic with a size going from 2 to 50 µm.
-The device should be able to take both microscope images and do spectrometer analysis.
-The device must have a precision <5cm-1

5 Choice of the design

5.1 Excitation source

To be able to detect the Raman scattering, the excitation source must be a monochromatic light, usually lasers with wavelengths in the visible to the near infrared range [1]. The choice of appropriate technical specifications (wavelength, line width, optical power...) for the excitation source is crucial for obtaining good quality Raman spectra [3]. The optimal laser might differ from one sample to the other, depending on the material you want to analyze, this is why many Raman microscopes have several lasers that can be easily and quickly changed [7]. However, to keep this Raman microscope low-cost, only one laser will be chosen.

The first parameter to take into account to chose a laser is its wavelength. The most commonly used lasers in Raman spectroscopy and microscopy are green laser at 532nm, blue laser at 488nm, red laser at 633nm and infrared laser at 785nm [7].

The laser wavelength is important as the intensity of Raman scattered light (and the spectral intensity measured) varies inversely to the fourth power of the excitation wavelength (see equation 4) [3, 1]. So for an equal acquisition time, lasers with lower wavelength (UV and visible) produce a better Raman signal than the IR light sources. This issue can be overcome with a prolonged acquisition time. Laser wavelength also influences the spatial resolution (Rayleigh criterion, see eq.3). A shorter wavelength gives a better spatial resolution [1].

$$d_{(\text{Rayleigh })} = 0.61 * \frac{\lambda}{NA}$$

Figure 3: Rayleigh criterion for the diffraction limited spatial resolution (d), λ: wavelength, NA: numerical aperture (Anger et al. 2018) [1]

Because of these reasons, one might think that a lower wavelength is better to get a good Raman signal. However, one of the main limitation to Raman microscopy (and spectroscopy) is fluorescence. And laser with lower wavelengths have higher photon energy, which might be high enough to transfer a molecule to an excited electronic state and induce fluorescence [1]. The light emitted by fluorescence is much more intense than the weak Raman effect, making the Raman peaks difficult to distinguish from the noise or making the detector signal saturated by the fluorescence [7, 1].

$$I_R \propto \nu^4 * I_0 * N * \left(\frac{\partial \alpha}{\partial Q}\right)^2 \text{ with } \nu = \frac{c}{\lambda}$$

Figure 4: Equation of the reliance of the intensity of Raman scattered light (I_R) from the intensity (I_0) and wavelength (λ) of the radiation source, N: number of scattering molecules, $\partial\alpha/\partial Q$: change in polarizability, ν: frequency of the light of the radiation source, c: speed of light (Larkin 2011) [1]

The power of the laser must also be taken into account as the spectral intensity is proportional to the laser power (see eq.4) [1]. However, the laser power cannot be set randomly high for both safety reasons and because it might lead to sample destruction. Reported laser powers range from 0,4 mW (633 nm) to 25,2 mW (532 nm) [1]. And as said before, signal intensity can be increased by longer acquisition time.

Finally, the spectral bandwidth of the laser in important.In fact, the laser should have a bandwidth as narrow as possible as the bandwidth fixes a limit to the spectral resolution of the Raman signal. However, if the bandwith of the laser is not narrow enough, adding a narrow bandpass filter can solve the problem. One should just keep in mind that if the bandwidth of the laser is very broad, the narrow bandpass filter will diminish a lot the power of the light that will reach the sample.

For this design, the choice was made to select a laser with a ”high” wavelength to avoid fluorescence as much as possible, but staying in the visible range for safety reasons as this project aims to be open source and reproducible. The cost of the laser was also a criteria and near IR laser are much more expensive than visible lasers and so do powerful lasers. In addition, it is not easy to find a cheap narrow bandpass filter with a wavelength that matches the laser wavelength. So the choice of the laser was also made taking into account the narrow bandpass filters that could be found. This is why the excitation source in this design is a red laser with a wavelength of 639nm and a power of 4,5mW (see B). In addition, two narrow bandpass filters are used : Wavelength 636nm and 10nm bandwidth and 640nm and 10nm bandwidth.

5.2 Microscope

5.2.1 Illumination path

To be able to image the sample with the camera of the microscope and to do the focus on the sample, the latter is illuminated using a LED light source. There are two options to illuminate the sample: transmission or reflection (see figure 5).

In transmission illumination, the light shines upward and passes through the sample, creating a contrast to see internal composition and structure of thin samples. The drawback with using a transmission light path to do Raman microscopy of microplastics is that the capillary structure and opacity of the filter membrane on which the sample is might cause some interference to the microscopic imaging of the sample [2]. In reflective illumination, the light shines downwards onto the sample and bounce off the sample surface, enabling you to analyze topography and exterior features. Reflective illumination eliminates the potential interference of the capillary structure of the filter membrane when imaging, which is very suitable for nontransparent objects [2].

Therefore, the design developed here is based on a reflective illumination path instead of a transmission one (see fig.6). In order to avoid having to add extra components to the design in order to remove the light emitted from the LED light, it was decided that the microscope part of the Raman microscope (to do imaging of the sample or to do the focus) should not be used simultaneously with an acquisition of the Raman signal through the detector. This way, the LED light source can just be turned off when doing the acquisition of the Raman signal.

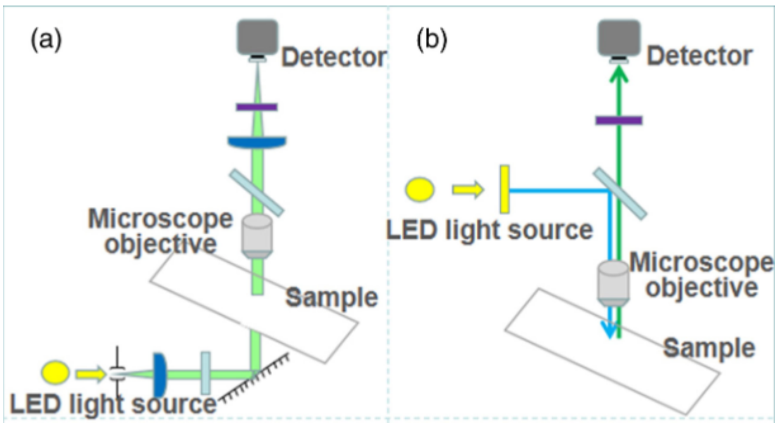

Figure 5: Comparison of two kinds of illumination light paths. [2] (a) Transmission illumination light path; (b) reflective illumination light path

5.2.2 Microscope objective lens

The objective lens of the microscope has an impact on the spatial resolution of the Raman microscope. More precisely, the numerical aperture of the objective lens is determinant (Rayleigh criterion, see eq.3) [7, 1]. The higher the numerical aperture of the objective lens, the higher the spatial resolution of the Raman microscope [7]. Barbara E. Oßmann reports that "microplastics down to a size of μm were analyzed with a objective with a magnification of 20x (NA=0.55) and the smallest reported microplastics down to a size of 1 μm were analyzed with an objective with a magnification of 50x (NA= 0.75)" [1].

The most prevalent criteria for the choice of the objective lens in the design presented in this report is the price of the objective. The possibility of using coupled lenses instead of buying an objective lens but making the objective with spares lenses add lots of works and it is really easier to use and adapt the system with a spare objective we found, this idea was rejected in favour of an already built objective lens.

5.2.3 Microscope stage

The microscope stage must allow to move the sample in the 3 axis. Moving the sample in the x and y axis allows to select the region of interest for analysis of the Raman signal. Moving the sample in the z axis allows to do the focus on the sample.

For the Raman microscope to be as low-cost as possible, it was decided to use an existing open-source design for the microscope stage and to 3D print it. The stage chosen is the block stage from OpenFlexure [14] because it has a sub-100 nm resolution. This other reason that motivated the choice of this stage design is that another MAKE project (SensUS) already 3D printed it few years ago and confirmed that it worked properly.

5.3 Detector

5.3.1 Grating

The grating in a Raman spectrometer is responsible for separating light by wavelength before detection. Typically, a Raman instrument contains multiple gratings, each with different line densities. The line density determines how much the light spreads across the detector, which in turn affects the spectral resolution, or the ability to distinguish individual peaks on the Raman spectra[7]. Higher line densities lead to higher spectral resolution, allowing for detailed examination of specific parts of the spectrum or fine-tuning experiments for different lasers. However, higher line densities also result in longer measurement times, as the entire spectral range cannot be covered in a single position of the grating. Generally, gratings with 600 to 1200 lines per millimeter are used[1] depending on the width of the CMOS camera, providing sufficient resolution for identifying common polymers and covering the necessary spectral range in one acquisition,

5.3.2 Line CMOS for Spectral measurments

To measure the Raman spectrum, a really good sensor should be used to detect all the shifted light. To do so, a Hamamatsu S10077 line CMOS sensor is used. This sensor has its peak sensitivity around 750nm which matches this requirement perfectly. We uses for this Project a Linear sensor without any filter to have a better detection. As the setup and particullary the grating and the mirror inside the spectrometer parts split the lights in 1D according to their Wavelength, we don't need an array detector but just a line. Typically, the Raman spectrum appears $10-200$ nm above (Stokes) and below (anti-Stokes) the excitation wavelength.[3]. Therefore we need to pay some particular attention to have The wavelength of the laser getting on one side of the sensor and the other side of the sensor corresponding to the 200nm shifted light.

5.3.3 Microcontroller Arduino

We use a microcontroller Arduino Nano to make the interface between the CMOS sensor and the user interface made by the Raspeberry PI. The Fast clock and the architecture makes it possible to Generate the Clock and Start signal. After that the microcontroller manages the output data and transmit the information from the sensor to the raspberry pi via the Serial port.

5.3.4 Raspberry Pi

A raspberry pi 5 is used as the Central computer of the setup. It is used to drive both the Camera and the linear sensor. The camera is directly a Raspberry camera module 3 to do the microscope imaging. In other hand, it makes the interface with the Linear sensor through an USB PORT. It has also spare GPIO usable if we want to use an electronically driven stage.

6 Final design

The final design is presented in figure 6. The list of materials as well as their costs is presented in table B.

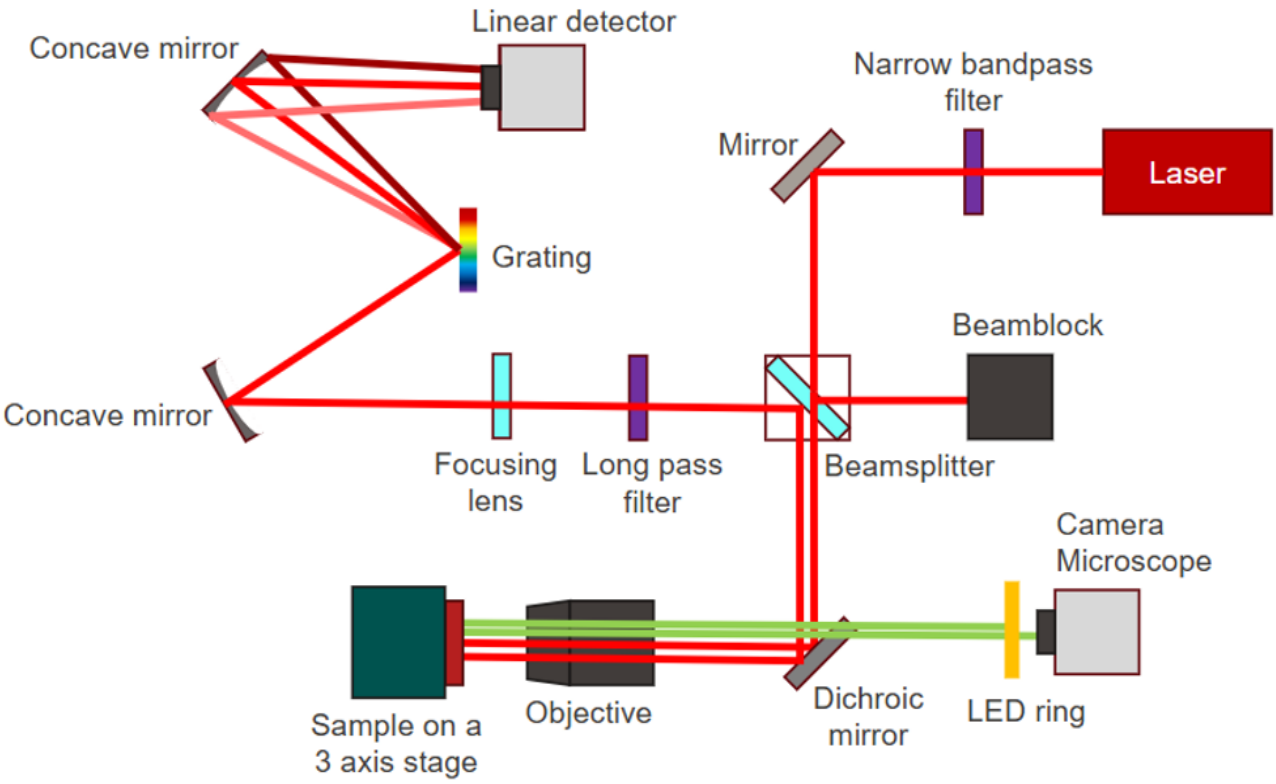

Figure 6: Schematic of the final design

The light from the laser first goes through a narrow bandpass filter which removes most of the unwanted wavelengths produced by the laser. Once the laser light has a very narrow bandwidth, is it directed towards the objective lens by optic components (it is redirected by a mirror, it goes through a beamsplitter and it is redirected again by a dichroic mirror). The excitation light goes through the objective lens and arrives on the sample which is placed on a microscope 3 axis stage.

In order to do the focus before a Raman measurement or to do microscopy imaging of the sample, a LED ring can illuminate the sample. This LED ring is placed between the camera of the microscope and a dichroic mirror that only transmits the light below a wavelength of 600nm but reflects higher wavelengths (including the laser wavelength and the wavelength of the scattered light).

Back to a Raman measurement, the laser light is scattered on the sample and collected through the objective lens. At this point, the light collected includes the Raman signal (both Stokes and anti-Stokes scattering) and the Rayleigh scattering at the same wavelength than the laser. This is why this collected light is redirected by the dichroic mirror and the beamsplitter towards a longpass filter that will only transmit the wavelengths higher than 650nm. This way, the Rayleigh scattering is removed from the light beam. Note that the anti-Stokes scattering is also removed in the process.The light is then focused on the grating thanks to a focusing lens and a concave mirror. The grating splits the different wavelengths of the scattered light which are then focused on a linear detector by a concave mirror. From there, the Raman spectrum can be recovered and analyzed.

Note that a beamblock is also added near the beamsplitter for safety reasons.

7 Results and discussion

For now only results present were about the shape of the laser. The first results considering the setup showed that the filter were not enough for our utilisation. The laser signal should be around 10^{-10} to not saturate the raman signal. Therefore a second Filter was added to get a better.

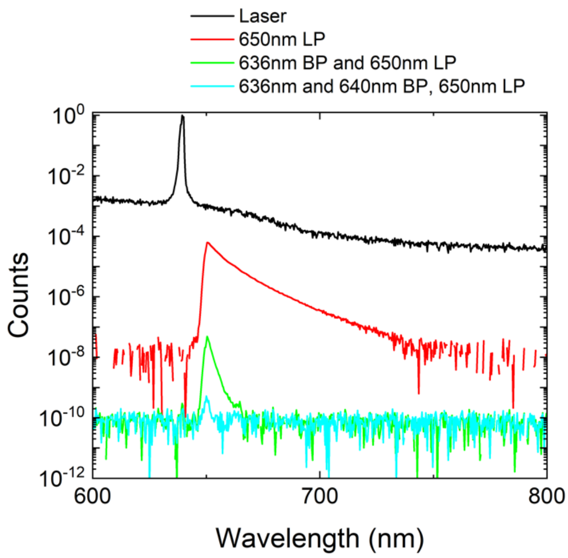

Figure 7: Curve Showing the different counts of photon against the wavelength

Finalizing the setup is crucial for acquiring our first Raman measurement and ensuring accurate data through calibration. No conclusion can be drawn about the rest of the setup for now.

7.1 Future improvements

To transform this project into a highly effective spectrometer, several key improvements are necessary. The primary enhancement involves completing the integration of all components into a single, user-friendly enclosure. Additionally, finalizing the software and user interface is crucial, and making these resources available on GitHub will allow others to replicate the system. It is also essential to develop comprehensive protocols and instructions for operating the spectrometer.

8 Conclusion

In conclusion, this project has shown the possibility of making a Low-Cost Raman Microscope. By trying to be cost effective and open source, we managed to reduced the global cost of the project to about 2000.- which is way less than commercially available solutions. The project has significant potential for further development but still need a lot of work to be completed. This project was also really interesting in the context of the Master because it covered lots of different aspect from conception to realisation.

Acknowledgement

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A Example of Raman spectra obtained in the literature

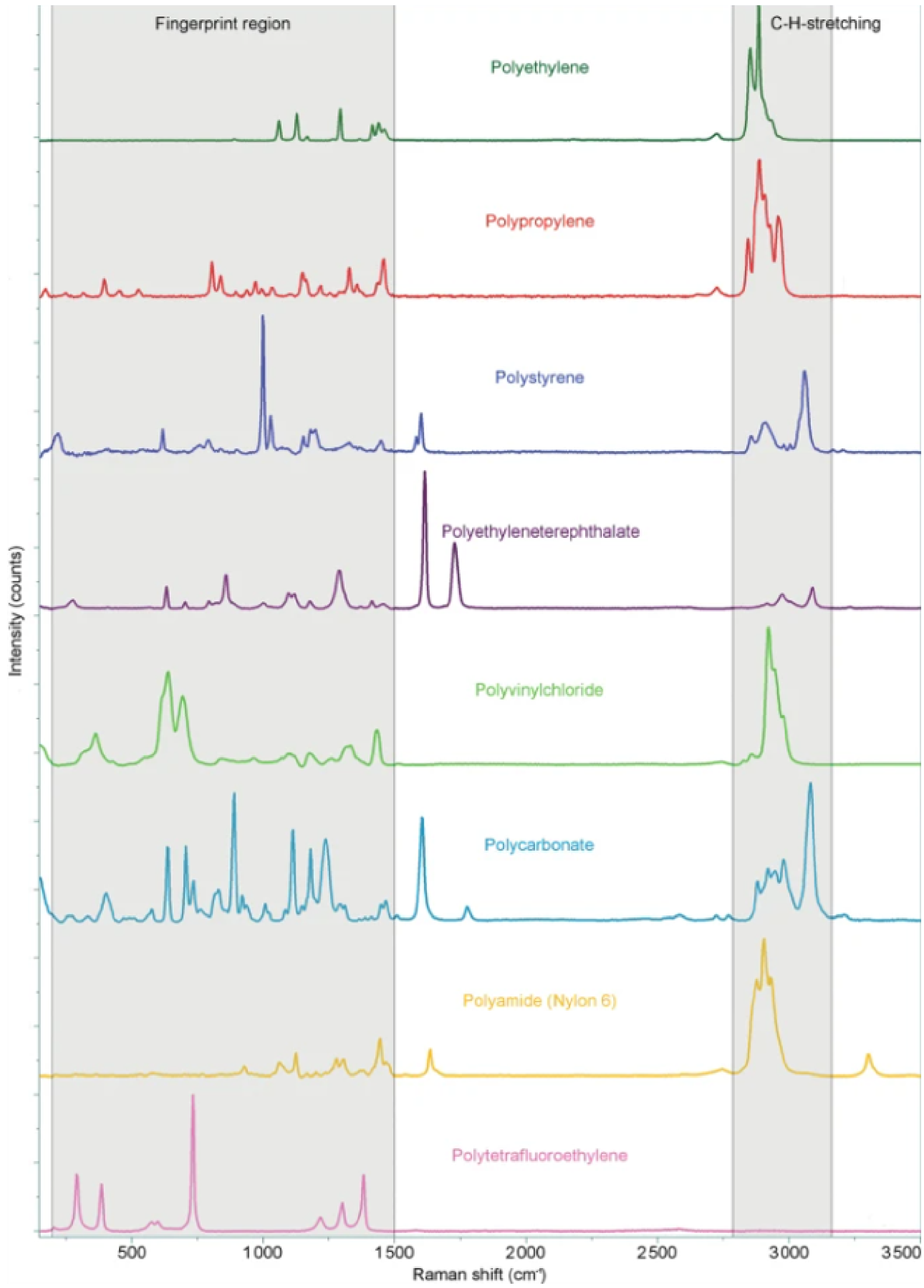

Figure 8: Raman spectra of different polymers, normalized by maximum, fingerprint region and C-H-stretching vibrations are marked in grey areas [1]

B List of the parts used and bill of materials

Below is the full lists of all parts which were bought for building this Project.

Pièce	Référence	Prix unitaire	Quantité	Prix total
Laser	Thorlabs - PL252 Compact Laser Module with USB Connector, 639 nm, 4.5 mW (Typ.)	154,94 €	1	154,94 €
Narrow bandpass filter	Edmund Optics (Techspec) - 640nm CWL, 12.5mm Dia., Hard Coated OD 4.0 10nm Bandpass Filter	165,00 €	1	165,00 €
Mirror	Thorlabs - PF10-03-P01 - Ø1" Protected Silver Mirror	51,14 €	1	51,14 €
Beamsplitter	Thorlabs - BSW28 - Ø1/2" 50:50 UVFS Plate Beamsplitter, Coating: 600 - 1700 nm, t = 3.0 mm	83,41 €	1	83,41 €
Objective	Edmund Optics (Zeiss) - 40X ZEISS A-Plan Objective	478,00 €	1	478,00 €
Longpass filter	Thorlabs - FELH0650 - Ø25.0 mm Longpass Filter, Cut-On Wavelength: 650 nm	130,72 €	1	130,72 €
Grating	Edmund Optics - 1200 Traits, 25 x 25mm, 750nm, Réseau Or pour IR Proche	160,00 €	1	160,00 €
Linear detector	Hamamatsu - S10077 - Line sensor	110,00 €	1	110,00 €
Concave mirrors	Edmund Optics - 25mm Dia. x 50mm FL Protected Aluminum, Concave Mirror	47,50 €	2	95,00 €
Dichroic mirror	Edmund Optics (Techspec) - 600nm, 12,5mm de Diamètre, Filtre Passe-Bas Dichroique	123,00 €	1	123,00 €
3 axis stage (3D printed parts)	OpenFlexure Block Stage	10,18 €	1	10,18 €
LED ring	EverythingPi - EP00299 - NeoPixel Ring - 16 x WS2812 5050 RGB LED Ring Board for Arduino Raspberry Pi	4,20 €	1	4,20 €
Raspberry Pi camera	Raspberry Pi - SC0872 - Camera Module 3 / Standard 75°	43,00 €	1	43,00 €
Raspberry Pi 5 + kit	HutoPi - KITPI58GB - Kit de démarrage Raspberry Pi 5 - version 8 Go	149,00 €	1	149,00 €
Cable for Raspberry Pi camera	Raspberry Pi - RASP CAM FPC 30 - Câble de caméra Raspberry Pi [1x CSI - 1x CSI] 0.30 m	16,20 €	1	16,20 €
Arduino Nano	Arduino Nano	24,50 €	1	24,50 €
Electrical board + Cables		14,50 €	1	14,50 €
Focusing lens			1	0,00 €
Additional bandpass filter	Edmund Optics (Techspec) - 636nm CWL, 12.5mm Dia., Hard Coated OD 4.0 10nm Bandpass Filter	165,00 €	1	165,00 €
Mountings				0,00 €
Screws				0,00 €
Prix total				**1 977,79 €**