Bacterial detection using Raman spectroscopy

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1. Introduction

During a two-month period at EPFL's Laboratory of Quantum and Nano-Optics (LQNO), I closely collaborated with a team whose work combines physics and the biomedical field. The objective of the project is to revolutionize bacterial detection in large food chains, with potential expansion into areas such as pharmaceuticals.

The project seeks to address the inefficiencies of conventional bacterial detection methods, which are often cumbersome and involve multiple, labor-intensive steps that can sometimes lead to inaccurate or inconsistent results. These traditional techniques, such as culture-based methods or PCR, are not only time-consuming but can also be impractical in high-demand environments like food safety, where timely detection is crucial. The delays and inaccuracies inherent in these processes can compromise food safety, highlighting the need for a faster, more reliable solution.

The gold standard of bacterial detection approaches is **culture-based testing**. This method consists of growing food samples taken of the production lines in a laboratory environment to observe the bacterial colonies which develop. While relatively reliable, this approach is greatly time-consuming as bacteria need time to multiply enough for detection, typically taking 24 to 48 hours (Saravanan et al., 2020).

Due to slow innovation in the field, amongst other factors, bacterial infections and diseases caused by food contamination remain a major health issue, responsible for both high morbidity and mortality. According to reports from the World Health Organization (WHO), foodborne diseases lead to around 600 million cases of infection annually, which amounts to nearly 1 in every 10 people falling ill each year. These infections result in 420,000 deaths annually, with children under the age of 5 being disproportionately affected, accounting for 125,000 of these fatalities (World Health Organization: WHO, 2022).

Additionally, foodborne diseases have a deep socioeconomic impact, particularly in low- and middle-income countries. In these parts of the world, unsafe food leads to productivity losses

and medical costs which amount to about $110 billion annually (World Health Organization: WHO, 2022).

These reports underline the urgent need for more sophisticated, rapid, and accessible methods of bacterial detection to enhance food safety and public health.

1.1 Background on The Subject

The group at LQNO investigate the interaction between light and matter on the nano scale. In particular, they use several forms of spectroscopy to probe and control the properties of matter on the level of a single molecule. The study of the interaction between light and matter has led to countless practical applications in recent years. The technological ability that has been developing in recent years makes it possible to create a wide manifold of nanophotonic structures. These are patterned nanostructures made of different material and by which the light can be confined into tiny regions in the space. When electromagnetic radiation is confined into tiny regions, the interaction nature of the radiation with matter becomes of great interest from both the fundamental point of view as well as for many optical engineering applications. This interaction can be used to probe the properties of the material and even manipulating them in some circumstances. One of the LQNO group is to exploit this kind of interaction to develop quantum and nanoscale sensors.

In the context of bacteria detection, The LQNO group aims to employ Raman scattering to develop a new platform of sensing. Raman scattering is an optical phenomenon that is widely used for molecular detection. The LQNO group specifically intend to integrate highly sensitive structures with a powerful algorithms based on artificial intelligence (AI) to detect variety of bacteria species with high sensitivity and specificity. This is an innovative program that exploit a fundamental quantum phenomenon with a postmodern capability to create a practical sensing platform that should change the landscape of biomedical sensing.

1.1.1 Raman Scattering

Electromagnetic radiation, i.e. light, interacts with matter through multiple phenomena such as absorption, transmittance, and scattering. While absorption requires a particular match between the energy of the incident photons and that of the energy gap between successive electronic energy levels, the process of scattering involves virtual energy states. virtual energy states are short-lived quantum phenomena in which electrons are excited to an “imaginary” energy state (Robinson, 1985). Virtual states help explain many observed optical phenomena but are, unfortunately, outside the scope of this report.

Upon the decay of the virtual energy state, the electron returns to the to a real energy state emitting a photon in the process (Orlando et al., 2021).

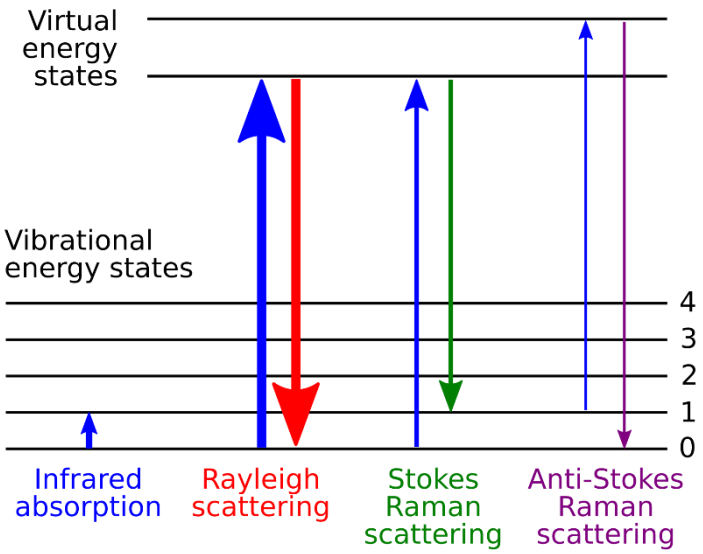

Figure 1; Energy-level diagram showing the states involvedin Raman Spectra, including virtual energy states (Wikipedia contributors, 2024).

If the energy of the emitted photon matches that of the incident photon (also called excitation photon) and the electron involved in the excitation returns to the same energy state, the scattered photon is termed “elastic”. Elastic scattering is also often referred to as “Rayleigh scattering” (see figure 1).

A second possibility is that the emitted photon has **less** energy (longer wavelength) than the excitation photon. This group of photons is called Stokes Raman Scattering. The Third and final case is that the emitted photon has **more** energy (shorter wavelength) than the excitation photon. This scattering category is referred to as Anti-Stokes Raman scattering. Figure 2 sums up the different categories of emitted photons.

When the energy of the excitation photon does not match that of the emitted photon, the scattering is termed “inelastic”. The energy difference between excitation and scattered photons is characterized by the “Raman shift” (Orlando et al., 2021).

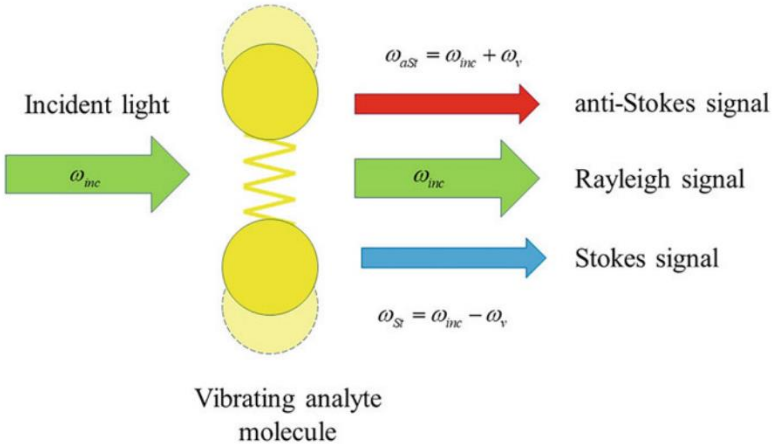

Figure 2, Light scattering by a vibrating an analyte molecule (Lisyansky et

It should be noted that Raman scattering, i.e. Stokes and anti-Stokes scattering, is a particularly weak phenomenon, as only 1 in about 100 million scattered photons are Stokes or anti-Stokes (Orlando et al., 2021). Practically, in Raman spectroscopy, the frequency of the scattered photons are measured, and from this one can easily deduce the vibrational frequency of the scattering molecule. Therefore, Raman scttaering is used as a spectroscopic tool for molecular detection.

1.1.2 Surface Enhanced Raman Scattering

A great disadvantage in any application of Raman spectroscopy results from the extremely weak signals of the Raman process, which is many orders of magnitude below other spectroscopic techniques such as fluorescence for instance. Therefore, in the 1970s, a discovery which showed extraordinarily high Raman signals of molecules on rough metallic surfaces attracted enormous attention. Experiments conducted across various laboratories have demonstrated that the enhancement in Raman signals is due to a genuine increase in the efficiency of Raman scattering itself, rather than being caused by a higher concentration of scattering molecules. Within few years, strongly enhanced Raman signals were verified for many different molecules which had been attached to various “rough” metal surfaces, and the effect was named “surface enhanced Raman scattering (SERS). The discovery showed promising to overcome the traditionally low sensitivity problem in Raman spectroscopy.

Estimated enhancement factors of the Raman signal started with modest factors 10^3 – 10^5 in the first reports on SERS. Later, many authors claimed enhancement factors of about 10^{10} – 10^{11} for certain molecules. About 20 years after the first discovery of the SERS effect, researchers were able to reach enhancement factors of about 10^{14} using special metallic nanostructures. This unexpectedly enhancement factors enabled the detection of single molecule or small number of molecules using the SERS effect (Kneipp et al., 1997b) (Moskovits, 1985).

The advancement in nanostructures fabrication makes SERS a spectroscopic technique which combines modern laser spectroscopy with the exciting optical properties of metallic nanostructures, resulting in strongly increased Raman signals when molecules are attached to nanometre-sized metallic structures. SERS combines the structural analysis capabilities

of Raman spectroscopy with ultra-sensitive detection limits, enabling the detection and identification of **single** molecules (Kneipp et al., 1997b).

Surface-Enhanced Raman Scattering occurs primarily due to electromagnetic effects triggered by the excitation of localized surface plasmons. Surface plasmons are oscillations of the free electrons in the metal that enhance the electric field near the metallic structure's surface, leading to stronger Raman signals (Lisyansky et al., 2024). In fact, the intensity of the Raman signal is proportional to the electromagnetic field raised to the fourth power. These Raman signals are so sensitive to the point where they can provide detailed molecular **fingerprints** for complex biological samples, including proteins, DNA, and pathogens.

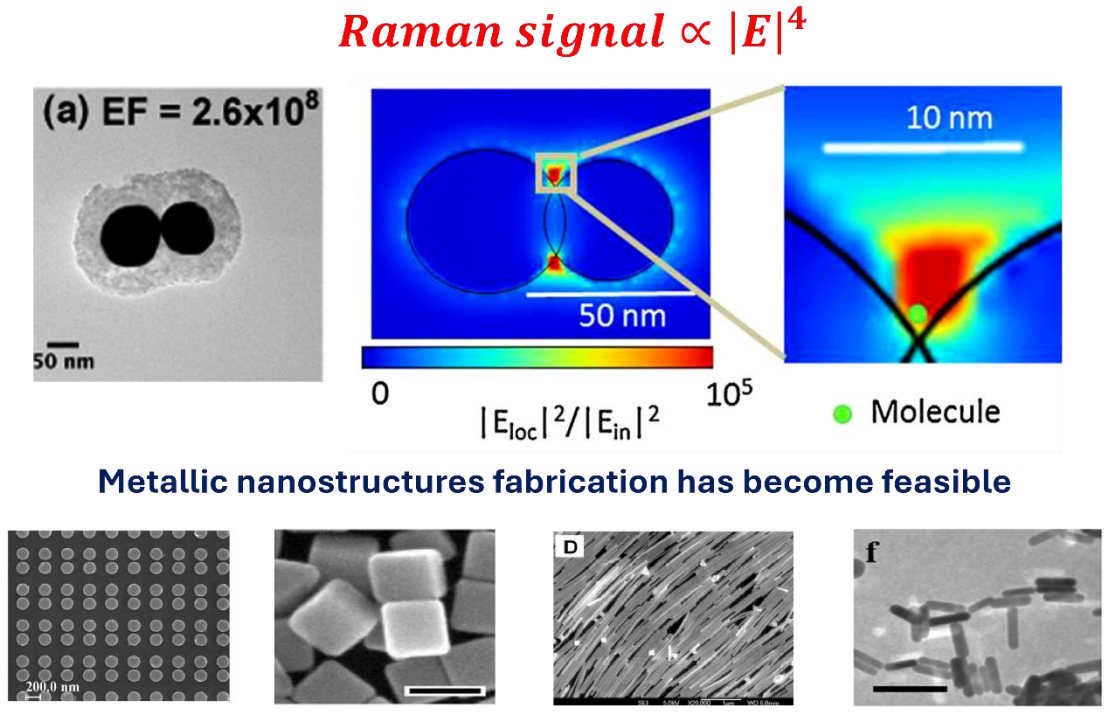

Figure 3. Upper section shows nanoparticles with illustration of the intensity of the electromagnetic field around these particles. Lower section shows a variety of nanostructures used in SERS detection. (Itoh et al., 2013c)(Banaee et al., 2010)(Camargo et al., 2009)(Tao et al., 2003)(Orendorff et al., 2005).

1.1.3 Microbiology

Since the main analytes in the context of the project were bacterial species, a profound understanding of the composition of these microorganisms had to be established, particularly the composition of the outer layers and structures of the bacteria.

When observing the outer layers of the bacteria, we can make a distinction two primary categories, Gram-negative species and Gram-positive ones. The main distinction happens

at the level of the bacterial cell wall. In Gram-positive bacterial species, the cell wall is composed of two layers, a peptidoglycan layer, and an inner membrane. While in their Gram-negative counterparts, the bacterial cell wall consists of three layers, an inner membrane, the periplasm, and the outer membrane (Swoboda et al., 2009).

Peptidoglycans are macro-molecules, i.e. giant chains of molecules, which are layered one over the other forming a “near” impenetrable barrier. The inner and outer membranes are composed of many organic molecules such as proteins, lipids, and phospholipids. Specifically, the outer membrane in Gram-negative species contain an interesting component called Lipopolysaccharides (LPS). These large chains of molecules are themselves composed of multiple parts some of which can be used as an identifier of the bacteria species, and often enabling the distinction of specific strains (Brun et al., 2013a).

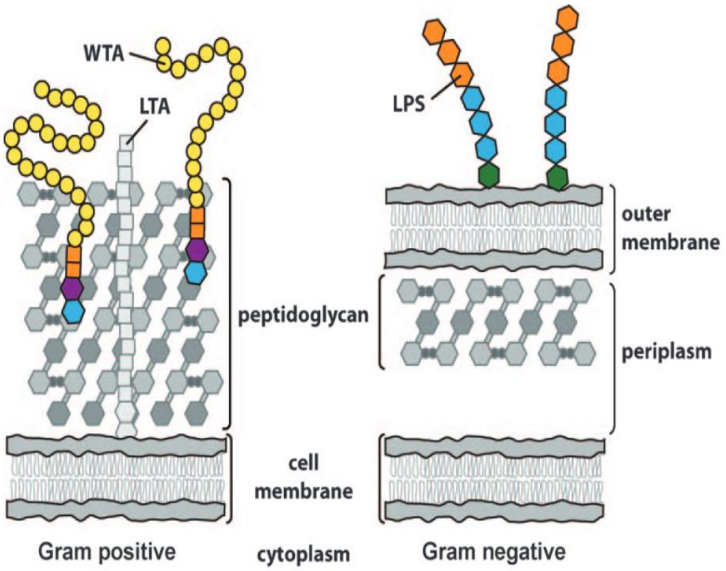

Figure 4, graphical illustration of the cell walls in Gram-positive and Gram-negative bacteria (Swoboda et al., 2009).

As presented above with the example of the LPS macro-molecules, on the molecular level, there exists numerous ways of bacterial identification. Therefore, continued investigation of these structures could drastically contribute to the development of new manipulation methods which target a certain well-studied component of the bacteria enabling rapid, reliable, and reproducible identification.

The cell wall is of particular interest since all metallic structures employed for SERS measurements will come into direct contact with it, thus disproportionately amplifying Raman signals sourced from that area.

1.1.4 Functionalization

As previously mentioned, SERS is a phenomenon that arises from the local electromagnetic fields around metallic structures with nano-features. The electromagnetic field around said metallic structures decreases with the square of the distance, meaning that if we increase the distance between the analyte and the metallic surface by a factor of x the field consequently decreases by a factor of x^2 (Moskovits, 1985).

In order to assure proximity between the analyte and the metallic structures used, we employ a chemical agent which bound to the nano-metallic structures and at the same time, bound to the bacterial surface. This forms a "physical link" between the metallic structures and the targeted organisms. This process is called "functionalization".

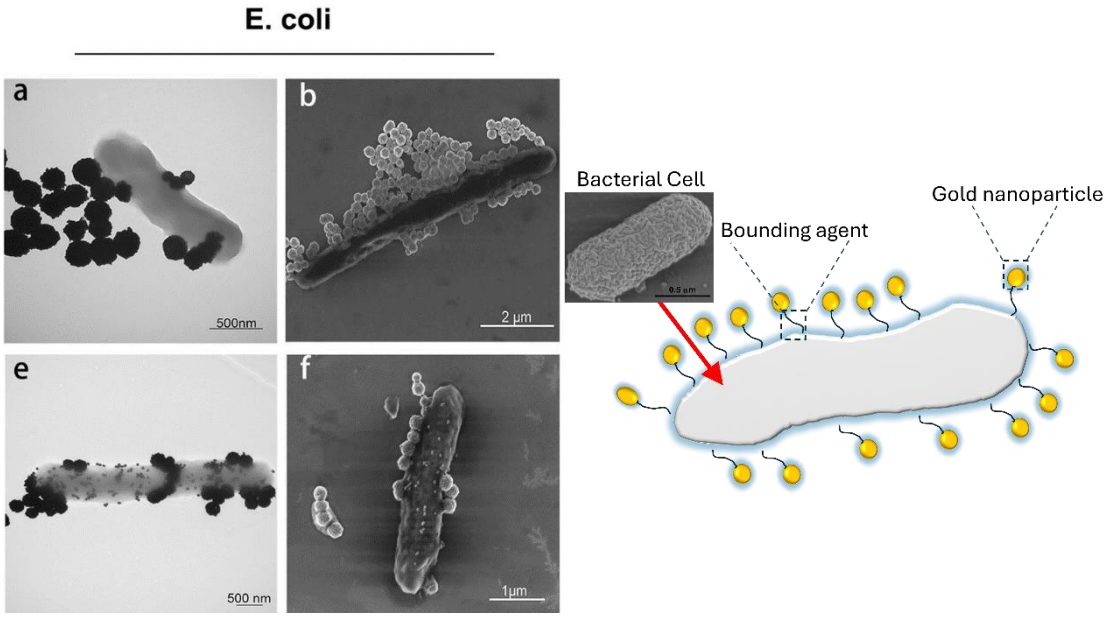

Figure 5. Illustration of the functionalized of gold nanoparticles bounding to the Bacteria (C. Zhang et al., 2018b).

2 Internship description

Due to the project's confidentiality, there is only a little that could be detailed about the objectives and achievements of the project so far as well as the tasks I completed and their results.

2.1 Bacterial detection using Raman spectroscopy

Considering the rich background described above, the aim and objectives of the project could now be explained and understood clearly.

In the context of our project, the aim is to use SERS to construct fingerprint spectra of bacteria. Using SERS over "normal" Raman scattering could help us surpass and ignore any inconsistencies that might be present in the sample. This is because, even with potential inconsistencies, the Raman signal coming out of the bacteria is amplified orders of magnitudes higher than any other signal coming from elsewhere in the sample.

As explained above, in order for SERS to be present, functionalized metallic nano-structures have to be present too. In the literature, there are many approaches to the introduction of metallic nano-structures, some use metallic wafers with nano-features and others use metallic nano-particles. The most popular approach however is to use gold nano-particles since gold is inert and unreactive which is especially important when considering that the metal chosen has to be biocompatible for it not to cause any secondary reactions within the bacterial samples.

For our project, we illuminated the bacteria using a visible-light laser and recorded the scattering spectra using a spectrometer. Again, in the literature multiple values of wavelengths were used across the visible spectrum. In our case, we experimented with multiple values of wavelengths which were 532nm, 633nm, and 785nm. An optical setup conceived by our team members directed the excitation laser rays towards the sample, and then guided the scattered photons towards the spectrometer. Sometimes, we initially conducted our measurements on a research-grade Raman microscope which was considered as the "reference" for the measurements done with our optical setup. The result treated by us is similar to the one displayed in figure 6.

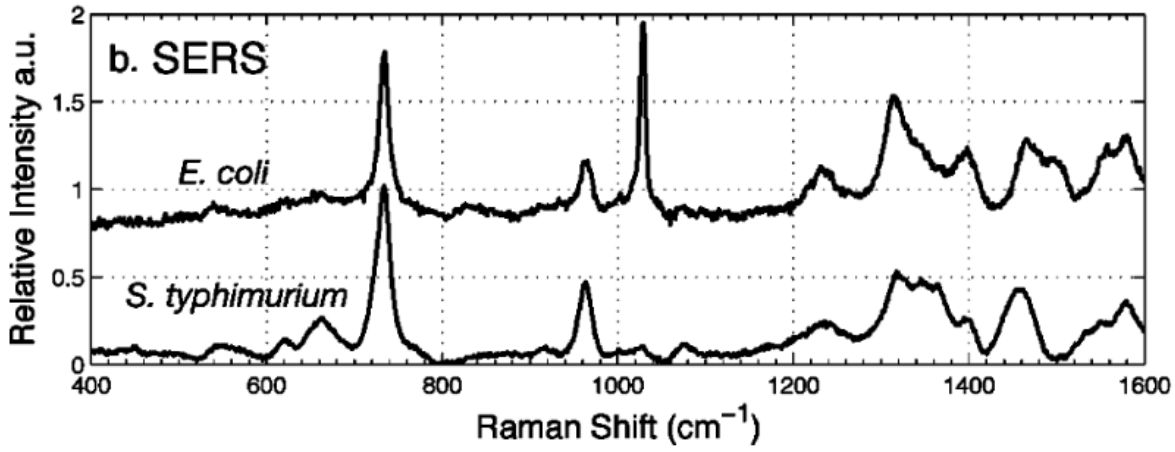

Figure 6. Representative SERS spectra of different bacteria. Upper spectrum is taken from a sample of Escherichia Coli, the lower was taken from a sample of Salmonella Typhimurium (Premasiri et al., 2004b).

Another part of the team was developing an artificial intelligence (AI) model which, as explained above, played a major role in in the data analysis part. After training sessions, the AI model was then given these spectra and based on multiple factors such as the Raman shifts of certain peaks and their respective intensities, it could make decisions regarding the bacterial strain in question.

2.2 my role in the project

During the course of two months, I got the opportunity to work on multiple fronts and engage in nearly every aspect of the project.

First, I created a detailed 40-page summary of the chemical composition of bacteria. I studied the structure of bacteria and the compositional characteristics that distinguish one species from another in hopes of better understanding the sometimes-subtle differences between the spectra of different bacteria.

The summary I carefully put together helped us make more pronounced decisions regarding the chemical agents we used in the functionalization process. Additionalsly, it also contributed to the understanding of the specific characteristic such as the location of peaks and their intensities in the Raman spectra that we collected. The knowledge gained also inspired the initiative to investigate certain chemical compounds esteemed to be of great importance to the process of identification of the bacterial strains.

These details, which mainly concern the microbiology of the bacteria, if well-understood and their implication on the Raman fingerprints correctly identified, and taught to our AI model, could be revolutionizing in the detection process.

Secondly, I worked closely with sample preparation as well as taking measurements. Our samples were prepared on reflective metal wafers in order to reduce the amount of absorbed excitation photons which could lead to undesired effects and potentially decreasing the detection efficiency. I prepared bacterial samples for conventional Raman scattering measurements and SERS measurements.

Additionally, I also prepared samples of pure chemical compounds whom Raman spectra were investigated to enhance our knowledge and understanding of their role in what we were observing in the spectra collected from bacteria. It is notable to mention that the chosen chemical compounds which were investigated were selected based on my judgement taking into account the information I collected about the bacterial structural composition.

Thirdly, I worked on gold nano-stars synthesis. These nano-stars were then experimented with in order to optimize functionalizing techniques for the context of our project. Optimization revolved around finding the perfect order to execute the steps required to achieve the functionalization process, finding the perfect environment in terms of temperature, pH, and reagent concentrations for each step, and finding the ideal storing conditions.

This optimization process is particularly important since at the time of experimentation with the prototype and even when using the final device, it would be important to have the functionalized metallic nanostructures ready beforehand to be able to directly introduce the food sample on them and proceed with measurements. In addition to being ready, the functionalization has to be stable and reproducible to guarantee consistent results.

3 Summary

Prior to completing most of the tasks I was responsible for, I had to develop a set of skills and gather information about the relevant topics required for the task's completion.

Firstly, I spent about 10 days reading in the literature about Raman scattering, SERS, bacterial detection, and the different approaches there are to it. Secondly, when compiling the microbiology summary, I had to study the domain from the ground up as I had practically had zero background in biology. Additionally, when taking measurements and preparing samples, I had to learn how to use the lab equipment such as the Raman microscope mentioned in section 2.1 of this report.

Moreover, within my experimentation with functionalization and gold nano-stars I underwent a training enabling me to correctly use the equipment present in the biology lab. Combining this training with the experience I gathered while working there has enabled me to build a wide skillset encompassing many domains that I, in my field of study, am not very familiar with.

Ultimately, this research internship exposed me to additional important aspects of research such as teamwork, defining goals and objectives, time planning, interpersonal communication, critical thinking in experiment design and in deducing conclusions.

3.1 Future outlook

After a very successful internship at LQNO, I look forward to staying in contact with the group for potential collaborations in the future.

4 References

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