Challenge Based Innovation CBI.ATTRACT program

Project Report

B-QuaD

Instantaneous, automatic, and precise Diagnosis of Bacterial Infections utilizing Computer Vision techniques on Fluorescent Imaging with Biosensors via integration of Confocal Microscope with Superconducting Nanowire Single-Photon Detector (SNSPD).

Team MicroQuaD

Academic Year 2022 - 2023

July 7, 2023

Eugeniu Miron[1]

Dipartimento di Scienze Aziendali - DISA
Alma Mater Studiorum - Università di Bologna

Fabio De Lisio[1]

Dipartimento di Economia e Management - DEM
Università degli studi di Ferrara

Francesca Canestra[1]

Dipartimento di Chimica “Giacomo Ciamician” - CHIM
Alma Mater Studiorum - Università di Bologna

Lorenzo Moretti[1]

Dipartimento di Fisica e Astronomia “Augusto Righi” - DIFA
Alma Mater Studiorum - Università di Bologna

Shankho Boron Ghosh[1]

Dipartimento di Ingegneria “Enzo Ferrari” - DIEF
Università degli studi di Modena e Reggio Emilia

Shola Oshodi[1]

Dipartimento di Informatica Scienza e Ingegneria - DISI
Alma Mater Studiorum - Università di Bologna

[1]Equal contribution.

Index

The Team

Figure 1: *The interdisciplinary team.*

From left to right, the interdisciplinary team is composed of:

- Shankho Boron Ghosh - Computer Engineering (Artificial Intelligence Applications) (*UniMoRe*);
- Lorenzo Moretti - Astrophysics and Cosmology (*UniBo*);
- Francesca Canestra - Photochemistry and Molecular Materials (*UniBo*);
- Shola Oshodi - Artificial Intelligence (*UniBo*);
- Fabio De Lisio - Green Economics and Sustainability (*UniFe*);
- Eugeniu Miron - Business Administration (*UniBo*).

Introduction

Throughout the *CBI.ATTRACT* program, the technology assigned to the team had been MicroQuaD (hence the team's name), which consists of two different components:

- a Superconducting Nanowire Single-Photon Detector (*SNSPD*), provided by the Dutch company *Single Quantum*® and capable of detecting single light particles (photons);
- a confocal microscope, provided by the German company *PicoQuant*®, designed to detect the fluorescent signals emitted by the analyzed samples.

According to the Tech Card given to the team by the research group, the integration of these two technologies has the aim of investigating material science and discovering new microscopy techniques.
The task of MicroQuaD had been to discover alternative and new potential applications of this technology by exploiting the strengths of both the SNSPD and the confocal microscope. In order to address the challenge, the team underwent three different stages of work, each marked by a milestone: the *Discovery, Design* and *Prototyping* phases.

The first section of this report will be dedicated to the explanation of the technology, while the following will be about the work carried out by the team, starting from the very first stage and up to the last.

The *Discovery* Phase

During this first phase, the technology had been studied in order to understand the physical processes that define its function and characterize its strengths. The study consisted of:

- researching the scientific literature to understand the state of the art of the technology and its current utilization in the market;
- online research about the scientific knowledge on which the *SNSPD* technology rests. This activity included research on the company's website, *Wikipedia* pages, and other sources of information;
- research concerning partners and competitors, but also on the *Single Quantum*® website, in order to understand the market sectors in which the company is operating and the opportunities they are keen on exploring.

The whole study had been documented on *Miro*®, a real-time collaboration platform, in which the team designed a mind map to collect and organize the information acquired.
Figure 2 shows the MicroQuaD team working to create and complete the mind map on *Miro*® in order to move to the second step: *diverge to innovate.*

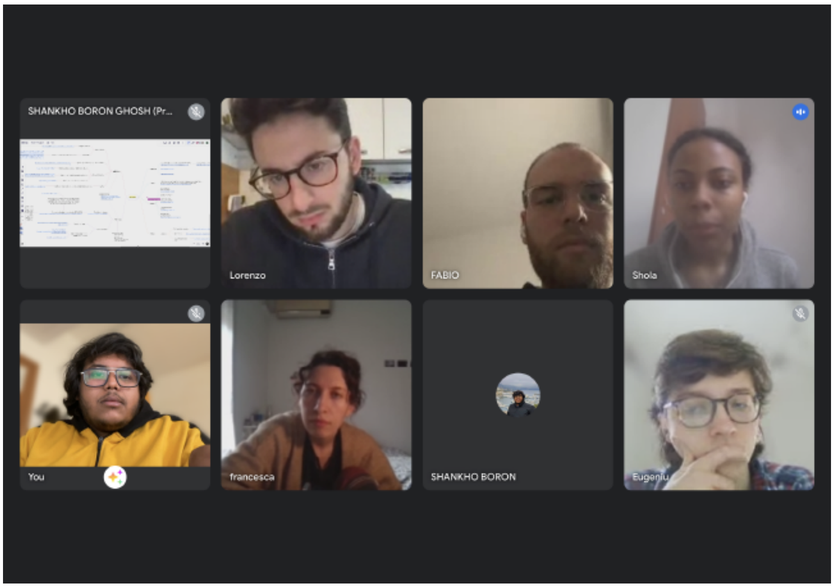

Figure 2: *The team working on the mind map on Miro*®.

During this phase of the project, *Mirco Tincani*, a research fellow at *UniMoRe* specialized in *Matter Physics*, gave crucial assistance on the understanding of the the technology. Thanks to his help, the team had been able to gain some useful insights on the physical functioning of SNSPDs.
After understanding the technology, the next challenge was to delve into the ideas to find alternative uses of the technology, taking into consideration its characteristics. To complete this task, the team designed a divergence map on *Miro*® to define the new possible fields of application and opportunities. Subsequently, the map was upgraded during the *Collision Week* at *IdeaSquare CERN* (which took place from 26/03/23 to 31/03/2023), where different team building dynamics activities were carried out to invoke exponential thinking (*Figure 3* and *Figure 4*). Moreover, the team had the opportunity of networking with some of the *CERN*'s scientists and staff (*Figure 5*), who gave useful insights on the technology.

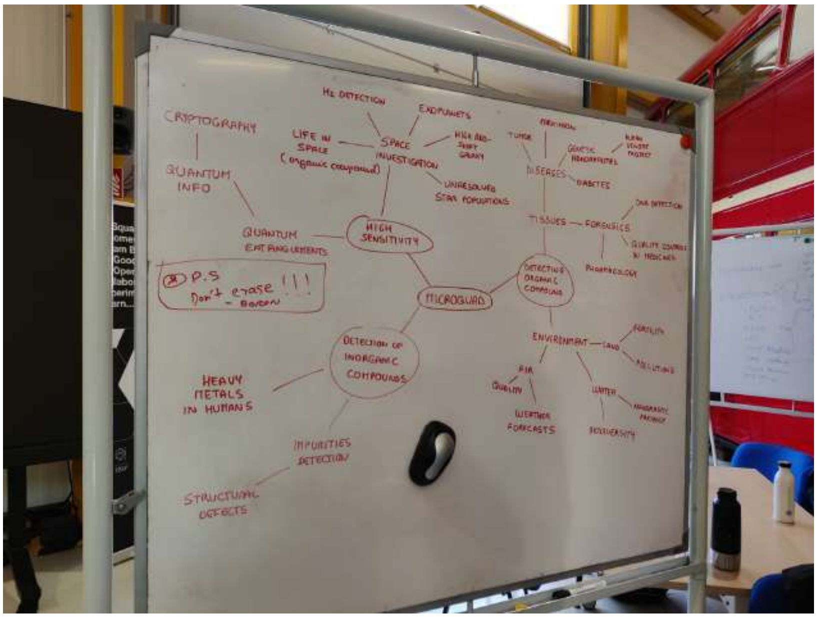

Figure 3: *The divergence map created at IdeaSquare.*

During the group dynamic activities, the ideas which had been thought of throughout the divergence process were presented to the whole *CBI.ATTRACT* student group, with the aim of receiving useful feedback and finding other opportunities worth exploring.

Figure 4: *The team working in the famous red double-decker bus at IdeaSquare.*

Figure 5: *The team networking with other students and the quantum expert Michael Doser at IdeaSquare.*

The Collision Week at *CERN* was fruitful in terms of ideas generated and knowledge acquired. In fact, it enabled the MicroQuaD Team to individuate the first five possible new applications of the technology (*Figure 6*): the detection life indicators in exoplanets (*Astrobiology* scenario), the identification of impurities in drugs (*Quality control in medicine* scenario), the assessment of the resistance of patients' immune systems to medicines (*Antimicrobial Resistance* scenario), the quality control of 3D printed organs (*3D bioprinted organs* scenario) and the monitoring of HIV in patients through their breath (*HIV monitoring* scenario).

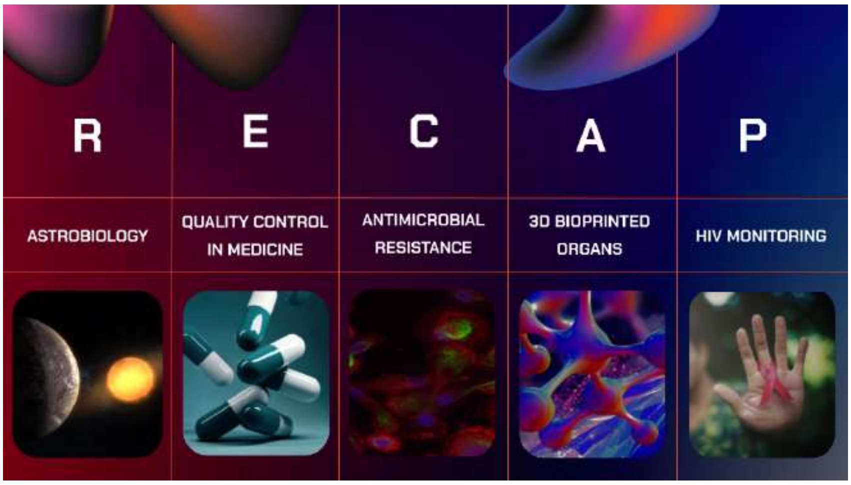

Figure 6: *The five opportunities presented during the first Milestone event.*

On the 14th of April 2023, the team interviewed *Fabio Del Sordo* (astrophysicist) to further understand the feasibility of the astrobiological application. He suggested exploring the opportunity of utilizing the SNSPD technology to detect auroras in exoplanets in order to infer the possibility of the latter to host life.

The team worked on the aforementioned opportunities up to the first milestone event (19/04/2023), during which they were presented to the *ATTRACT* group and to different researchers/representatives from the partner companies, such as *Benedetta Valerio* (Marketing & Business Development) from *Single Quantum®*. After a long discussion on the chosen five opportunities, she requested a summary of the scientific papers that led the team to present those applications in order to help individuate the most feasible one.

On the 21/04/2023, some of the team members interviewed *Vincenza Andrisano*, tenured professor at *UniBo* and coordinator of the *Pharmaceutical Chemistry* Master's degree course (*Figure 7*). The professor gave useful insights on the quality control (*QC*) of drugs application, and suggested utilizing the technology in the QC process instead of the *HPLC* process (*High-Performance Liquid Chromatography*). This change would avoid the dissolution of the drug and, thus, saving time for the preparation of the sample.

Figure 7: *Interview to professor Vincenza Andrisano.*

Later, on the 24/03/2023, the MicroQuaD team discussed with *Benedetta Valerio* and *Hein Zijlstra* (Application Engineer) from *Single Quantum®* the feasibility of the five potential applications, based on the literature summaries previously provided. The meeting narrowed us down to two applications: quality control in drug production and antimicrobial resistance (*AMR*).
On the 02/05/2023, the team interviewed *Simone Ambretti*, a researcher at *UniBo* and clinical microbiologist at the *Sant'Orsola Hospital* in Bologna, and discussed with him about the *AMR* opportunity. The discussion was useful to get both theoretical and practical insights into the field of antimicrobial resistance. The researcher was very interested in the technological opportunity proposed by the team since Italy is among the top *EU* countries in terms of antibiotic consumption (*ECDC Annual Epidemiological Report*, 2022). In fact, he suggested using the MicroQuaD technology to detect bacterial infections in blood samples to formulate more precise medical diagnoses and antibiotic treatments for patients.

The *Design* Phase

The aim of this phase is to converge to one of the two applications previously chosen and build a *pretotype*, which is a rough version of a future official prototype. To address these goals, the team started identifying potential stakeholders involved in the scope of the technology and identify their needs as well as how the way they would interact with it, for both applications.
For the quality control in drug production, the identified stakeholders were: the *CEOs* of pharmaceutical companies, the project managers, the supply chain control managers, the analytical/industrial chemists, the clinical engineers, the control technicians, and the procurement department managers. To identify their actual needs, on the 10/05/2023 an interview with a quality control analyst was carried out. The interviewed specialist, *Bruno Dalla Torre*, is a quality control analyst in *FIS* (*i.e. Fabbrica Italiana Sintetici*) in the production plant of Termoli. The discussion revealed the presence of some problems in the application of the technology to the quality control of drug production:

- the production of drugs takes place via different steps, each of them characterized by a different quality control protocol. Therefore, a problem would lie in precisely identifying the step of the production chain in which the technology can be applied;
- quality control protocols are very strict; indeed, they specifically dictate which instrument and substances the chemists must make use of. The former is written and approved by the *I.I.S.* (*i.e. Istituto Superiore di Sanità*), which defines specific standards. The protocols are contained in the *Official Pharmacopeia* of the Italian Republic. Then, a long amount of time would be required to introduce the technology into the market since it must undergo numerous tests and experiments, carried out by both the Government and private companies.

On the 12/05/2023, another meeting with *Simone Ambretti* took place at the *Clinical Analysis Laboratory* of the *Sant'Orsola Hospital* in Bologna. There, he explained how blood analyses are carried out, from the beginning to the formulation of the patient's results, also showing (partially) the laboratory and the machinery needed for this (*Figure 8*). The interview was also useful for understanding which actors would be involved in the utilization of the technology and how this can be physically implemented in a clinical laboratory.

Figure 8: *The Clinical Analysis Laboratory in Sant'Orsola Hospital, Bologna.*

The blood cultures' results are ready after approximately two to five days from the receiving of the sample. This implies that patients are treated with generic antibiotics in the meantime, leaving a high margin of uncertainty regarding the right treatment to administer. *Simone Ambretti* underlined the urgent need to contrast *AMR* by getting negative results from the blood cultures as well so that doctors can interrupt the patients' antibiotic treatments. Moreover, he highlighted the difficulty of the laboratory in processing a large amount of blood samples, since it is the only laboratory appointed in analyzing samples in the city of Bologna and its whole province.

From 15/05/2023 to 18/05/2023, the MicroQuaD team visited the *Single Quantum®* headquarters in Delft (NL) to discuss with the company's researchers and gain a broader understanding of the technology. In particular, on 16/05/2023 the researchers talked to the team about the capability of the technology and the feasibility of the presented opportunities. Furthermore, *Benedetta Valerio* and *Mario Castaneda* (Research Manager) gave a tour of the laboratories to show the SNSPD technology and its functioning (*Figure 9*). Moreover, they allowed the team to enter the R&D laboratory in order to present and discuss with *Martin Caldarola* (Application Scientist) the effective functioning of the MicroQuaD technology.

The visit was very fruitful, as it gave the physical perception of how the technology should be set up inside a laboratory: it requires space and full darkness and it is provided with a compressor which regularly produces a quite uncomfortable noise - thus it should be positioned outside the room or in a more convenient place, where it does not create discomfort. We

Figure 9: *The MicroQuaD team with the Single Quantum's staff in Delft.*

were also able to witness multiple steps conducted during the manufacturing and servicing of SNSPD and were shown a live process of calibration in a dark room, in which a bias current had to be adjusted to measure the dark count rate by aligning an attenuated light source to the SNSPD's input. This allowed the measurement of the efficiency of the device by creating a calibration curve relating the incident photon flux to the detector's output to calculate its noise and analyze the data to benchmark the device.

On the 24/05/23, the team started to work on the presentation for the second milestone by individuating the *user persona*, which is a general representation of a fictional character who will interact with the technology. The description of the user persona highlighted: the job and its related field, their goal and approach to the work, the personality, and a phrase that summarizes the strong points. Moreover, the desirability and feasibility of the two opportunities were considered in order to narrow down to one of the two (see *Table 1* and *Table 2*).
At the end of this phase, the team decided to focus on the fast diagnosis of bacterial infections, as it is the most promising application in terms of feasibility and desirability.

Desirability
It allows to bypass the dissolution phase
It reduces waste generated by drugs analysis
It enables the study of drugs with complex biological matrices
It enables the detection of even small quantities of impurities

Feasibility
MicroQuaD has the capability to analyze drugs that contain fluorescent components
BUT
Many safety protocols must be followed
Difficulties in integrating it into the quality control production pipeline

Tab. 2: *Desirability and Feasibility for the QC application.*

Desirability
The analyses can be performed on small quantities of blood
Less economic burden on healthcare infrastructures
Rapid, almost instantaneous diagnosis for bacterial infections
Saves lives by enabling patients to receive immediate and effective therapy

Feasibility
The binding of biosensors to bacteria results in emission in the near-infrared (NIR) range, which can be detected by MicroQuaD

Tab. 3: *Desirability and Feasibility for the AMR application.*

The *Prototyping* Phase

During the third and last phase of the project, the team started to elaborate and design a prototype to be presented during the Student Expo on the 16/06/2023, and to the final milestone 3 event on the 30/06/2023, both held in Bologna. Starting from the MicroQuaD technology, and throughout the previous working phases, a new name was given to the technological solution: *B-QuaD*, which stands for *Bacterial Quantum Detection*.
On the 16/06/2023, the *CBI.ATTRACT* students and other student teams from the *ATTRACT Academy* gathered together to present their current projects and achievements. Moreover, every team exhibited their prototype in order to get feedback from professors and experts (*Figure 10*).

Figure 10: *The team presenting their work and prototype at the Student Expo.*

After the *Student Expo*, the team started working on the final presentation for the 30/06/2023. During this last day, they presented their final work (*Figure 11*), enriched with data about AMR and information about the prototype.

Figure 11: *The MicroQuaD team during their final presentation.*

After the presentation, all the teams moved into the expo area in which they had the opportunity to showcase their final prototype (*Figure 12*).
The prototype exhibition was followed by a feedback session from the team's *ATTRACT* project partners in single breakout rooms, with the aim of discussing the future of the opportunity presented. The MicroQuaD team had a session with the *UniMoRe* Professor *Bernardo Balboni*, *Clio Dosi* and *Mirco Tincani*, in which they discussed the potential future of the *B-QuaD* technology and the possibility of acquiring new project partners.

The specifications of the prototype as well as some data concerning the AMR will be given in the next paragraphs.

Figure 12: *The MicroQuaD team demonstrating the prototype after final presentation.*

Data on Antimicrobial Resistance

According to the study published in 2022 by *The Lancet*, among the 13.7 million deaths that occurred in 2019 caused by infections, 7.7 million were attributed to antimicrobial resistance, placing AMR as a second cause of death globally behind ischemic heart diseases. The *OECD* (2018) estimates that, given the current AMR death rates, the United States, Italy, and France will have the highest absolute death rate by 2050.

In Europe, the estimated healthcare cost for fighting AMR is €1.1 billion (`https://encr.pw/Txdbm`). This high expenditure is explained by the inefficient response of an infection to a first-line antimicrobial treatment, which leads doctors to switch to more expensive and, hopefully, more efficient treatments, leading to longer hospital stays, further increasing healthcare costs. It follows that the estimated cost of a multi-resistant bacterial infection ranges between €8500 and €34000 (*OECD*, 2017 `https://encr.pw/2FHgJ`).

Better healthcare policies are required to fight the AMR challenge. The *OECD* (2019, `https://l1nq.com/YxHAl`) estimates that a per capita investment in a mixed package of healthcare policies of €1.5 per year would save 27,000 lives leading to reduction of €1.4 billion per year.

User Experience Mockup

A tangible demonstration was developed and presented at the *ATTRACT Student Expo* and Milestone 3 in order to showcase the capabilities of our diagnostic solution, focusing on simulating the user experience of a microbiologist dealing with the analysis of blood samples (*Figure 13*). The goal was to provide participants with an immersive experience that emulates the workflow and tools used by microbiologists and illustrates two key aspects: fluorescence simulation on a blood sample using fluorescein and result inference generated by a *Raspberry Pi*-based computing system.
The demonstration first involved showcasing the fluorescence of the blood sample through the microscope. This was achieved by implementing a simulated fluorescence effect on the sample, allowing participants to observe the characteristic fluorescence patterns associated with specific biomarkers or indicators. In particular, in order to do so, UV light was used to excite the fluorescein molecules and to observe the fluorescence emission resulting from the interaction between the fluorescein and the excitation light. Secondly, the experience focused on the use of the *Raspberry Pi*-based computing system, which played a crucial role in providing the result inference: it processed the data acquired from the microscope and generated the final diagnostic report. By interacting with this system, participants were able to gain insights into how the collected data could be analyzed and interpreted to yield meaningful diagnostic information.

Figure 13: *Setup of the user experience at the ATTRACT Student Expo.*

During the demonstration, the participants were instructed on the proper procedure for the preparation of slides. Synthetic blood was utilized for this purpose, and a drop was placed on the slide. To simulate the fluorescence produced by biomarkers, fluorescein was imployed, as it is a widely used fluorescent tracer in various applications.
After this first step, the prepared slide was meticulously positioned on the mechanical stage of a microscope, ensuring its stability and proper alignment for observation. To facilitate optimal visibility of the fluorescence, a key aspect of the demonstration, the room was deliberately dimmed. This adjustment created an environment where the fluorescein-induced fluorescence could be effectively observed (*Figure 14*). The dimmed room not only enhanced the visual impact for the participants, but also replicated a real-life scenario where fluorescence analysis is typically conducted. Furthermore, this environment is crucial for the use of Superconducting Nanowire Single-Photon Detectors (SNSPD) in practical applications, as it minimizes external light interference and enhances the sensitivity of the detector to capture even the faintest fluorescence signals. By replicating these conditions during the demonstration, participants gained an even more authentic experience and a deeper understanding of the significance of controlled lighting conditions in fluorescence analysis, particularly in conjunction with SNSPD technology.

Figure 14: *Fluorescent sample analyzed under the UV light at the ATTRACT Student Expo.*

Participants were subsequently encouraged to engage with a *Raspberry Pi*-based computing emulator, which played a crucial role in the demonstration (if interested in the implementation aspects of the *Raspberry Pi*-based computing emulator system, one can access the source code on *GitHub*; the repository is available at the following link: `https://github.com/growupboron/microquad-amr`). By pressing a button, this system accurately mimicked the behavior of a Superconducting Nanowire Single-Photon Detector (SNSPD). Leveraging the computational power of the *Raspberry Pi*, the system efficiently emulated the entire process of data processing and analysis, ultimately generating a comprehensive diagnostic report and teaching the participants the significance of computational data processing in the diagnostic process (*Figure 15*).

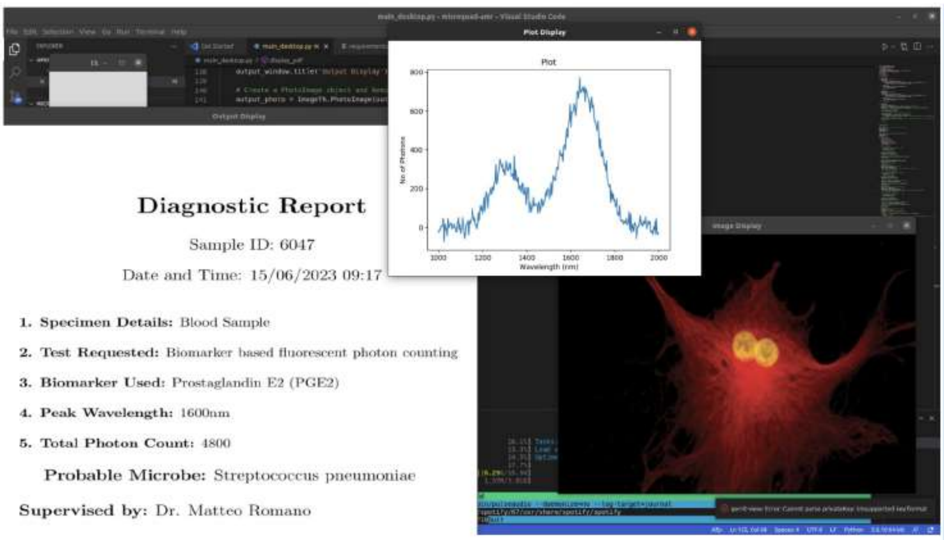

Figure 15: *User Experience front-end with Diagnostic report, SNSPD raw data, and Hyperspectral image.*

The demonstration aimed to address the current drawbacks associated with conventional blood culture and polymerase chain reaction (*PCR*) techniques, which are known for their time-consuming nature. Blood cultures, although widely used for detecting bacterial infections, often require several days for the growth and identification of microorganisms. This extended waiting period can delay the initiation of appropriate treatment, potentially leading to worse patient outcomes. Similarly, PCR, while highly sensitive and specific, typically involves multiple steps and can take hours to complete. This extended processing time can hinder the efficient diagnoses and management of infectious diseases. In contrast, the proposed techniques showcased in the demonstration offer the potential for faster and more efficient diagnostics. The fluorescence approach, using an integrated system of a confocal microscope and SNSPD combined with a computing system, enables real-time observation and rapid result inference. By reducing the time required for analysis and report generation, these techniques have the potential to revolutionize the field of diagnostics, enabling healthcare professionals to make informed treatment decisions more promptly. Furthermore, by mitigating the limitations of conventional techniques, the proposed alternatives have the potential to empower the fight against antimicrobial resistance.

Potential Implementation Framework

In conjunction with our vision of *B-QuaD*, a proposed implementation pathway was developed with a strong emphasis on real-time detection (*Figure 16*). This framework is designed to enable industrialization and scalability, addressing the challenges associated with the high procurement and operating costs of SNSPDs by leveraging economies of scale.

The implementation pathway begins with the acquisition of a patient blood sample awaiting diagnosis. To maximize efficiency and throughput, the sample is sub-sampled into multiple slides, each treated with different biosensors targeting specific regions of the near-infrared electromagnetic spectrum. This approach allows simultaneous analysis of multiple subsamples and reduces the time required for sample processing. The prepared subsamples, ready for fluorescence analysis, are then subjected to an integrated confocal microscope and SNSPD system. Once placed on the mechanical stage of the microscope, the relevant photon sources are imployed to induce fluorescence. The SNSPDs, strategically arranged in a spatial grid layout, detect and count the fluorescent photons emitted from each subsample across different wavelengths in real-time. This real-time detection capability allows for rapid and continuous monitoring of the fluorescence signals, significantly reducing the time required for analysis. To achieve this massive throughput, the outputs from the spatial grid layout are efficiently recombined across the multiple subsamples, enabling hyperspectral fluorescent imaging. This imaging technique provides a comprehensive view of the sample, with each channel input of the SNSPD corresponding to a specific biosensor and wavelength range. By capturing a wide range of fluorescence information simultaneously, the *B-QuaD* system accelerates the detection process and enhances the accuracy of bacterial identification.

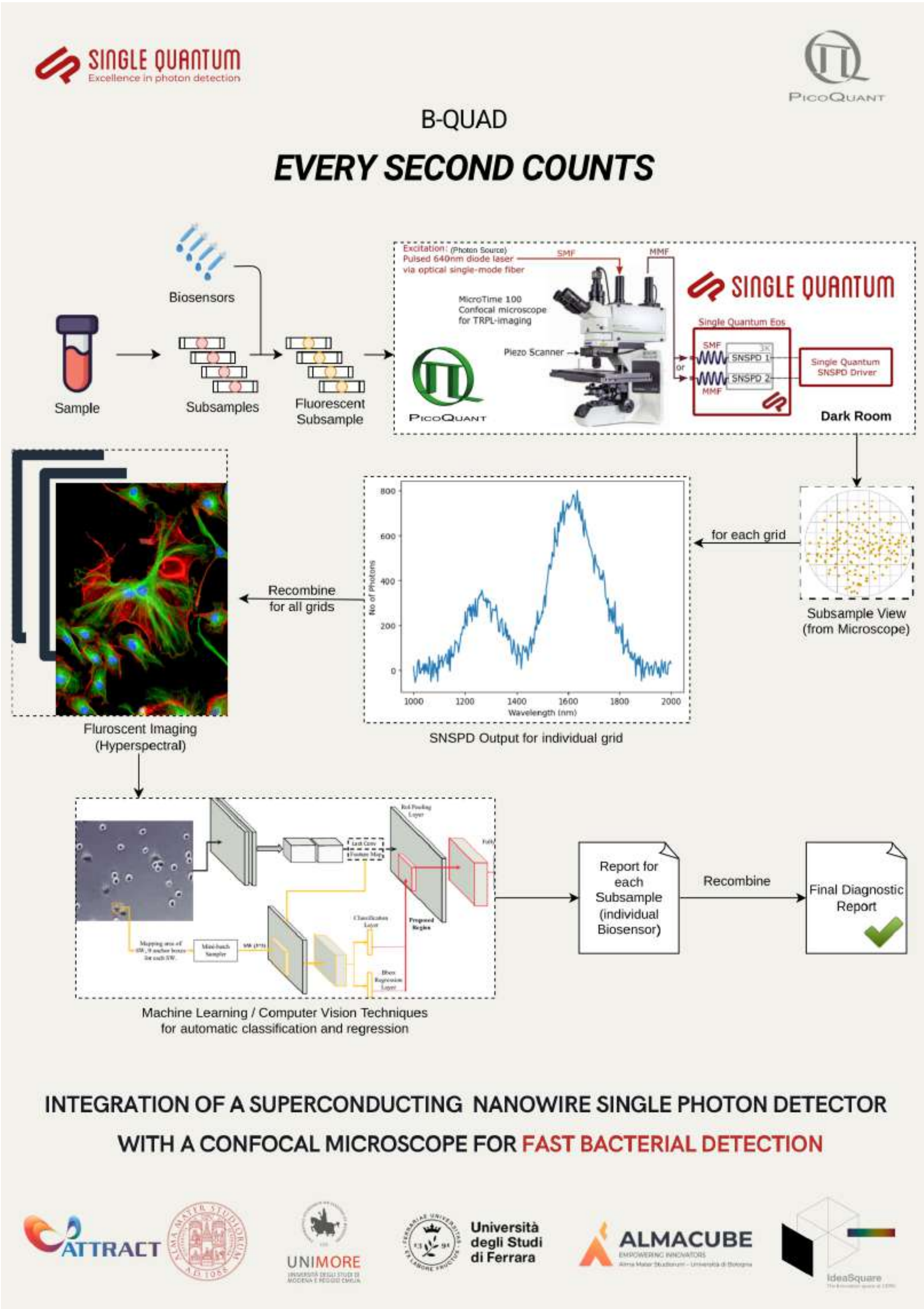

Figure 16: *Implementation pathway.*

Industrialization and scalability are critical aspects of the proposed implementation pathway for the *B-QuaD* system. To achieve these goals, the workflow is optimized and automation is leveraged to enable integration into a high-throughput diagnostic pipeline. The system utilizes multiple slides and biosensors, allowing for parallel processing and enhancing throughput. Real-time detection capabilities, combined with advanced computer vision techniques and deep learning algorithms, enable the rapid analysis of large volumes of data.
To facilitate industrialization, the *B-QuaD* system must incorporate modular components and standardized protocols. This approach ensures consistency and reproducibility across different laboratories, simplifying the adoption of the system and supporting its widespread implementation in clinical settings. By continuously optimizing the system, making iterative improvements, and collaborating with industry partners, the proposed implementation pathway establishes a foundation for the industrial-scale deployment of *B-QuaD*. This deployment has the potential to revolutionize bacterial detection by providing rapid and accurate results, empowering the fight against antimicrobial resistance.

Technical Considerations for Future Improvements

1. **Biosensor selections and compatibility**: one important research gap is determining the compatibility and combinability of different biosensors used in the *B-QuaD* system. Investigating the compatibility of various biosensors for simultaneous use in detecting multiple bacterial species or strains can help optimize diagnostic capabilities. Additionally, careful selection of the photon source for fluorescence excitation is crucial to ensure efficient and specific excitation of the biosensors. Exploring different photon sources and evaluating their effectiveness in exciting biosensors is an important area of research.

2. **Spatial resolution of each grid**: the spatial resolution of the grid layout used by the SNSPD is another technical constraint to consider. Determining the optimal resolution of each grid on the SNSPD system is crucial to capture fine details of the fluorescent signals emitted by the biosensors. Balancing the resolution requirements with the practical limitations of the SNSPD technology is an ongoing challenge.

3. **Photon count and imaging constraints**: understanding the limitations associated with photon count and its impact on imaging is an

important consideration for the *B-QuaD* system. SNSPDs are highly sensitive to individual photons, enabling precise detection. However, the count rate and limitations on the number of detectable photons per unit of time should be taken into account when designing the system. Evaluating the trade-off between photon count, imaging speed, and signal-to-noise ratio is crucial to optimize the imaging capabilities of the system. Additionally, the imaging constraints, such as the dynamic range and signal saturation, must be carefully addressed.

4. **Complexity of hyperspectral image analysis**:

   - the analysis of hyperspectral fluorescent images generated by the *B-Qua*D system can be technically challenging due to the high dimensionality and complexity of the data. Developing efficient algorithms and computational methods to extract meaningful features and classify bacterial infections accurately from these images is an ongoing research area of research;
   - the performance of supervised machine learning algorithms, such as convolutional neural networks (*CNNs*) and transformer-based models, heavily rely on the availability of large and diverse labeled datasets. However, creating comprehensive and representative datasets for bacterial infections can be challenging, as they require extensive data collection, annotation, and expert knowledge. Bridging this gap by establishing standardized datasets can contribute to more robust and generalizable models;
   - while fluorescence analysis has proven to be a valuable tool in bacterial detection, there may be research gaps regarding the distinct fluorescence patterns exhibited by different bacterial species or strains. Further research is needed to enhance our understanding of these patterns and their correlation with specific bacterial infections;
   - development of deep learning model for automatic bacterial classification:
     - in particular, *CNNs* and unsupervised learning algorithms can be imployed to classify bacterial infections directly from the hyperspectral fluorescent images generated by the system. *CNNs* have demonstrated remarkable performance in image classification tasks, and by training them on a dataset of labeled images, they can learn to recognize specific bacterial patterns and provide accurate classifications in real-time.

Alternatively, unsupervised learning algorithms can extract meaningful features from the data without the need for labeled examples, allowing for the discovery of patterns and relationships that may not otherwise be apparent;

- one such option is transformer-based models, such as the Vision Transformer (*ViT*) architecture. Transformers have demonstrated exceptional performance in various computer vision tasks, including image classification. By leveraging self-attention mechanisms, transformers can capture global dependencies within the hyperspectral images, enabling accurate classification of bacterial infections. These models can be trained using supervised learning techniques on labeled datasets, allowing them to learn complex patterns and achieve high classification accuracy;
- another option is generative adversarial networks (*GANs*) combined with unsupervised learning. *GANs* consists of a generator and a discriminator network that work in tandem. The generator network produces synthetic images that aim to resemble real bacterial samples, while the discriminator network evaluates the authenticity of these generated images. By iteratively training the networks, *GANs* can learn to generate realistic bacterial samples and also identify genuine bacterial patterns from the real hyperspectral images. This unsupervised approach enables the discovery of hidden features and relationships in the data, enhancing the system's ability to accurately classify bacterial infections;
- by exploring these state-of-the-art computer vision techniques, including transformer-based models, *GANs*, and other emerging algorithms, the *B-QuaD* system opens up avenues for advanced classification and analysis directly from the hyperspectral fluorescent images. This diversification of options allows for the selection of the most suitable approach based on the specific requirements and characteristics of the bacterial detection problem at hand.

5. **Benchmarking and Validation Parameters**: defining appropriate benchmarking and validation parameters is essential to assess the performance and reliability of the *B-QuaD* system. These parameters may include metrics such as sensitivity, specificity, accuracy, and positive predictive value. Establishing standardized benchmarking protocols and performance metrics will enable meaningful comparisons between

different implementations of the *B-QuaD* system and facilitate the objective evaluation of its diagnostic capabilities.

6. **Standardization and reproducibility of experimental protocols**: ensuring consistent and reproducible experimental protocols is crucial for the industrial-scale deployment of the *B-QuaD* system. Establishing standardized protocols for slide preparation, biosensor utilization, imaging, and data analysis can help minimize variations and ensure the reliability of results across different laboratories and settings.

7. **Cost and accessibility of SNSPDs**: the SNSPD technology plays a vital role in the *B-QuaD* system, but can be associated with high procurement and operating costs. Overcoming these cost constraints and making SNSPD technology more accessible to a wider range of research and healthcare institutions is an important consideration for scaling up the system.

8. **Comparison with low-cost implementation using Photomultiplier Tubes (PMT)**: comparing the performance and cost-effectiveness of the *B-QuaD* system with a low-cost implementation using *Photomultiplier Tubes* (PMT) is a relevant consideration. While SNSPD offers superior sensitivity and detection capabilities, PMTs can be a more affordable alternative for certain applications. Conducting comparative studies to evaluate the performance, cost, and scalability of the *B-QuaD* system with SNSPD and PMT-based implementations can provide valuable insights for different resource settings and budget constraints.

9. **High-temperature SNSPDs**: the current implementation of the *B-QuaD* system utilizes conventional SNSPD technology. However, there is ongoing research and development focused on high-temperature SNSPDs, which operate at higher temperatures and can offer improved performance and cost-effectiveness. Exploring the use of high-temperature SNSPDs in the *B-QuaD* system could address the challenges associated with cooling requirements and potentially enhance the scalability and practicality of the system.

10. **SNSPD in other electromagnetic ranges enabling more biosensors**: the selection of biosensors used in the *B-QuaD* system is currently limited to specific regions of the near-infrared (*NIR*) electromagnetic spectrum. However, advancements in SNSPD technology and the development of novel materials may enable the extension of detection capabilities to other electromagnetic ranges. By incorporating SNSPDs

that operate in different spectral regions, the *B-QuaD* system could support a broader range of biosensors, allowing for the simultaneous detection of multiple bacterial markers or indicators. Expanding the range of detectable biomarkers could improve the accuracy and specificity of bacterial identification, further empowering the fight against antimicrobial resistance.

11. **Integration with existing laboratory infrastructures**: integrating the *B-QuaD* system into existing laboratory infrastructures may pose technical challenges, especially when it comes to compatibility with different imaging systems, data management platforms, and electronic medical record systems. Seamless integration with existing workflows and infrastructure requires careful planning, coordination, and potential development of standardized interfaces.

In summation, the research and development of the *B-QuaD* system is not merely an opportunity, but an imperative in the context of antimicrobial resistance (AMR), which has emerged as a significant global health challenge. AMR poses a threat to the efficacy of antibiotics and the management of bacterial infections, necessitating the development of innovative solutions. *B-QuaD*, thanks to its advanced technology and disruptive capabilities, has the potential to revolutionize bacterial detection and diagnosis, playing a crucial role in combating AMR.
Finally, investing in the research and development of the *B-QuaD* system is not only a strategic choice but a necessary step in addressing the urgent challenge of AMR. By leveraging its advanced capabilities, healthcare professionals can accurately identify bacterial infections, enabling targeted treatment strategies and reducing the reliance on broad-spectrum antibiotics. The technical considerations, such as biosensor compatibility, spatial resolution optimization, photon count and imaging constraints, and algorithm development, are critical in maximizing the diagnostic potential of the *B-QuaD* system. By advancing the *B-QuaD* technology and overcoming technical challenges, we strengthen our ability to combat AMR effectively and safeguard public health.

Reflections

The aim of the project required design thinking, teamwork, and innovation: the MicroQuaD team excelled in this environment and worked hard to tackle any issue that arose. Despite some initial struggles in discovery phase, the interdisciplinarity of the team proved to be a winning factor for the success of the project. The project was organized allowing each member to flourish by employing and developing their various skills, while simultaneously learning how to trust and collaborate with one another. As none of the members were knowledgeable in design thinking, the concepts of user persona, feasibility, and other technicalities were a thought provoking endeavour. This project cultivated skills that the team will continue to practice throughout their careers, such as problem solving and collaborative skills; it also helped to refine the expertise already held by the team members. The MicroQuaD team had the chance to grow up together, with many ups and downs, while always focusing on the scope of the project and how to respect each other.

Figure 17: *The MicroQuaD team rejoicing after milestone 3.*

Conclusion

Taking on the challenge they were given, the team delved into understanding the strengths, weaknesses, and opportunities presented by the MicroQuaD technology, which combines a SNSPD and a confocal microscope, in order to identify innovative and advantageous new applications that they finally found in the *B-QuaD* system. Through engaging discussions with various stakeholders, including their tutors, their PhD mentor, their Tech Partner and professors, and researchers within and outside the university, they gained valuable insights. Additionally, extensive individual research complemented these discussions, benefiting from the team's multidisciplinary approach. This collaborative effort allowed them to narrow down different scenarios and identify a highly promising and urgent opportunity aligned with the principles of sustainable development: fast diagnostics for bacterial infection and the fight against antimicrobial resistance. AMR has emerged as a significant global health challenge, jeopardizing the effectiveness of antibiotics and bacterial infection management. Leveraging the advanced capabilities of the *B-QuaD* system, such as real-time detection, advanced computer vision techniques, and deep learning algorithms, healthcare professionals could promptly and accurately identify bacterial infections. This technology has the potential to revolutionize bacterial detection and diagnosis, playing a crucial role in addressing the urgent challenge of AMR. While topics such as biosensor compatibility, spatial resolution optimization, photon counting, imaging constraints, and algorithm development merit further consideration, they believe that investing in the research and development of the *B-QuaD* system is not only a strategic choice, but also takes a necessary step toward safeguarding public health.

Figure 18: *The CBI.ATTRACT consortium at milestone 3.*