

Design, Fabrication, and Testing of Graphene Oxide-Based Biosensing Platforms

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Statement of Contributions

Alex Xu was the sole author for Chapters 1, 3, and 5, which were written under the supervision of Dr. Mustafa Yavuz and were not written for publication.

This thesis consists in part of two manuscripts written for publication. Exceptions to sole authorship of material are as follows:

Research presented in Chapter 2:

This research was conducted at the University of Waterloo by Alex Xu under the supervision of Dr. Mustafa Yavuz. Alex Xu primarily designed and conducted the experiments in the project with consultations from Dr. Mustafa Yavuz, Ilya Belyakov, Mehrdad Irannejad, Sarah Labas, Bersu Bastug Azer, Ahmet Gulsaran, Dogu Ozyigit, Joel Pennings, and Dr. Juewen Liu, who contributed to experimental feedback, data analysis and material characterization. Alex Xu, Xenia Medvedeva, and Anna Klinkova were responsible for the synthesis of the gold nanoparticles. Alex Xu conducted the fabrication of the nanocomposite with the aid of Reza Karimi and Dr. Joe Sanderson. Alex Xu, Liena Zaidan, and Saeed Takaloo wrote the manuscripts, with intellectual input from all co-authors.

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Research presented in Chapter 4:

This research was conducted at the University of Waterloo by Alex Xu under the supervision of Dr. Mustafa Yavuz. Alex Xu primarily carried out the research and analysis, with consultations from Dr. Mustafa Yavuz, Dr. Michal Bajcsy, Liena Zaidan, and Saeed Takaloo. Alex Xu also primarily drafted the manuscripts, with support from Liena Zaidan and Saeed Takaloo for revisions and editing. This manuscript has not yet been published but is planned for submission within the next year.

As the lead author of these two chapters, I contributed to the conceptualization of the study design, conducted data collection and analysis, and prepared and submitted the manuscripts. My coauthors offered guidance and support throughout the research process and provided feedback on manuscript drafts.

Abstract

Timely and accurate disease diagnosis remains a cornerstone of effective healthcare delivery, but many current diagnostic tools, such as PCR and ELISA, require centralized laboratory infrastructure, trained personnel, and extensive processing times. These constraints limit their accessibility and scalability, especially in resource-limited settings. Biosensors offer a promising alternative; providing rapid, label-free detection with potential for point-of-care deployment. However, despite extensive academic research, most biosensor platforms struggle to transition beyond proof-of-concept due to fabrication complexity, low yield, and limited integration into scalable systems.

This thesis explores two nanomaterial-based strategies designed to address these challenges. First, I use femtosecond laser ablation to fabricate a reduced graphene oxide and gold nanocomposite substrate for surface-enhanced Raman spectroscopy (SERS). Using a 24-mer DNA sequence as a target analyte, this platform achieves a limit of detection (LOD) of 10^{-7} M of its complementary DNA. Compared to conventional colloidal SERS substrates, this nanocomposite demonstrates improved hotspot distribution and substrate uniformity, indicating its promise for scalable nucleic acid detection.

Second, I investigate boron nitride-doped reduced graphene oxide field-effect transistor (FET) biosensors, focusing on device yield and fabrication reproducibility. Across multiple batches of devices, I identify failure modes including fabrication inconsistencies, gel synthesis variability, and passivation requirements. By analyzing these trends and benchmarking our device performance against current market diagnostic tools for COVID-19, I propose practical modifications to enhance reliability and consistency.

Collectively, these projects demonstrate how laser ablation-based defect engineering of graphene materials can advance biosensor platforms from experimental prototypes toward scalable, clinically relevant technologies. By emphasizing fabrication scalability, electrical reliability, and molecular sensitivity, this work contributes to the growing effort to align high-performance biosensing with real-world applicability.

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List of Abbreviations

AuNP gold nanoparticle ix, 12, 14, 16, 18–26, 28, 29, 58

BN boron nitride x, 3, 12, 30–38, 41, 42, 47, 53–58

cDNA complementary DNA 20, 25, 28

DI deionized 18, 19, 32, 33

DNA deoxyribonucleic acid ix, 3, 7, 12–14, 18, 20, 24–28, 58

EF enhancement factor 19, 20, 25

ELISA enzyme-linked immunosorbent assay 1, 2, 27

FET field-effect transistor ix, x, 3, 5, 8, 12, 30–35, 38–42, 47, 53–59

GO graphene oxide ix, x, 8–11, 18–20, 22–24, 27, 33, 35–38, 41, 44

HEPES 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid 18, 20

LOD limit of detection 5, 31, 43, 50, 53–56

LSPR localized surface plasmon resonance 15–17, 22

POC point-of-care 2, 7, 27, 29, 47, 49, 54–57

rGO reduced graphene oxide ix, 3, 8–10, 12, 14, 18–26, 28–31, 33–35, 38, 41, 42, 47, 53–58

RT-PCR reverse transcriptase-polymerase chain reaction 1, 2, 27, 49–51, 53–57

SEM scanning electron microscopy [18–20](#)

SERS surface-enhanced Raman spectroscopy [3](#), [12–17](#), [20](#), [24–29](#), [58](#), [59](#)

TEM transmission electron microscopy [18](#), [19](#), [21](#)

UV ultraviolet [ix](#), [10](#), [17–19](#), [22](#), [23](#)

Chapter 1

Introduction

Disease diagnosis and monitoring are foundational to modern healthcare, guiding clinical decision-making, treatment planning, and ultimately influencing patient outcomes [1]. When diseases such as cancer or cardiovascular conditions are detected early, they are often more manageable and associated with significantly lower morbidity and mortality rates [2, 3]. Conversely, delayed diagnosis, particularly in chronic and infectious diseases, can lead to severe complications, reduced treatment efficacy, and increased transmission risk. Accurate diagnostics are also essential for distinguishing between clinically similar conditions, helping ensure that patients receive appropriate, targeted therapies while minimizing the risk of misdiagnosis and associated harm [1]. In addition to improving clinical care, effective diagnostic systems contribute to the cost-effectiveness of healthcare systems by reducing the need for late-stage interventions and unnecessary treatments, supporting more efficient allocation of medical resources [4]. On a larger scale, timely diagnosis plays a pivotal role in public health infrastructure by enabling reliable disease surveillance, outbreak response, and epidemiological research [5]. As such, there is a critical need for diagnostic technologies that deliver results both accurately and rapidly, especially in settings where timely intervention can dramatically alter clinical and systemic outcomes.

While gold-standard diagnostic tools such as [reverse transcriptase-polymerase chain reaction \(RT-PCR\)](#) and [enzyme-linked immunosorbent assay \(ELISA\)](#) offer exceptional sensitivity and specificity, their deployment is often limited by practical and infrastructural constraints. These techniques typically require centralized laboratories, trained personnel, and sophisticated equipment, which can hinder their accessibility, particularly in rural or resource-limited settings [6, 7, 8]. Although innovations such as cartridge-based [RT-PCR](#) systems have improved decentralization, many clinical environments still struggle with delays in sample transport, high costs per test, and limited throughput [9, 10]. Furthermore,

their reliance on controlled laboratory conditions makes real-time, on-site decision-making difficult in contexts such as emergency care, field diagnostics, and low-income regions [6]. On the other hand, there are lateral flow assays, which are rapid, low-cost, and user-friendly diagnostic tools for [point-of-care \(POC\)](#) use and mass distribution. Unlike [RT-PCR](#) and [ELISA](#), which require complex instrumentation and trained personnel, lateral flow assays enable on-site testing without specialized equipment. However, this simplicity comes at the expense of analytical performance, as they generally exhibit lower sensitivity and specificity, are often limited to the detection of a single analyte, and can be affected by sample matrix effects and subjective visual interpretation [11, 12, 13]. As a result, there is growing interest in alternative diagnostic platforms that can bridge the gap between the analytical performance of [RT-PCR](#) and [ELISA](#) and the operational simplicity of lateral flow assays.

1.1 Biosensors

Biosensors are defined as analytical devices that detect specific biological targets by converting a biochemical interaction into a measurable signal. These systems typically consist of three core components: a biorecognition element that selectively binds to the target analyte, a transducer element that converts the binding event into a signal, and a signal processor that interprets the data and converts it into a readable output [14, 15, 16]. This foundational architecture enables the engineering of biosensors for a wide range of applications, facilitating the detection of a variety of biomolecules. Their potential for rapid detection, minimal sample preparation, and device miniaturization, alongside their adaptability, makes them well-suited for decentralized testing and point-of-care applications [17]. Ongoing advances in material science, micro-fabrication, and low-power electronics have further broadened the capabilities of biosensor platforms, bringing them closer to replicating the diagnostic performance of laboratory processes in real-world settings [14, 18].

Biosensor Classifications

Biosensors are often classified by the sensing principle underlying the signal transduction process. A wide variety of biosensing platforms have been developed, with each relying on distinct sensing principles that offer specific advantages depending on the target analyte, detection environment, and intended application. Each transducer type offers unique properties in terms of sensitivity, multiplexing capability, or integration potential. These classifications not only influence the design and fabrication of biosensor platforms but also dictate their suitability for particular clinical applications [17, 19].

The major classes include electrochemical, optical, piezoelectric, thermal, and magnetic biosensors, with each category offering distinct advantages depending on the sensing environment, analyte type, and operational requirements [20]. Among the electrochemical biosensing platforms, FET biosensors have gained increasing attention for their ability to enable label-free, real-time detection while leveraging scalable semiconductor fabrication techniques [21, 22]. Among the optical biosensing platforms, plasmonic biosensors are also promising, particularly those based on surface-enhanced Raman spectroscopy (SERS). SERS biosensors offer exceptionally high sensitivity and molecular specificity, supported by a well-established theoretical and experimental foundation [23, 24]. Together, these two biosensor subtypes offer promising pathways toward achieving high analytical performance alongside design flexibility and device integration. The following chapters will examine these platforms in greater detail, beginning with the development of a SERS platform, followed by the fabrication and optimization of a BN-rGO FET biosensor, and concluding with a comparative analysis of the FET biosensor against clinically approved COVID-19 diagnostics.

1.1.1 Common Biomarkers & Bioreceptors

Biosensors function by converting a selective interaction between a biological target (biomarker) and a recognition element (bioreceptor) into a measurable signal. Biomarkers may include nucleic acids, proteins, small molecules, and cells, each representing a distinct class of diagnostic targets relevant to a wide range of diseases and physiological conditions. The selection of an appropriate bioreceptor depends on the molecular characteristics of the biomarker, as well as the required specificity, stability, and surface compatibility. Effective immobilization of the bioreceptor onto the sensor surface is crucial for maintaining binding activity and enabling reproducible signal transduction, making surface functionalization a key factor in biosensor performance [14, 15, 16, 20].

Nucleic Acids

Nucleic acids such as DNA and RNA are key biomarkers in the detection of genetic disorders, infectious diseases, and certain cancers. Their sequence-specific nature enables highly selective detection via hybridization with complementary strands. Common bioreceptors for nucleic acid targets include single-stranded DNA probes and aptamers. These bioreceptors can be immobilized onto sensor surfaces through a variety of chemical linkages, including amine or thiol functional groups, as well as non-covalent interactions such as $\pi - \pi$ stacking or electrostatic adsorption. Nucleic acid-based detection benefits from high

specificity and ease of probe design, though performance can be affected by temperature and nonspecific binding in complex samples [25, 26].

Proteins

Proteins are among the most targeted biomarkers due to their roles in immune response, inflammation, hormone regulation, and a wide range of diseases. Examples include the prostate-specific antigen in prostate cancer, troponin I and T for myocardial infarction, and the spike and nucleocapsid protein in SARS-CoV-2 [27, 28, 29]. Antibodies are the most established bioreceptors for protein detection, offering high specificity and affinity, though they are sensitive to denaturation and often require orientation-controlled surface functionalization via protein A/G or linker molecules, like EDC/NHS coupling. Peptides, while generally less specific than antibodies, are more chemically stable and can be synthesized at a lower cost. However, the superior binding affinity and diagnostic reliability of antibodies often outweigh the greater stability and ease of synthesis offered by peptides [30, 31].

Small Molecules

Small molecules such as glucose, lactate, and cortisol are commonly monitored in metabolic and endocrine disorders. These targets are typically recognized by enzyme-based bioreceptors, such as glucose oxidase or lactate dehydrogenase, which catalyze reactions that produce measurable byproducts. While enzyme-based biosensors allow for signal amplification, their activity is often sensitive to pH, temperature, and cofactor stability, limiting long-term performance in some applications [20, 32, 33].

Cells and Whole Organisms

Whole-cell detection relies on the recognition of surface-bound markers, such as membrane proteins or glycan structures, to identify cells. Some common targets include bacteria, viruses, and cancer cells. Antibodies, lectins, and aptamers are all commonly used for cell-specific targeting. These bioreceptors must be immobilized in a manner that preserves accessibility and orientation, often requiring additional passivation steps to minimize non-specific adhesion. Due to the complexity and size of these biomarkers, whole-cell biosensors typically demand high surface coverage and strong binding interactions [34, 35, 36].

Focus of This Thesis

While a wide range of biomolecules can serve as targets in biosensing, this thesis focuses specifically on the detection of nucleic acids and proteins, given their diagnostic relevance and their compatibility with the fabrication and transduction mechanisms explored in the subsequent chapters.

1.1.2 Limitations of Current Biosensor Technologies

To systematically assess the maturity of technology from concept to deployment, organizations such as academic institutions, startups, and regulatory agencies often rely on a framework known as the Technology Readiness Levels (TRLs), first created by NASA [37]. This framework consists of a nine-level scale used to manage development, assess risks, and guide financial and resource planning. In the context of biosensors and diagnostic tools, TRLs 1–3 may involve identifying key biomarkers and establishing theoretical feasibility, while TRLs 4–5 include proof-of-concept testing using buffer-based or in vitro samples. TRLs 6–7 generally encompass integration into functional prototypes and early-stage field testing, while TRLs 8–9 mark the transition to commercial production and real-world deployment [38, 39]. A general breakdown of TRLs is shown in Figure 1.1.

Despite significant advances in biosensor design and sensing performance, the vast majority of biosensor technologies remain confined to academic research or early-stage prototyping. Most published studies fall within TRLs 4 or 5, indicating that the devices have only been validated using buffer-based or partially complex biological matrices such as blood or saliva. While these conditions offer controlled environments for evaluating analytical performance, they do not reflect the complexity of real-world diagnostic scenarios. Advancing to TRL 6 and beyond, where the biosensor is tested in an integrated system under real-world conditions, poses significant technical and regulatory challenges [39].

One key technical barrier is the sensor’s performance degradation in complex media. Real biological samples contain a diverse array of interfering substances, including nonspecific proteins, metabolites, and organic matter that can compromise both the sensitivity and selectivity of the biosensor. For instance, a study by Hwang et al. on a deformed graphene-based FET demonstrated an [limit of detection \(LOD\)](#) of 600 zM in idealized conditions, but only 20 aM in complex samples, a three-order-of-magnitude decrease in sensitivity [40]. This drop-off demonstrates how even the most advanced sensing platforms must contend with issues such as matrix effects, nonspecific adsorption, and signal drift when applied outside the lab.

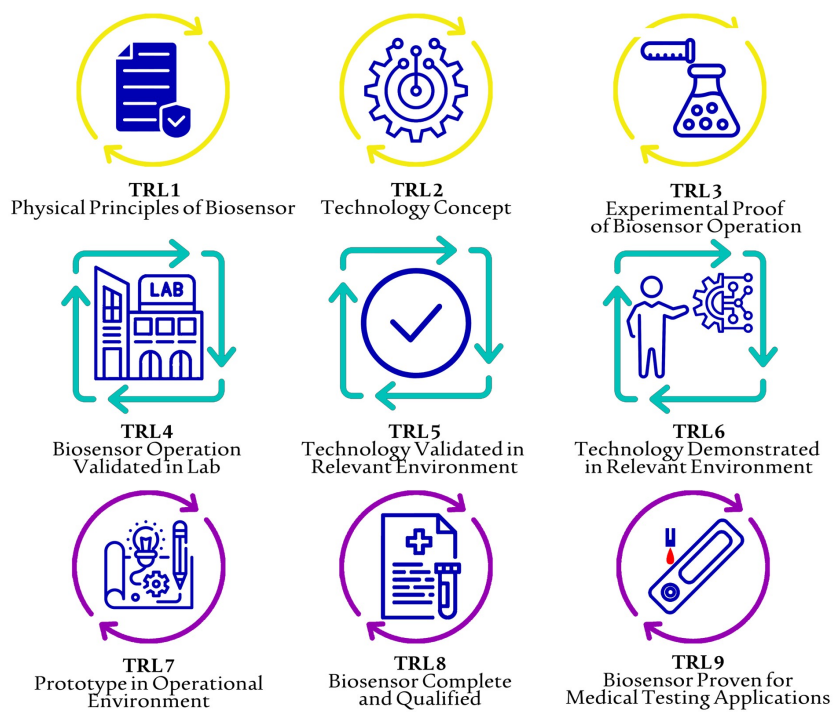


Figure 1.1: A general outline of the Technology Readiness Levels

Beyond sensing performance, biosensors face additional hurdles that limit their readiness for clinical or commercial deployment. These include the stability and shelf life of biorecognition elements, long-term biocompatibility, challenges with consistent surface functionalization, and limited device-to-device reproducibility [41, 42]. Biological components such as antibodies and enzymes are often susceptible to denaturation or degradation over time, especially under varying temperature or pH conditions, which are common in decentralized or POC environments [31]. Furthermore, maintaining performance over repeated use or during extended storage remains a critical barrier to practical adoption.

Even when technical hurdles are overcome, regulatory and manufacturing barriers remain. Reaching TRL 6 or higher requires clinical validation and approval by regulatory agencies such as the Food and Drug Administration (FDA) and European Medicines Agency (EMA) [41, 43]. The integration of biosensors into diagnostic workflows must also address quality assurance, batch reproducibility, and user safety. These additional demands often require interdisciplinary collaboration across biology, engineering, clinical research, and regulatory affairs, which are resources that are frequently inaccessible to academic labs or small startups [41].

To address these barriers, this thesis focuses on evaluating and improving the market optimization potential of two nanomaterial-based biosensing platforms. Rather than solely targeting analytical sensitivity in controlled environments, the work presented here emphasizes the importance of fabrication reproducibility, material stability, and system integration, highlighting key criteria for advancement towards higher TRL stages.

1.2 Graphene

Graphene, first discovered in 2004 by Novoselov and Geim, consists of a single layer of carbon atoms arranged in a two-dimensional hexagonal lattice [44]. It combines remarkable mechanical strength with high flexibility and minimal weight, while exhibiting exceptional electrical and thermal conductivity, making it highly suitable for innovative electronic and sensing technologies [45]. In the context of biosensing, graphene demonstrates excellent chemical tunability and biocompatibility, as its surface chemistry permits non-covalent functionalization through $\pi - \pi$ interactions and electrostatic forces, facilitating the immobilization of DNA and antibodies [46]. Other desirable qualities of graphene include ambipolar charge transport, large surface area, and optical transparency. [44, 46, 47]. Despite these advantages, the practical application of pristine graphene remains constrained by key fabrication and processing limitations.

Despite these strengths, several practical and electronic limitations restrict the implementation of pristine graphene in biosensing platforms. One major challenge lies in its fabrication. Methods such as mechanical exfoliation or chemical vapour deposition can yield high-quality graphene but are often expensive, time-consuming, or difficult to scale reliably across large surface areas [48]. Additionally, graphene is intrinsically hydrophobic and chemically inert, making it poorly suited for aqueous biological environments and difficult to functionalize with bioreceptors without thorough surface modification [46, 49]. A crucial electronic limitation is that graphene lacks a bandgap, effectively behaving like a zero-bandgap semimetal. This prevents the material from achieving the well-defined on/off switching behaviour required for biosensing applications such as FETs [50]. As a result, these limitations have motivated the development of chemically modified derivatives, particularly GO, which sacrifice some electrical performance in exchange for vastly improved processing, aqueous compatibility, and surface functionalization potential.

1.2.1 Graphene Oxide

Graphene oxide is a chemically modified derivative of graphene that contains oxygen-rich functional groups such as hydroxyl, carboxyl, and epoxy units distributed across its basal plane and edges. The oxygenated functional groups interfere with the electronic structure of graphene, lowering conductivity and charge carrier mobility when compared to pristine graphene [46]. However, these structural changes enable several advantages for biosensing applications. The presence of diverse surface functional groups enables GO to facilitate the covalent and non-covalent attachment of a wide range of biomolecules, making it highly compatible with various surface chemistries [49, 51]. Furthermore, GO exhibits a tunable bandgap dependent on the degree of oxidation and reduction, allowing for some control over its electronic properties [46]. Unlike pristine graphene, GO can be synthesized at a large scale through solution-based processes. These methods can yield nanoflake suspensions that are easily deposited via drop-casting or spin-coating techniques [52]. GO’s improved solubility, ease of functionalization, and scalable processing make it a compelling functional material for biosensing platforms, despite its comparatively lower charge carrier mobility and electrical performance.

Reduced graphene oxide is derived from graphene oxide through chemical, thermal, or electrochemical reduction processes that partially restore the sp^2 carbon network. This restoration partially recovers its electrical conductivity and charge carrier mobility compared to GO, while maintaining compatibility with biosensor functionalization strategies. As a result, rGO offers a middle ground between pristine graphene and graphene oxide [53]. Additionally, rGO tends to exhibit a smaller bandgap than GO due to partial restoration

of the sp^2 network, although precise control over this property remains a challenge [54]. To fully harness the tunable properties of rGO, its synthesis and post-treatment processes must be carefully optimized and refined, making effective defect engineering critical to its performance in biosensing applications [55].

1.3 Defect Engineering of Graphene Oxide

1.3.1 Overview of Defect Engineering Methods

The properties of rGO are highly dependent on how its oxygen-containing functional groups are removed and how its sp^2 carbon network is restored. This process, commonly known as defect engineering, plays an impactful role in tailoring rGO for biosensing applications. Various reduction methods, such as chemical, thermal, or electrochemical processes, enable partial or full restoration of the carbon lattice, each uniquely influencing the overall properties of the final material [55]. Chemical reduction using agents such as hydrazine or ascorbic acid is widely employed due to its simplicity and scalability. However, it often leads to heterogeneous defect distributions, residual contaminants, and limited reproducibility. These methods also tend to suffer from safety risks, unwanted environmental impacts, and batch-to-batch variation. Thermal and electrochemical methods offer improved purity and tunability, but can cause lattice damage or require more complex operational conditions [55, 56].

For biosensing applications, the ideal rGO aims for partial restoration, mainly to retain enough oxygenated groups for bioreceptor immobilization while restoring some conductivity for effective transduction. Recent advances have explored more precise and scalable reduction techniques, including laser irradiation, which allow for localized, controllable, and additive-free modification of GO surfaces [57]. The following section will explore the use of femtosecond laser ablation as a tool for rGO fabrication and how the laser parameters can be optimized to engineer rGO with tailored properties for biosensor integration.

1.3.2 Femtosecond Laser Ablation

Femtosecond laser ablation has emerged as a powerful and precise method for the defect engineering of GO to produce rGO. Unlike thermal or chemical reduction approaches that rely on bulk heating or chemical reagents, femtosecond laser ablation produces ultrashort pulses (10^{-15} seconds) with extremely high peak intensities. This enables cold,

non-thermal processing, where electronic excitation occurs faster than heat transfer to the lattice [58]. This enables the reduction and patterning of GO without prolonged thermal exposure, minimizing structural damage and often preserving key nanoscale features [59]. Furthermore, the maskless and additive-free nature of femtosecond laser ablation makes it promising for the scalable fabrication of functional materials that require precise control of their structural properties [58].

Femtosecond laser reduction is highly tunable, with key parameters influencing both the degree of GO reduction and the resulting material morphology. These include:

- **Pulse duration and repetition rate:** Short pulses allow for high-intensity excitation without thermal diffusion, while moderate repetition rates balance processing speed and heat accumulation
- **Wavelength:** The infrared range (800 nm) offers deeper penetration into the sample compared to UV and more efficient multi-photon absorption into the GO
- **Laser power:** Controls the fluence delivered to the GO nanoflakes; low power yields low reduction rates, while high power may cause substrate damage
- **Depth of focal point:** Positioning the focal point just beneath the GO surface concentrates reduction and nanostructuring, while directing it deeper results in less uniform processing
- **Stirring speed:** Controlled agitation ensures uniform exposure of GO nanoflakes to the laser, preventing aggregation and heterogeneous reduction

Careful modification of these parameters is essential for engineering rGO structures with desirable electronic and surface properties, which are critical in biosensing applications [59]. Figure 1.2 illustrates a schematic of the Ti:Sapphire laser setup that is used for the reduction of all GO solutions.

1.4 Research Objectives

The primary objective of this thesis is to bridge the gap between high-performance biosensor prototypes and the practical demands of real-world diagnostic deployment. While numerous platforms have demonstrated impressive sensitivity and selectivity in controlled

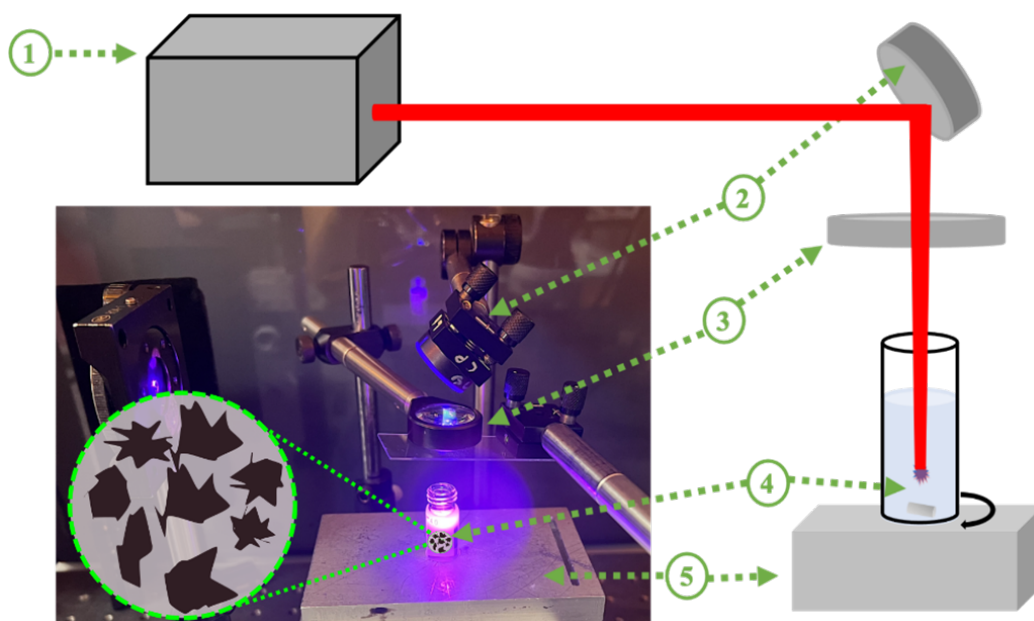


Figure 1.2: Femtosecond laser ablation set-up for reduction of GO solutions consisting of the (1) Ti:Sapphire laser, (2) mirror, (3) focus lens ($f=50$ mm), (4) GO solution with a magnetic stir bar, and (5) magnetic stir plate. Adapted from [60]

environments, few offer the fabrication reproducibility, material robustness, and integration potential required for commercial translation. To address these limitations, this work focuses on the design, fabrication, and characterization of nanomaterial-based biosensing platforms that utilize **rGO** as a foundational sensing element.

Specifically, the research is guided by the following two goals:

- To develop a scalable and reproducible **SERS** biosensing platform using laser-ablated **rGO** and **AuNP** nanocomposites. This platform is designed to detect short nucleic acid sequences with high sensitivity, leveraging the plasmonic enhancement of **AuNPs** and the molecular affinity of **rGO**. Emphasis is placed on using femtosecond laser ablation as a precise, additive-free method for **rGO** reduction and **AuNP** integration, with detailed characterization of the resulting nanostructures.
- To investigate the fabrication and optimization of **BN-doped rGO**-based **FET** biosensors for protein biomarker detection. This includes analyzing device yield, identifying failure modes, and comparing device performance against clinically approved diagnostics. By focusing on reproducibility, gel uniformity, and passivation techniques, the work aims to align **FET** biosensor development with market-readiness standards.

By addressing both the material science and fabrication strategies underpinning biosensor development, this thesis demonstrates how tailored defect engineering, alongside promising transduction methods, can enhance biosensor functionality and scalability. These concepts are explored in depth across three chapters: the development of a **SERS** platform for **DNA** detection, the fabrication and optimization of a **BN-rGO FET** biosensor for protein detection, and a comparative performance analysis of the **FET** biosensor against established COVID-19 diagnostic technologies.

Chapter 2

Reduced Graphene Oxide & Gold Nanocomposite for SERS Detection of DNA

2.1 Introduction

The rising demand for biosensing technologies capable of rapid and sensitive detection of small biomolecules, such as nucleic acids and biomarkers, has spurred the development of plasmonic sensing platforms [61]. These biosensors leverage the unique optical properties of noble metal nanoparticles, such as gold and silver, to enhance weak molecular signals. This enables label-free detection of analytes at extremely low concentrations [62]. Among these platforms, SERS is recognized as a highly promising technique due to its molecular specificity, non-destructive nature, and capacity for multiplexed analysis [23, 63]. SERS is particularly attractive for clinical diagnostics and point-of-care applications because of its ability to distinguish between closely related molecules and detect subtle structural or compositional changes [23, 63]. By generating a unique detailed spectral profile, SERS can facilitate the structural, compositional, and conformational characterization of biological samples with exceptional precision [64, 65, 66]. As a result, extensive research efforts are focused on optimizing SERS platforms, particularly in substrate design and signal reproducibility, to fully harness their potential for reliable, scalable, and real-world biosensing applications.

Short DNA sequences such as 24-mer oligonucleotides play a pivotal role in molecular diagnostics due to their optimal length for achieving both high specificity and stable

hybridization with complementary strands [67]. These sequences are widely used in the design of probes for hybridization-based assays, enabling the detection of genetic mutations, pathogen-specific DNA regions, and microRNA biomarkers [68]. Because they often correspond to conserved or functionally significant genomic regions, 24-mer targets are particularly effective for identifying clinically relevant nucleic acid signatures without the need for full-length gene analysis [69, 70]. In biosensor applications, 24-mer DNA offer a practical advantage due to their manageable size, which facilitates efficient surface immobilization and rapid hybridization kinetics—both critical for sensor performance and reproducibility. Their use is especially advantageous in point-of-care diagnostics, where minimal assay complexity and fast turnaround are essential [71]. Techniques that can detect these sequences label-free and without amplification, such as SERS-based platforms, simplify workflows, lower costs, and enable the use of diagnostic tools in resource-limited or decentralized settings [72, 73].

This chapter focuses on the development of a rGO and AuNP nanocomposite platform for the SERS-based detection of a 24-mer single-stranded DNA sequence. The rGO not only provides a large surface area for AuNP functionalization, but also as an effective medium for π - π stacking interactions with bases in nucleic acids, facilitating efficient DNA adsorption. The resulting nanostructures leverage the synergistic effects of gold’s plasmonic enhancement and rGO’s molecular affinity for label-free detection of short DNA sequences with high sensitivity. By integrating material synthesis, substrate assembly, and spectroscopic analysis, this work aims to demonstrate a versatile, scalable approach for constructing reliable SERS-active biosensors for nucleic acid target sequences.

2.2 Background

2.2.1 Surface-Enhanced Raman Spectroscopy (SERS)

Raman spectroscopy is a powerful analytical technique that provides detailed molecular fingerprints through the inelastic scattering of monochromatic light, typically from a laser source [74]. This method offers several key advantages over other sensing platforms, including minimal sample preparation, non-destructive analysis, and the ability to characterize a wide range of chemical species in solid, liquid, or gas phases [61]. However, its application in practical settings is often limited by the inherently weak intensity of Raman signals, especially for small or single-molecule samples [74]. Due to its low scattering efficiency, adequate signal acquisition often requires higher laser powers or longer exposure times. These adjustments, however, can be damaging to sensitive samples [75]. Additionally,

fluorescence from the sample or surrounding media can easily overwhelm the weak Raman signals, a problem that becomes more pronounced at shorter excitation wavelengths [76]. To overcome these limitations and enhance sensitivity, **SERS** was introduced as a significant advancement in Raman-based biosensing platforms.

SERS enhances weak Raman signals by several orders of magnitude, typically ranging from 10^4 up to 10^{11} , when the analyte molecules adsorb onto or near nanostructured metallic surfaces [63]. The enhancement factor is influenced by two main mechanisms: electromagnetic enhancement and chemical enhancement. The electromagnetic mechanism is dominant and is driven by the excitation of **localized surface plasmon resonance (LSPR)** in metallic nanostructures when illuminated by incident light [77]. These resonances generate intense localized electric fields near the metal surface. This effect is especially strong at hotspots, such as the gaps between nanoparticles, which significantly amplify the Raman signals of nearby molecules [74, 78]. The chemical mechanism, while typically smaller in magnitude, involves charge-transfer interactions between the analyte and the metal surface [78]. These interactions can alter the polarizability of the molecule or introduce resonance conditions that lead to additional signal enhancement. Together, these mechanisms allow **SERS** to detect analytes at trace or even single-molecule levels. A schematic of both of these mechanisms can be seen in Figure 2.1.

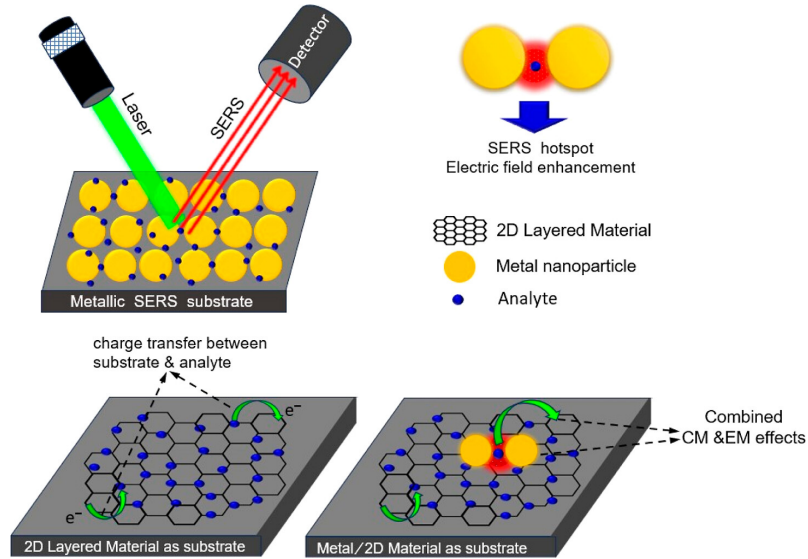


Figure 2.1: Schematic of the electromagnetic and chemical mechanisms for signal enhancement in **SERS**. Adapted from [78]

2.2.2 Plasmonic Nanomaterials

Noble metal nanomaterials, particularly gold and silver nanoparticles, are widely employed in plasmonic sensing platforms due to their strong optical responses, surface accessibility, and compatibility with many fabrication techniques [24]. In SERS applications, their geometry-dependent plasmonic behaviour enables fine-tuning of both the enhancement factor and the spatial distribution of electron hotspots. By adjusting particle size and shape, their resonance wavelengths, field localization, and coupling behavior can be manipulated to match specific analyte and equipment requirements [79]. These tunable parameters are central to the design of SERS substrates, allowing performance optimization across various laser excitation wavelengths and applications. Moreover, noble metal nanoparticles integrate easily with both solid and flexible substrates, enabling the development of scalable and portable plasmonic systems [24, 62].

While silver nanoparticles are known to produce stronger electromagnetic enhancements and sharper LSPR peaks, they suffer from significant chemical instability under ambient conditions. Oxidation and nanoparticle aggregation can degrade their plasmonic properties over time, reducing reproducibility and limiting use in biosensing or field-deployable systems [80]. AuNPs, though often providing lower maximum enhancement, offer a more favourable profile for real-world applications. They exhibit excellent chemical inertness, stability in biological and buffered environments, and compatibility with thiol-based functional groups for biomolecule functionalization and adsorption [63]. Although silver stability can be improved with passivation or alloying, such modifications often introduce new variables that complicate hotspot accessibility or surface chemistry [80]. Therefore, the decision to use gold reflects a deliberate tradeoff of prioritizing chemical robustness, reproducibility, and integration potential over the theoretical maximum enhancement achievable with silver. This choice aligns with the demands of biosensing platforms, where consistent and reliable performance often outweighs absolute signal strength.

In this project, 40 nm AuNPs were chosen based on both theoretical considerations and support in literature. AuNPs of this size exhibit a strong and well-characterized LSPR peak centered around 530 nm, closely matching the 532 nm excitation wavelength used in the Raman spectroscopy setup [81]. This spectral overlap enables effective resonance coupling, therefore maximizing local field enhancement and increasing the Raman signal intensity. Although larger AuNPs with sizes ranging from 80 - 100 nm can sometimes provide stronger enhancements, they are more prone to scattering losses, sedimentation, and aggregation. These factors reduce substrate stability and uniformity of the SERS substrate [63]. The choice of 40 nm spheres represents a balanced compromise between providing sufficient enhancement and maintaining colloidal stability, reproducibility, and

ease of functionalization. Furthermore, the synthesized particles were experimentally validated via UV-Vis spectroscopy to confirm that their absorbance peak aligns with the laser excitation, ensuring optimal plasmonic performance under our specific SERS conditions.

2.2.3 Challenges in SERS Substrate Design

While noble metal nanoparticles are essential for producing the local electromagnetic fields required for SERS, purely metal-based platforms, particularly those relying on colloidal or drop-cast methods, often suffer from fabrication and reproducibility challenges [78]. The synthesis of noble metal nanoparticles frequently results in batch-to-batch variability in particle size, shape, and dispersion, all of which directly influence plasmonic behaviour. When transferred to solid substrates, achieving uniform surface coverage and consistent hotspot distribution is particularly difficult. Even slight variations in drying conditions or solvent interactions can lead to uncontrolled aggregation or uneven nanoparticle distribution, which degrade signal reliability and exhibit elevated background noise [79]. Although top-down fabrication methods such as nanoimprint or electron-beam lithography can circumvent these issues, they are typically expensive, low-throughput, and poorly suited for flexible or disposable sensing platforms [82]. As a result, there is a strong need for hybrid systems that retain the plasmonic performance of noble metals while improving structural stability, dispersion control, and scalability.

To address these limitations, researchers have increasingly explored hybrid platforms that integrate semiconducting materials with noble metals. Semiconductors such as WS₂, MoS₂, and carbon-based nanostructures offer several complementary advantages [83]. They enhance mechanical stability, improve chemical robustness, and can facilitate analyte adsorption through increased surface affinity. In certain systems, they also contribute to the chemical enhancement mechanism by enabling charge transfer at the analyte–substrate interface, which modifies molecular polarizability and boosts Raman scattering intensity. For example, ZnO–Ag systems have demonstrated the ability to photocatalytically regenerate their sensing surface under UV illumination, allowing for the removal of adsorbed analytes and reuse of the substrate without significant loss of SERS performance [84, 85, 86]. Alternatively, MoS₂-Au hybrid platforms offer enhanced SERS sensitivity through the synergistic combination of the LSPR from gold and interfacial charge-transfer interactions from MoS₂ [87, 88]. Beyond enhancement, these materials help regulate nanoparticle distribution, suppress unwanted aggregation, and expand compatibility with flexible substrates [83]. The integration of plasmonic and semiconducting components reflects a broader trend toward multifunctional SERS systems that seek to balance sensitivity, structural integrity,

and fabrication scalability. In our platform, **rGO** was selected as the semiconductor component due to its high surface area, tunable chemistry, and ability to anchor **AuNPs** in a stable and spatially uniform manner [89].

2.3 Methodology

2.3.1 Materials and Equipment

Single-layer **GO** solution (purity > 80%, concentration 6.2 g/L, flake size 0.5–5 nm) was purchased from Graphene Supermarket (Graphene Laboratories Inc., USA). **4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES)** salts, NaCl, and MgCl₂ were also purchased from Sigma-Aldrich. **deionized (DI)** water was used to prepare all salt solutions, samples, and buffers. The buffer composition included 50 mM **HEPES**, 5 mM NaCl, and 2 mM MgCl₂. The **DNA** used in this work was purchased from Integrated DNA Technologies; the specific sequence used (002A) was 5'-ACG CAT CGA CAA AGA GAA CCT GGG-3'.

The **scanning electron microscopy (SEM)** and **transmission electron microscopy (TEM)** images were acquired on a JEOL JSM-7200F and JEOL JEM-F200 (JEOL Ltd., Japan), respectively. All **UV-Vis** spectroscopy was done using the UV-2501PC spectrometer (Shimadzu Co., Japan). All Raman Spectroscopy tests were carried out using the Horiba HR800 Spectrometer (HORIBA Jobin Yvon SAS Co., Canada), with a laser wavelength of 532 nm. Laser irradiation was done using a Ti: Sapphire regenerative amplifier. The stirring of the solution was performed by the 04638-Series magnetic stirrer (Cole Parmer, USA). To determine the zeta potential of the **GO** and **rGO**, the Malvern Zetasizer Nano series instrument (Malvern Panalytical Ltd., Massachusetts, USA) was used at 25 °C and 40% moisture. The ultrasonic bath (Cole-Parmer, USA) was used to disperse the **GO** and for pretreatment.

2.3.2 Synthesis of Gold Nanoparticles

The **AuNPs** were synthesized via chemical reduction using NaBH₄ and HAuCl₄. First, NaBH₄ was diluted in **DI** water to prepare a 0.1 M solution. Separately, HAuCl₄ was diluted in deionized water to obtain a 1 mM solution. Next, the NaBH₄ solution was slowly added to the HAuCl₄ solution under constant stirring to prevent rapid nanoparticle formation. The reaction proceeded for two hours to allow for complete nanoparticle synthesis. After

formation, citrate was added to stabilize the AuNPs. Finally, the solution was centrifuged to remove excess reagents.

2.3.3 Nanocomposite Synthesis and Assembly

The GO solution was prepared by diluting 6.2 mg/mL aqueous GO to a final concentration of 5 mg/mL with DI water, followed by ultrasonication for 30 minutes at room temperature. Then, 250 μ L of AuNP (2.5 mg/mL) was added to the 5 mL GO solution to form the GO@AuNP suspension.

To synthesize the rGO@AuNP nanocomposite, the suspension was irradiated using a Ti:Sapphire pulsed laser (wavelength: 800 nm, pulse duration: 35 fs, repetition rate: 1 kHz). The beam was focused through a 5 cm lens onto a spot area of 7.85×10^{-4} cm², producing an intensity of 3.5×10^{15} W/cm². The GO@AuNP suspension was exposed to 1 W for 1 hour under ambient conditions, with continuous magnetic stirring to maintain homogeneity.

2.3.4 Characterization

To characterize the rGO@AuNP nanocomposite, the material was spin-coated onto silicon substrates and analyzed in two stages. First, SEM and TEM were employed to examine the morphology of the nanocomposite and determine the size distribution of the embedded AuNPs. These imaging techniques also served to evaluate the effectiveness of the femtosecond laser irradiation process in reducing GO and spatial distribution of the AuNPs. SEM images were captured at a magnification of 10,000 $\times$, while TEM images were acquired at magnifications of 20,000 $\times$ and 100,000 $\times$.

In the second stage, the optical properties of the synthesized nanocomposite were assessed using UV-Vis spectroscopy. Raman spectroscopy was also performed to analyze structural changes in the material, using a 532 nm excitation laser with a spectral resolution of 0.3 cm⁻¹ and a maximum power output of approximately 1 mW. Spectra were collected over the 200–3000 cm⁻¹ range, to fully convey the physical changes from GO to rGO.

2.3.5 SERS Enhancement

The enhancement factor (EF) parameter in Raman spectroscopy analysis is calculated using Equation 2.1 [63].

$$EF = \frac{I_{SERS}/N_{SERS}}{I_n/N_n} \quad (2.1)$$

I_{SERS} and I_n represent the Raman scattering intensities measured on the **SERS**-active substrate and the reference (pure silicon) substrate, respectively. Similarly, N_{SERS} and N_n are the number of probed molecules present on each substrate. Given that the same volumes and concentrations of **DNA** probe and target strands were used for both conditions, it is reasonable to assume that $N_{SERS} \approx N_n$. This allows the **EF** to be simplified to the ratio of I_{SERS}/I_n , isolating the contribution of the substrate’s plasmonic enhancement to the observed Raman signal.

2.3.6 DNA Detection and Probe Functionalization

A 1 nM **DNA** probe solution prepared in 50 mM **HEPES** buffer containing 5 mM NaCl and 2 mM $MgCl_2$ was drop cast onto the **rGO@AuNP** substrate and incubated at room temperature for 2 hours to facilitate **DNA** adsorption and surface immobilization. The immobilization likely occurs through a combination of $\pi - \pi$ interactions between the **DNA** bases and the **rGO** surface, as well as possible electrostatic attraction to the nanocomposite interface. Following immobilization, **complementary DNA (cDNA)** strands at concentrations ranging from 10^{-7} to 10^{-5} M were introduced to the substrate to enable hybridization. After hybridization, the **SERS** signal was measured.

2.4 Results & Discussion

2.4.1 Nanocomposite Characterization

Microscopic Characterization

Figure 2.2 shows **SEM** images of **rGO@AuNP** samples synthesized via laser irradiation of an **GO** solution functionalized with **AuNPs**. Figure 2.2 (left) provides a tilted view of the sample, revealing its layered morphology, while Figure 2.2 (right) presents a top-down, perpendicular perspective. The images clearly show spherical **AuNPs** immobilized within the stacked **rGO** flakes. These nanoparticles are uniformly distributed across the **rGO** surface, which is a structural characteristic known to result in enhanced **SERS** signals due to the generation of consistent electromagnetic hotspots.

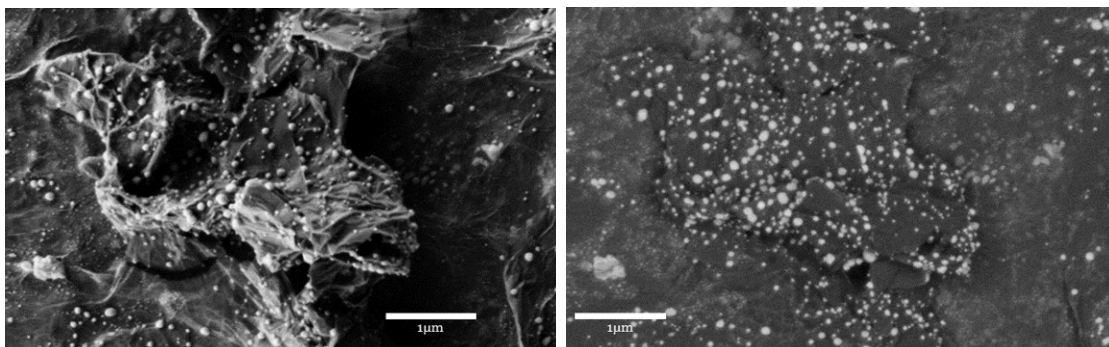


Figure 2.2: SEM images of rGO@AuNP nanocomposite after laser irradiation with magnification factor of 10,000 $\times$

Figure 2.3 presents TEM images of rGO@AuNP samples at magnifications of 20,000 $\times$ and 100,000 $\times$, shown in (a) and (b), respectively. In Figure 2.3 (left), AuNPs are visible as dark spherical contrasts distributed across the lighter rGO sheets. These particles exhibit a relatively uniform spatial distribution along the surface of the rGO flakes. Higher magnification in Figure 2.3 (right) confirms this morphology, and the AuNPs are observed to have diameters ranging from approximately 10 - 40 nm.

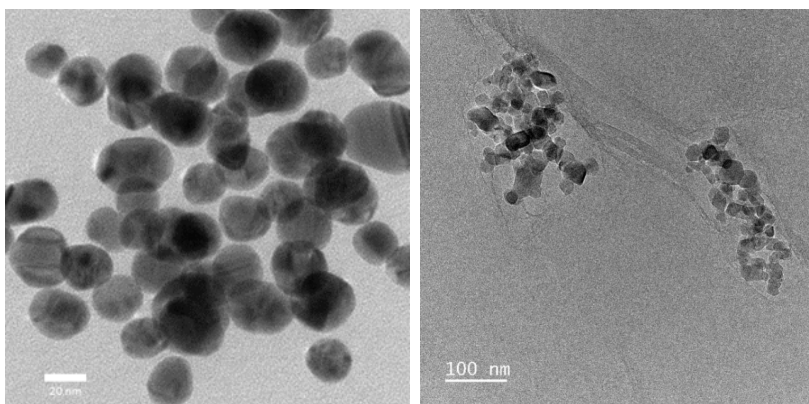


Figure 2.3: TEM images of (left) gold nanoparticles after synthesis at a magnification factor of 500,000 $\times$ and (right) rGO@AuNP nanocomposite after laser irradiation with a magnification factor of 100,000 $\times$

A quantitative size distribution analysis was conducted based on the TEM images to

assess the uniformity of the synthesized AuNPs. As shown in Figure 2.4, the particle diameters ranged from 10 - 100 nm, with an average size of approximately 40 nm, demonstrating the success of the synthesis method. As demonstrated later by UV–Vis spectroscopy, the optical response of the nanocomposite confirms the suitability of these size distributions for this application.

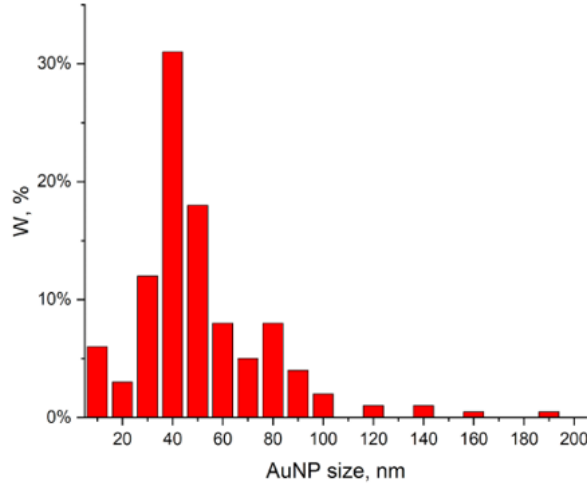


Figure 2.4: Histogram of AuNP sizes in the range of 0 – 50 nm

Optical Characterization

UV–Vis spectroscopy was used to monitor the optical characteristics of the nanocomposites and to confirm the presence of both AuNPs and rGO. As shown in Figure 2.5, the GO spectrum displays characteristic absorbance peaks at 228 nm and 302 nm, corresponding to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions, respectively. Following laser-induced reduction, the primary peak red-shifts from 228 nm to 268 nm, indicating partial restoration of the sp^2 carbon network, which is characteristic for rGO. The absorption peak at 531 nm, associated with the AuNPs, remains unchanged before and after reduction, confirming that the plasmonic properties of gold are preserved throughout the process. Additionally, a slight increase in the intensity of the 531 nm peak was observed, suggesting improved surface exposure or dispersion of AuNPs on the rGO nanoflakes. The stability of the LSPR peak also indicates minimal plasmon damping due to interactions with the rGO, implying effective and stable nanoparticle immobilization. Crucially, the absorption peak at 531 nm

closely aligns with the 532 nm excitation wavelength used in Raman spectroscopy, which is expected to enhance the Raman signal significantly due to resonance coupling.

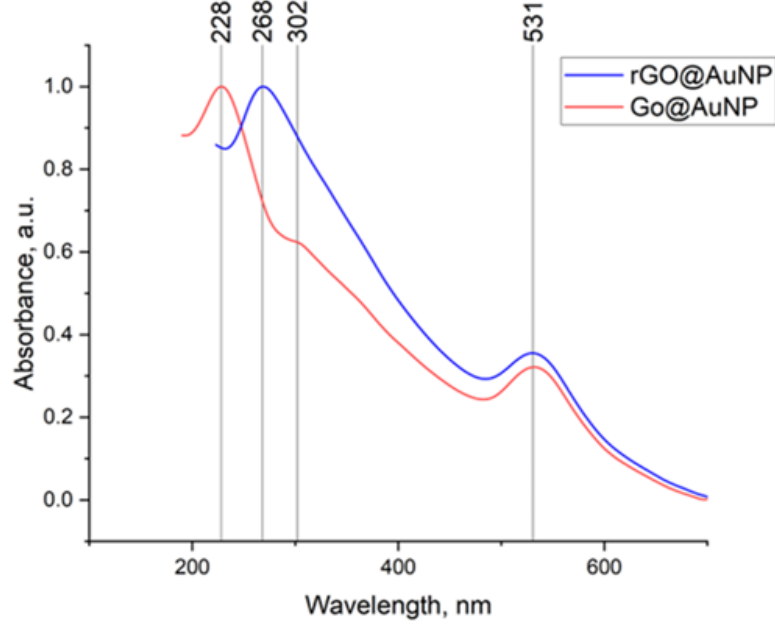


Figure 2.5: UV-Vis spectra of GO@AuNP and rGO@AuNP nanocomposites

Raman spectroscopy was used to analyze the structural evolution of the nanocomposite before and after laser-induced reduction. As shown in Figure 2.6, both GO@AuNP and rGO@AuNP nanocomposites exhibit two prominent peaks at approximately 1350 cm^{-1} and 1598 cm^{-1} , corresponding to the D and G bands, respectively. The D band (1350 cm^{-1}) arises from the breathing modes of sp^3 -hybridized carbon atoms associated with lattice defects, while the G band (1598 cm^{-1}) reflects the in-plane vibrational mode of sp^2 carbon networks. After laser irradiation, the D band intensity increases relative to the G band, leading to a higher I_D/I_G ratio in the rGO@AuNP spectrum. This increase suggests the formation of smaller and more disordered sp^2 domains due to partial restoration and restructuring of the carbon lattice [90]. Additionally, a weak and broad feature appears in the $2700 - 2900\text{ cm}^{-1}$ range in the rGO spectrum, corresponding to the 2D band. This band, although subtle, is indicative of partial graphitic ordering and π - π stacking interactions in the rGO [91]. The spectral changes collectively confirm that laser irradiation effectively induces the reduction of GO, introducing structural rearrangements while preserving the

overall carbon structures.

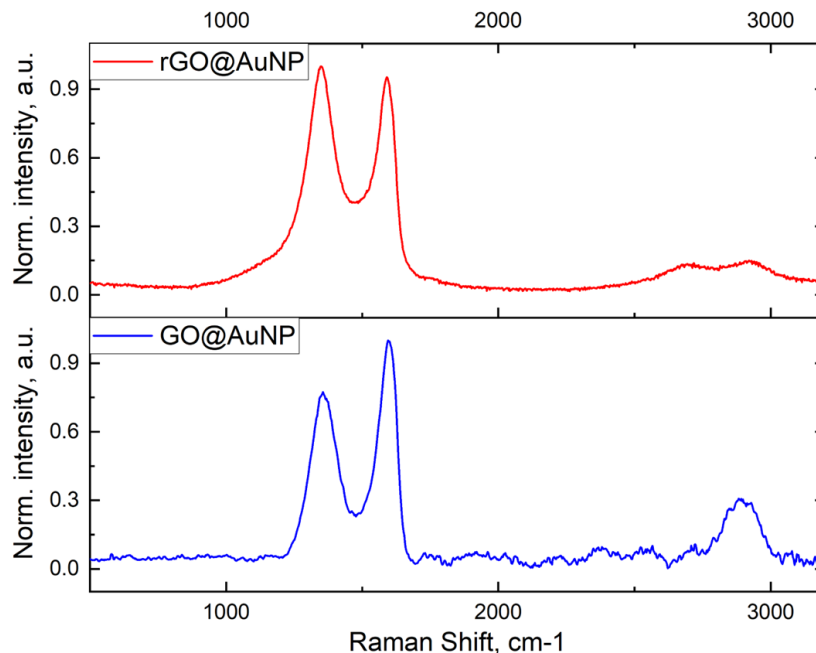


Figure 2.6: Raman spectra of **GO@AuNP** (before laser irradiation) and **rGO@AuNP** (after laser irradiation) nanocomposites

2.4.2 Detection of 24-mer DNA

Raman spectroscopy was employed to assess the ability of the **rGO@AuNP**-functionalized substrate to detect **DNA** at a concentration of 10^{-3} M via **SERS**. Figure 2.7 presents the Raman spectra of **DNA** deposited on a bare silicon substrate (red trace) and on the **rGO@AuNP** nanocomposite (black trace). The silicon-only substrate shows minimal Raman activity, while the nanocomposite-enhanced substrate yielded five well-defined peaks at around 969, 1281, 1350, 1424, and 1598 cm^{-1} . Notably, the peaks at 1350 and 1598 cm^{-1} correspond to the D and G bands of **rGO** and does not correlate to any common **DNA** indicators. However, the other peaks are attributed to vibrational modes of **DNA** nucleobases and their structural components. Specifically, the peak at 969 cm^{-1} corresponds to N–H bending in guanine, the 1281 cm^{-1} peak is associated with in-plane C–H bending and ring breathing of cytosine and thymine, and the 1424 cm^{-1} peak is linked to ring stretching and C–N vibrations in guanine [92, 93]. The appearance and intensity of these features confirm

successful DNA immobilization and hybridization on the SERS-active substrate. Using the EF equation defined earlier, an EF of approximately 10^4 was calculated, demonstrating the nanocomposite's effectiveness in amplifying DNA-specific Raman signals.

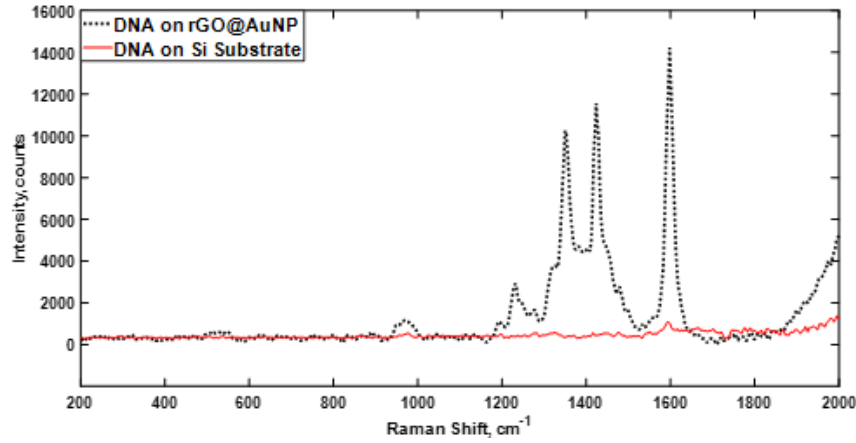


Figure 2.7: Raman spectra of DNA adsorbed onto rGO@AuNP and pure silicon substrates

A concentration test was performed on another functionalized substrate using solutions ranging from 10^{-7} to 10^{-5} M to examine the effect of target concentration on Raman intensity. Based on SERS spectral analysis, changes in peak intensities at approximately 970, 1282, and 1425 cm^{-1} were attributed to the adsorption of cDNA onto DNA probes immobilized on the AuNPs and rGO surface. As shown in Figure 2.8, increasing the cDNA concentration led to a significant amplification in the Raman signal at 1281 cm^{-1} with measured intensities of 1525, 2616, and 3145 counts for 10^{-7} , 10^{-6} , 10^{-5} M, respectively. This trend reflects the increasing likelihood of hybridization events as the concentration of cDNA molecules in the solution increases. At 969 cm^{-1} , a more modest increase in signal intensity was observed, with values of 1667, 1795, and 2000 counts across the same concentration range.

In addition to the peaks observed in both spectra, two additional features emerged in this SERS spectra compared to the first one. A prominent peak at 520 cm^{-1} was attributed to the underlying silicon substrate, where its Raman peak becomes visible due to insufficient deposition and spin-coating of the nanocomposite layer. This signal suggests that there are regions of thin material coverage, where the laser is able to penetrate to the substrate. The appearance of a peak around 809 cm^{-1} corresponds to vibrational modes of the DNA phosphate backbone [92]. The emergence of this peak further confirms successful

DNA adsorption onto the material surface. However, the spectrum overall exhibited increased background noise compared to the first baseline measurement, which is a result of variability in nanocomposite morphology and uneven DNA hybridization efficiency. These results emphasize the need for improved deposition consistency and surface uniformity to ensure reproducible SERS, especially in the context of market viability.

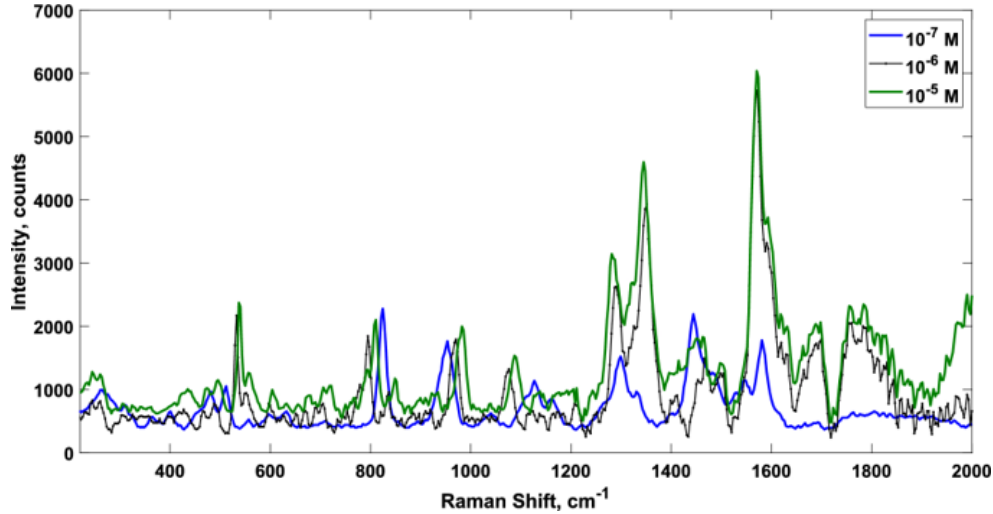


Figure 2.8: Raman spectra of DNA adsorbed onto rGO@AuNP with concentrations ranging from 10^{-5} to 10^{-7} M

2.5 Future Work & Outlook

2.5.1 Next Steps for Current Material

Building on the promising performance of the nanomaterial, several avenues remain to further improve material performance, reproducibility, and sensitivity. One important direction is the exploration of alternative AuNP morphologies. Unlike spherical nanoparticles, anisotropic structures, such as nanorods, nanostars, or nanocubes, can create sharper and more localized electromagnetic hotspots, leading to enhanced SERS activity [63]. However, these shapes also pose challenges in synthesis reproducibility and colloidal stability, particularly under biological conditions, and may require better control of reaction kinetics and surface functionalization strategies [94]. In parallel, hybrid plasmonic structures combining gold and silver could be explored to leverage the superior enhancement properties of

silver while retaining the chemical stability of gold. A gold-shell/silver-core configuration is promising, as it allows for a tunable plasmonic response while minimizing silver oxidation through the protective gold shell. These nanostructures can be optimized to balance performance with environmental stability, making them desirable for biosensing platforms operating under varying conditions.

Another potential avenue is tuning the reduction level of GO by varying the duration or intensity of laser irradiation during synthesis. Since the degree of reduction influences the surface chemistry, conductivity, and defect density, adjusting this parameter may allow for further optimization of SERS enhancement and DNA adsorption capabilities. Systematic experiments measuring laser irradiation time versus SERS performance could help identify the ideal reduction point of the semiconducting material. Additionally, improving the uniformity of the nanocomposite deposition remains a priority. As demonstrated through the SERS spectra, the current drop-casting method leads to uneven material distribution, resulting in spectral variability and regions with exposed silicon. Future work could explore other methods that can achieve more consistent surface coverage across the substrate and better hotspot distribution. These scalable and reproducible deposition techniques could significantly enhance substrate uniformity and measurement repeatability, which are crucial considerations for market viability.

2.5.2 SERS Market Considerations

While SERS biosensors demonstrate strong potential for label-free and highly sensitive detection of DNA, transitioning these platforms into real-world diagnostic settings requires careful consideration of several market factors. SERS is most promising in POC environments, where rapid and accurate molecular diagnostics are extremely important. However, practical translation demands more than proof-of-concept performance, as it must also address affordability, usability, reliability, and compatibility with complex biological matrices.

A key barrier to implementation is the cost and complexity of the instrumentation. Although portable and handheld Raman systems are commercially available, high-performance systems capable of reliable SERS measurements still carry a significant cost. These systems typically require precision lasers and sensitive optical alignment, which can raise both initial investment and maintenance demands [95]. While not as demanding as RT-PCR or ELISA set-ups, Raman instruments still require calibration and occasional servicing. In POC environments, especially in low-resource or decentralized settings, this necessitates either user-friendly, easy-to-use equipment or trained personnel, both of which can limit scalability.

Another critical factor is the quality and reproducibility of the SERS-activated substrates. Colloidal or drop-cast nanostructure-based platforms often suffer from batch-to-batch variation and non-uniform hotspot distribution, which can lead to inconsistent signal enhancement, as shown in the experimental data. This variability diminishes both analytical sensitivity and regulatory approval pathways. A transition to large-scale manufacturing would require standardized, shelf-stable substrates with minimal variability in signal response. Even though the rGO@AuNP platform shows good dispersion and signal enhancement, further work on uniform coating and stabilization is essential for reliable results.

Finally, biological fluids such as blood, saliva, and sweat present further challenges. These matrices are spectrally complex and often exhibit a multitude of overlapping Raman bands due to proteins, salts, and other biomolecules. In many cases, high background fluorescence or peak congestion can obscure signals from low-concentration targets such as short DNA sequences. As a result, successful detection in these real-world samples will depend on the development of standardized reference spectra and well-optimized detection protocols that distinguish target signals from biological noise [96]. This not only includes spectral calibration but also potentially integrating machine learning or artificial intelligence for accurate peak separation and target identification [97, 98].

In summary, while the technical viability of SERS biosensors is well-supported, the path to market depends on thoughtful optimization at both the material and system levels, ensuring not only high-performance detection, but also user-friendliness, reliability, and robustness in complex real-world conditions.

2.6 Conclusion

This chapter demonstrated the synthesis and application of a rGO@AuNP nanocomposite for the label-free detection of 24-mer DNA sequences via SERS. By leveraging both the plasmonic enhancement from the AuNPs and the chemical properties of rGO, this hybrid platform exhibited distinct Raman signals corresponding to DNA vibrational modes. The platform was sensitive to varying DNA concentrations, with a notable amplification in spectral intensity at 1281 cm^{-1} as the cDNA concentration increased. An enhancement factor of 10^4 was achieved, confirming the material’s capability to boost weak molecular signals. The emergence of DNA-specific peaks across multiple spectra validated successful hybridization, though the observation of background silicon peaks and signal variability outlined challenges in coating uniformity and deposition.

While the material shows promise, further refinements are required to meet the demands of practical application. Uniformity of the material on substrates must be improved to minimize background interference and to ensure reproducible signal production. Additionally, translating these platforms into diagnostic or **POC** contexts will necessitate scalable fabrication procedures, robust substrate design, and integration with compact, user-friendly Raman instrumentation. Despite these challenges, the **rGO@AuNP SERS** platform offers a compelling start towards viable implementation of **SERS**-based biosensors in molecular diagnostics.

Chapter 3

Optimization of BN-rGO FET Biosensors

3.1 Introduction

In recent years, FET biosensors have gained increasing attention as promising platforms for label-free, real-time detection of a wide range of analytes. Their inherent electrical sensitivity, low power consumption, and compatibility with miniaturization make them suitable for diagnostics, especially in point-of-care and wearable devices [21]. Among the variety of channel materials explored for FET biosensing, rGO stands out for its high electrical conductivity and tunable surface chemistry. Furthermore, when doping rGO with BN can exhibit further improvements in defect modification, charge carrier mobility, and sensor signal stability [29, 57, 99]. The outlined material properties, combined with the high sensitivity of FET devices, enable sensitive and selective detection of biomolecular targets at attomolar to zeptomolar concentrations. The synergy between rGO and FETs positions this platform as a promising innovation in biosensor design.

In our lab, a BN-doped rGO FET biosensor capable of detecting a range of clinically relevant biomarkers, including the spike (S) and nucleocapsid (N) proteins of SARS-CoV-2, biomarkers associated with Lyme disease, and Escherichia coli [29, 34]. The sensor surface has been functionalized with both antibodies and aptamers, with varying passivation protocols that may contribute to inconsistencies observed across fabrication batches. While the sensor's ability to detect a broad range of analytes demonstrates its versatility and potential for clinical use, further work is required to improve its reproducibility and readiness for market integration. This chapter focuses on addressing these translational challenges

by conducting a batch-to-batch yield analysis, identifying common failure modes, and optimizing fabrication parameters to improve device consistency. In the following chapter, a comparative analysis is performed between the lab-developed biosensor and commercially available COVID-19 diagnostic platforms, evaluating performance metrics such as LOD, response time, sensitivity, selectivity, and cost. It concludes by assessing the TRL of the BN-rGO FET biosensor and proposing strategies for market optimization. Collectively, these efforts aim to bridge the gap between innovative biosensor development and real-world application, offering a potential pathway toward commercialization of graphene-based FET platforms.

3.2 Background

Before presenting the core analyses in this chapter, it is important to outline the operational principles behind FET biosensors. FET biosensors detect molecular interactions by transducing surface binding events into electrical signals. FETs typically consist of three main terminals: source, drain, and gate, with a semiconducting channel connecting the source and the drain electrodes. The conductivity of the channel is modulated by an applied voltage to the gate electrode. When charged biomolecules adsorb onto the channel, they change the local electrostatic environment, leading to measurable changes in the device’s output characteristics, such as the drain current or threshold voltage. This mechanism enables real-time, label-free detection of analytes at low concentrations [100].

When rGO is used as the channel material, the FET exhibits ambipolar behaviour in the form of a Dirac point, defined as the gate voltage corresponding to minimum conductivity, where electron and hole carrier concentrations are equal. The resulting transfer (I_d - V_g) curve is V-shaped, and can be described with Equation 3.1, where μ_n is the carrier mobility, C_{ox} is the oxide capacitance, W and L are the channel width and length, V_g is the applied gate voltage, V_{th} is the operational threshold voltage, and V_d is the drain-source bias [101].

$$I_d = \mu_n C_{ox} \frac{W}{L} [(V_g - V_{th})V_d - \frac{V_d^2}{2}] \quad (3.1)$$

As mentioned in the introduction, rGO’s defective structure and surface tunability make it highly responsive to changes in surface potential [21]. Adsorption of target molecules, such as proteins or nucleic acids, alters the local charge environment, resulting in a measurable shift in the Dirac point. These shifts can be quantitatively correlated with analyte concentration, allowing for accurate biosensing at various concentrations [100]. Figure 3.1

illustrates this mechanism, showing representative transfer curves before and after molecular adsorption.

The magnitude of the Dirac shifts at each tested concentration can be plotted to generate a calibration curve, with the response often normalized to its maximum value. This relationship is commonly fit using the Hill-Langmuir binding model, as shown in Equation 3.2, where C is the analyte concentration, K_D is the dissociation constant, and n is the Hill coefficient describing binding cooperativity [100, 102].

$$\frac{\Delta V_{Dirac}}{\Delta V_{max}} = \frac{C^n}{K_D^n + C^n} \quad (3.2)$$

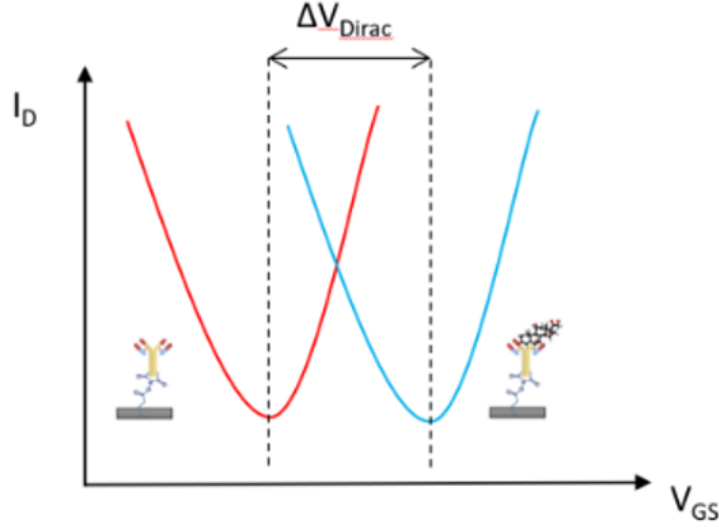


Figure 3.1: Example transfer curve of an FET device during binding event of a biomolecule

3.3 Methodology

3.3.1 BN-rGO Gel Synthesis

BN nanopowder (Graphene Supermarket) was added to a 1:1 mixture of DI water and ethanol (1.24 mg/mL) and ultrasonicated for 30 minutes at room temperature. The suspension was irradiated using a Ti:Sapphire femtosecond laser (800 nm, 35 fs pulse duration,

1 kHz repetition rate), with the beam focused 2 mm below the solution surface using a lens with a 50 mm focal length. Laser irradiation was carried out at a power of 2 W for 55 minutes, with continuous stirring to maintain suspension uniformity. After laser irradiation, the solution was transferred into smaller vials and centrifuged for 15 minutes at 3000 RPM. The supernatant, containing laser-irradiated BN nanoparticles, was carefully extracted for subsequent mixing with the GO solution.

The GO solution was prepared by diluting 6.2 mg/mL aqueous GO (Graphene Supermarket) to a final concentration of 5 mg/mL with DI water, followed by ultrasonication for 90 minutes at room temperature. The BN nanoparticles were added to the GO solution after ultrasonication. The BN-GO suspension was subsequently irradiated at 1.85 W for 90 minutes using the same laser setup. Evaporation during this process was allowed to proceed until the solution volume was reduced to approximately 50% of its initial value to obtain gel-like viscosity.

3.3.2 FET Fabrication and Device Layout

All FET devices were fabricated using commercially available p-doped silicon/silicon dioxide (Si/SiO₂) wafers, consisting of a 500 μm thick p-type silicon substrate with thermally grown SiO₂ layers of varying thicknesses (300 nm, 280 nm, and 240 nm). Metal contacts were patterned using a shadow mask approach, with electrode and channel regions defined by carbon-based wiring and the metal frame. A 20 nm titanium adhesion layer followed by an 80 nm gold layer was deposited via e-beam evaporation (Angstrom Engineering). The resulting FET layout featured a channel width of 80 μm , with source and drain electrodes measuring 0.9 cm $\times$ 0.4 cm, and gate electrodes measuring 0.4 cm $\times$ 0.2 cm.

Following metal deposition, 5 μL of BN-rGO gel was drop-cast onto the channel region and uniformly distributed by spin-coating at 500 RPM for 10 seconds, followed by 2250 RPM for an additional 10 seconds. FETs were dried at 95°C for 2 minutes for drying of the channel. A schematic representation of the device layout is provided in Figure 3.2. While fabrication in this work primarily relied on shadow masking for simplicity and throughput, more conventional micro-fabrication techniques such as photolithography and cleanroom-based patterning were intentionally avoided. The rationale behind this choice will be discussed in the analysis section.

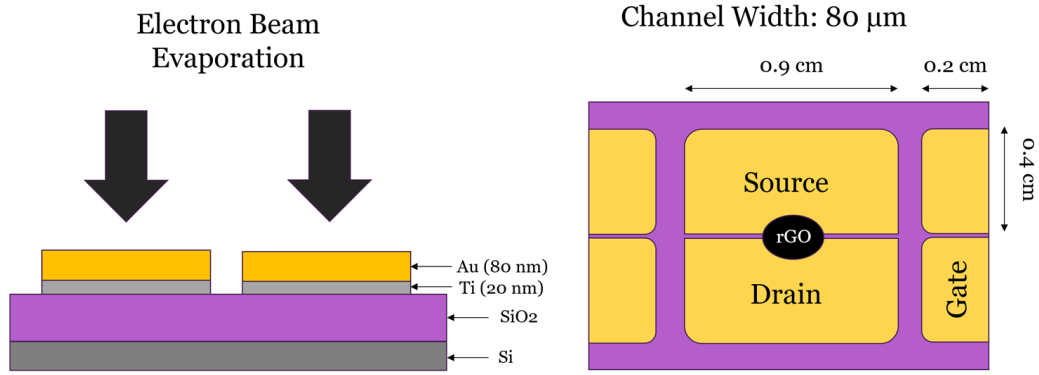


Figure 3.2: Schematic of fabricated FET device with film thicknesses and electrode dimensions

3.3.3 Bioreceptor Functionalization and Passivation Strategies

Bioreceptor functionalization of the BN-rGO FET devices was performed using both antibodies and aptamers specific to the target analytes. All antibodies were diluted in commercially available buffer solutions (Thermo Scientific, pH = 7) and subsequently functionalized onto the channel region by drop-casting 5 μ L of the antibody solution directly onto the BN-rGO surface. Devices were incubated at 4°C for 48 hours to facilitate strong binding between the antibodies and the carboxyl functional groups present on the channel surface. Aptamer-functionalized devices followed a similar process. However, an additional passivation step was required to block non-specific binding sites, which was achieved using an ethanolamine treatment following aptamer immobilization. Successful immobilization of both antibodies and aptamers was confirmed by a measurable shift in the Dirac point, indicating a change in the local electrostatic environment near the channel surface.

3.3.4 Biosensing Setup

Electrical measurements were conducted using a probe station (Everbeing C-2 Mini Probe Station) connected to a source measure unit (SMU)(KEYSIGHT B2900A Series). Devices were tested using supporting software. To generate calibration curves, each target analyte was serially diluted across a defined concentration range and applied to the bioreceptor-functionalized FET surface. Before analyte detection, each bioreceptor-functionalized de-

vice was subjected to a buffer stability test to confirm baseline consistency and rule out nonspecific electrical fluctuations before biosensing.

3.4 Optimization and Yield Analysis

3.4.1 Optimization of Fabrication Parameters

The fabrication of the BN-rGO FET biosensors in this project was carried out over a series of production batches, with the primary objective centered on achieving functional devices for biosensing evaluation. Rather than employing a formal design-of-experiments framework with predefined parameters, the optimization process followed an adaptive, iterative model. Fabrication adjustments were made reactively, driven by the identification of failure modes or performance issues that interfered with device functionality, such as unstable transfer curves or poorly defined Dirac points. Whenever a batch yielded a relatively high number of failed devices, specific fabrication steps were revisited and modified based on empirical observations and practical constraints. This approach allowed for continuous refinement of the process, guided by the end goal of achieving reliable bioreceptor functionalization and measurable electrical responses to target analytes. The following subsections examine each major fabrication stage in detail, highlighting observed issues, adjustments made over time, and their impact on device yield and performance.

BN-GO Gel Synthesis

The base procedure for fabrication of the BN-rGO gel was preserved across batches, as it had demonstrated high reproducibility and functionality in prior work [29, 34, 99]. Parameters such as the BN-to-GO ratio, solvent composition, and ultrasonication time were retained, as further modification proved no observable benefit within the scope of the project. As a result, fabrication inconsistencies were attributed primarily to the laser irradiation step rather than the chemical composition of the gel itself.

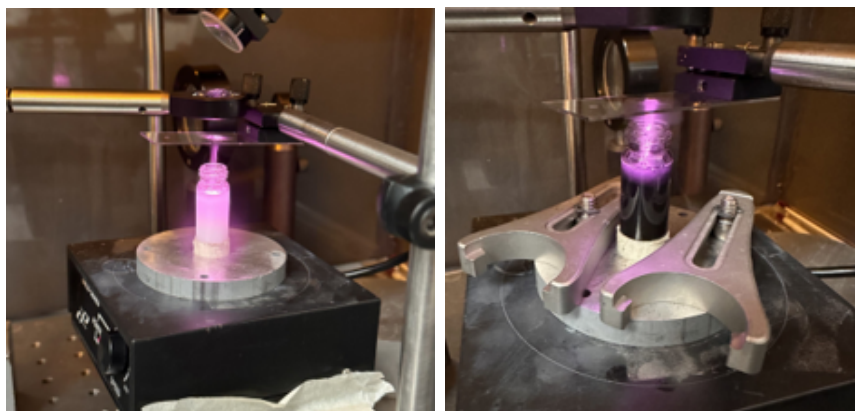


Figure 3.3: Femtosecond laser irradiation of BN suspension (left) and BN-doped GO suspension (right)

One key challenge associated with the first few batches was maintaining a constant laser focal point within the suspension as the solution volume gradually decreased due to evaporation. Initially, the femtosecond laser was focused 2 mm below the surface using a fixed stage setup. However, as the liquid level dropped throughout laser irradiation, the focal point had to be realigned by loosening a mechanical clamp and lowering the lens, an approach that resulted in a couple of drawbacks. First, it introduced the possibility of shifting the alignment between the laser beam and the lens, leading to power losses and beam defocusing. Second, the adjustment process required beam-blocking to comply with safety standards, further lengthening the fabrication time and increasing the likelihood of user-introduced variability.

To address this limitation, a fine-tuned adjustable knob mechanism was introduced in later batches, which enabled gradual and precise vertical translation of the focal lens without having to halt the irradiation process. This setup allowed for better tracking of the evaporation and ensured that the laser remained focused at a consistent depth within the suspension throughout the reduction process. This modification improved safety, accuracy, process efficiency, and eliminated the risk of defocusing due to lateral shifts in the lens during adjustment. A comparison of the original clamp-based setup and the upgraded adjustable knob configuration is shown in Figure 3.4, highlighting the mechanical differences and improvements in focus control.

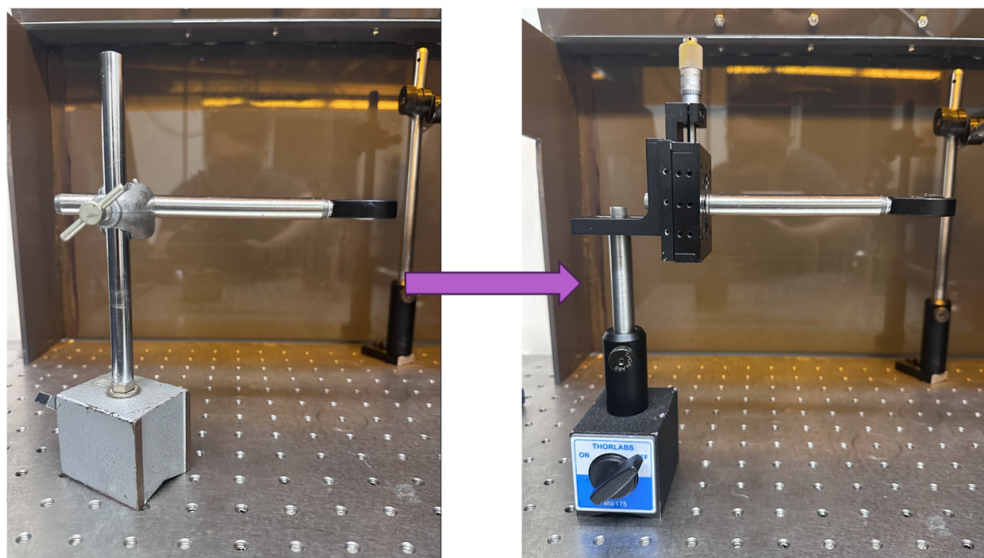


Figure 3.4: Mechanical clamp (left) vs. adjustable knob (right) for focus lens adjustment

Maintaining accurate focus is critical in this context for sufficient laser irradiation of the [BN](#) and [GO](#) suspensions. If the focal point is set too high above the liquid, little to no interaction occurs, as the beam mainly interacts with the air-liquid interface, contributing only to the evaporation. Conversely, if the focal point is positioned too deep within the suspensions, the beam encounters increased optical scattering and absorption, especially in more concentrated solutions. These effects attenuate the intensity of the femtosecond laser pulses before they reach the desired focal volume, resulting in reduced energy density and suboptimal reduction. While this phenomenon was not quantitatively measured, visual inspection of the gel quality across early batches suggests that focal depth plays a critical role in determining the extent of the laser-induced reduction.

In parallel, another critical factor influencing focal point stability was the optical condition of the glass slide used to protect the focus lens from the evaporation of the sample. Over time, solvent vapours and [GO](#) particulates accumulated on the underside of the glass slide, forming a layer that scattered or absorbed the incoming beam. If not regularly cleaned, this contamination altered the effective focal distance or reduced laser transmission, leading to inconsistent gel formation. To maintain optical clarity, it became necessary to block the laser beam and manually wipe the underside of the glass slide every 30 seconds for the first 30 minutes of irradiation, every 1 minute for the following 30 minutes, and

every 5 minutes for the last 30 minutes. While this routine was essential for ensuring consistent laser focus and energy delivery, it added significant time and labour to the process, highlighting a practical bottleneck in the current fabrication process.

The age of the commercial GO solution also emerged as another factor influencing the viscosity and cohesion of the final product. Older dispersions, subject to gradual water evaporation over time, exhibited a higher effective concentration of GO nanoflakes at the time of solution preparation. Upon reduction, these concentrated suspensions formed more cohesive, gel-like materials due to enhanced $\pi - \pi$ stacking and van der Waals interactions between the partially reduced GO sheets. This behaviour is driven by the progressive removal of oxygenated functional groups during laser irradiation, which increases hydrophobicity and promotes self-assembly into layered networks. These gels qualitatively exhibited better spreadability and adhesion during spin-coating, whereas gels synthesized using newer commercial GO solutions demonstrated less viscosity and lower device adhesion. The practical differences observed during deposition suggest that solution concentration should be carefully observed and noted to ensure more consistency between each vial.

Finally, a small but impactful optimization was implemented to reduce material waste and improve batch efficiency. Each laser-treated BN nanoparticle suspension yields approximately 750 μL of usable supernatant. Given that the nanoparticle suspension maintains stability and biosensing effectiveness for up to two weeks, producing two gel vials per BN batch, rather than just one, was found to be a more resource-efficient strategy. This ensured that more of the synthesized material was utilized within its usable window, minimizing unnecessary repeat runs and material costs.

The BN-rGO gel fabrication process, while chemically consistent across batches, required considerable precision and care to maintain reproducibility. Tasks such as tracking the laser focal point, managing the evaporation rate, and maintaining the cleanliness of the glass slide demanded sustained attention throughout the reduction period. These process sensitivities, if neglected, can often result in incomplete reduction, poor gel morphology, and batch-to-batch variability. Despite these challenges, several key refinements, such as improved focal point control, synthesizing two vials per synthesis to optimize BN usage, and better understanding of GO aging effects, helped stabilize the gel's properties and its production. These improvements established a more reliable foundation for subsequent stages of biosensor fabrication, particularly in FET construction and device integration.

FET Fabrication

While many aspects of the FET fabrication process evolved throughout the project, several foundational parameters were intentionally preserved across all batches to ensure baseline consistency. These included the deposition rates and thickness of the metal layers, specifically, the 20 nm titanium adhesion layer and the 80 nm gold contact layer deposited via electron beam evaporation. This also includes the geometry of the device: a fixed channel width of 80 μm , and electrode dimensions at 0.4 cm x 0.9 cm for the source and drain, and 0.4 cm x 0.2 cm for the gate. These parameters were selected based on early benchmarking and were not altered throughout the fabrication process. Each batch typically consisted of approximately 45 devices fabricated on p-doped silicon substrates with thermally grown silicon dioxide layers of varying thickness.

The most notable improvements in fabrication occurred in the methods used to define electrode geometry. Initially, pieces of the Si/SiO₂ wafers were masked using manually cut tape segments to cover regions during deposition. However, this approach introduced high variability in electrode placement and channel width, as manual application often led to misalignment or uneven masking. To address this issue, a custom-designed metal shadow mask was implemented, providing uniform channel and electrode definition across each wafer piece. A visual comparison of the tape-masked approach and the metal mask method is shown in Figure 3.5, with devices fabricated using both methods. The metal mask significantly improved dimensional consistency and reduced user-dependent variability in electrode geometry.

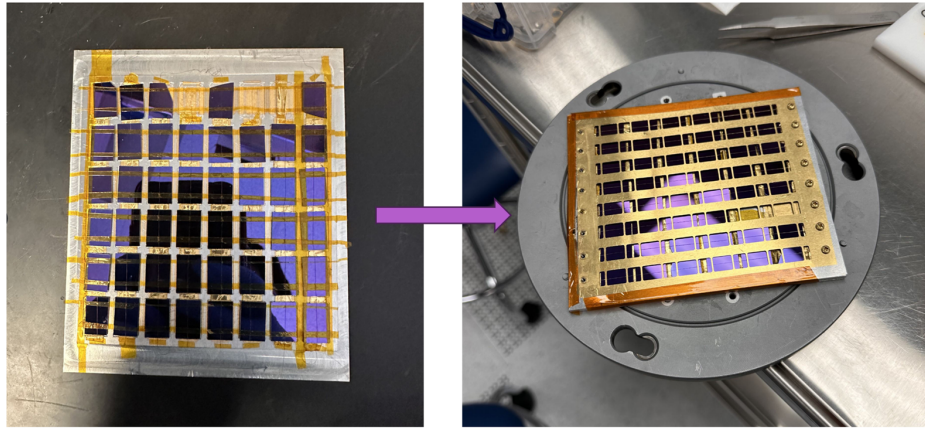


Figure 3.5: Tape shadow mask (left) vs. metal shadow mask (right) for FET device fabrication

The dicing method used to segment wafers also changed during the project. Early on, a dicing saw was used to cut the wafers into individual chips. While this produced uniformly sized devices, the water cooling step introduced during cutting compromised the adhesion properties of the gel in later deposition stages. Manual cleaving was adopted as a cost-effective alternative that avoided water exposure, albeit at the expense of some dimensional variability. Given the tradeoff between precision and material compatibility, manual cleaving ultimately offered better downstream biosensor performance despite its drawbacks.

Another critical factor in channel definition was the choice of material used to mask the channel during metal deposition. Carbon-based materials, specifically strands of human hair, ultimately proved to be the most effective option. They provided the correct diameter for channel width, were compatible with the vacuum conditions of electron beam evaporation, and left no residual contaminants on the wafer. Alternative materials posed significant drawbacks: metallic wires, while easy to handle, often left behind trace metal particles that disrupted the electrical behavior of the channel and contributed to high device failure rates. Polymer wires were considered, but they would not withstand the elevated temperatures of the deposition process. To ensure consistent channel definition, the hair had to be replaced and tightly re-taped before each deposition run, as the thermal environment caused them to curl or relax after a single use. While this added a small layer of manual effort, it was a necessary tradeoff for clean, reproducible channels that supported reliable biosensor operation. Negligence in this step resulted in poorly defined channels and unwanted connections between the source and drain electrodes.

Lithography was considered a potential alternative to manual masking and cleaving due to its superior resolution and reproducibility. However, its implementation would have introduced significant costs, labour demands, and procedural complexity, which are factors that outweighed its prospective benefits within the scope of this project. Additionally, lithography would involve variability in each step (resist coating, exposure alignment, and development), each introducing new opportunities for device failure and yield inconsistency. Given these trade-offs, the shadow mask strategy provided a more practical and sufficiently precise solution for consistent [FET](#) fabrication.

The choice of substrate also contributed to yield variability. Early batches used commercially sourced wafers with a 300 nm SiO₂ layer from Graphene Supermarket, which produced only moderate yields. Each wafer in this batch costs approximately \$67.40 (USD), making them a relatively expensive option. In contrast, a transition to university-provided 280 nm SiO₂ wafers not only yielded a noticeable improvement in electrical consistency and biosensor performance but also offered a substantial cost reduction, with each wafer priced at \$31.61 (CAD). However, a subsequent order from the same commercial supplier

led to a dramatic drop in yield, potentially due to unreported differences in substrate doping levels or intrinsic defect densities. A final test using 240 nm SiO₂ wafers resulted in the poorest outcomes, likely due to insufficient dielectric isolation or altered field-effect behavior. While other variables may contribute to the overall yield, observed yield drops are strongly correlated with wafer source and oxide thickness, as will be demonstrated later in the chapter. These results underscore that both the electrical and economic performance of the FET biosensors were closely tied to the physical and electronic properties of the silicon dioxide substrate, making substrate quality and thickness key parameters in ensuring device yield and stability.

Of all fabrication steps, yield analysis hinged most significantly on the FET fabrication process. Unlike conventional FETs, which often treat leakage currents as an indication of device failure, this biosensor platform demonstrated optimal signal transduction only when a small, consistent level of leakage current was present. As such, minor variations in channel uniformity, substrate quality, or electrode definition could dramatically affect yield. While the BN-rGO gel fabrication and bioreceptor functionalization steps also contributed to overall performance, it was the precision and consistency of FET fabrication that most reliably predicted whether a device would pass or fail the electrical screening required for biosensing use. With a refined electrode layout and improved substrate handling protocols in place, the next step in the process focused on the deposition of the BN-rGO gel and ensuring a stable, uniform channel coating that preserved electrical function while supporting bioreceptor immobilization.

BN-rGO Deposition and Channel Properties

Achieving a thin and uniform film of the BN-rGO gel on the FET channel region was essential for reliable biosensor function. If the deposited layer was too thick, electrical detection of bioreceptor binding events became impaired, as signal transduction is attenuated by the presence of excessive reduced GO material. Conversely, if the gel was too thin or inconsistently spread, the channel would be undercoated or patchy, leading to unreliable conductivity and reduced bioreceptor attachment. Throughout the batches, the spin-coating protocol remained largely consistent, consisting of a two-step program: 500 RPM for 10 seconds followed by 2250 RPM for 10 seconds. However, when gels were visually assessed to be overly viscous, the second spin step was increased up to 2800 RPM to promote more even radial spreading. This minor adjustment helped maintain surface uniformity without introducing procedural complexity.

Gel viscosity was found to play a critical role in coating performance. Less viscous suspensions tended to spread too thinly or completely off the device surface, while ex-

cessively viscous gels often clumped near the center, resisting the centrifugal force of the spin-coater. To ensure compatibility with the standard protocol, gels were qualitatively screened based on spreadability during trial runs on blank substrates. A comparison of effective versus ineffective coatings is provided in Figure 3.6, illustrating the visual characteristics of successful and failed spin-coated films. This approach allowed the spin-coating parameters to remain fixed for the majority of the project, simplifying fabrication and reducing batch-to-batch variability.

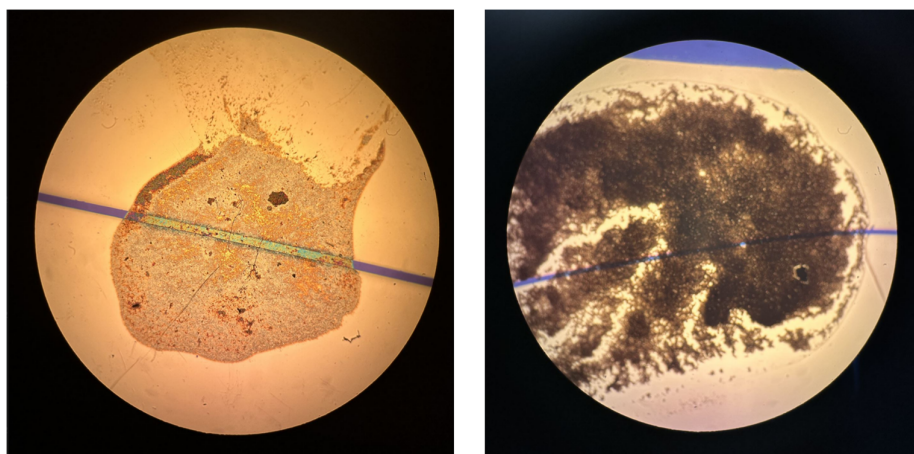


Figure 3.6: Microscopy images of gel deposited onto device channel with good deposition (left) and poor deposition (right)

Bioreceptor Functionalization and Passivation

The immobilization of bioreceptors onto the [BN-rGO FET](#) surface is a crucial step in the biosensor workflow, and the effectiveness of this process can vary significantly depending on the molecular properties of each target biomolecule. Rather than applying a single universal protocol, the functionalization strategies were adapted based on the specific bioreceptor type, each introducing unique challenges and optimization parameters. This section highlights key distinctions and considerations observed during the course of the project.

Antibody-based probes targeting Lyme disease and the SARS-CoV-2 N protein demonstrated high reliability in terms of surface attachment and signal consistency. These antibodies were drop-cast in buffer solution and incubated at 4°C for 48 hours. Following incubation and washing, a clear Dirac point shift was observed across around 50% of devices in each batch, and subsequent buffer tests showed electrical stability, suggesting successful

and reproducible immobilization. Notably, these antibodies did not require any additional surface passivation, streamlining the functionalization process and minimizing potential sources of error.

Similarly, aptamer-based functionalization generally achieved effective immobilization under the same incubation conditions. However, unlike antibodies, the aptamers required a post-functionalization passivation step with ethanolamine to block unreacted carboxyl groups and suppress non-specific interactions. While effective, this extra step introduced additional variability into the process. Inconsistencies in ethanolamine incubation time, concentration, or rinsing protocols could lead to either incomplete blocking or excessive surface coverage, which could inhibit aptamer sensor performance. Additionally, aptamers are structurally dynamic molecules whose function is tightly linked to their 3D folding, which is highly sensitive to changes in pH, ionic strength, and surface environment [25]. This structural dependence increases the probability of performance variation due to slight environmental or procedural inconsistencies during fabrication.

A direct comparison of antibodies and aptamers was made in the context of detecting SARS-CoV-2 S and N proteins, as both bioreceptor types were used to functionalize devices against viral targets. Aptamer-functionalized devices exhibited significantly higher sensitivity, achieving a LOD down to 3 zg/mL, compared to 10 ag/mL for antibody-functionalized devices [29]. This enhanced sensitivity is attributed to the smaller size, higher target specificity, and tunable conformational flexibility of aptamers, which can produce stronger signal shifts upon binding. Despite these advantages, aptamers are rarely used in commercial biosensors. Their production, though often cheaper at scale, involves more complex synthesis and folding validation protocols. Furthermore, their environmental sensitivity, as noted above, can hinder reliability under variable field conditions [25]. In contrast, antibodies are better understood, more stable in storage, and have a longer track record in regulatory approvals, making them a more commercially viable option despite their comparatively lower sensitivity. The transfer curves using both bioreceptors for detecting SARS-CoV-2 proteins can be seen in Figure 3.7.

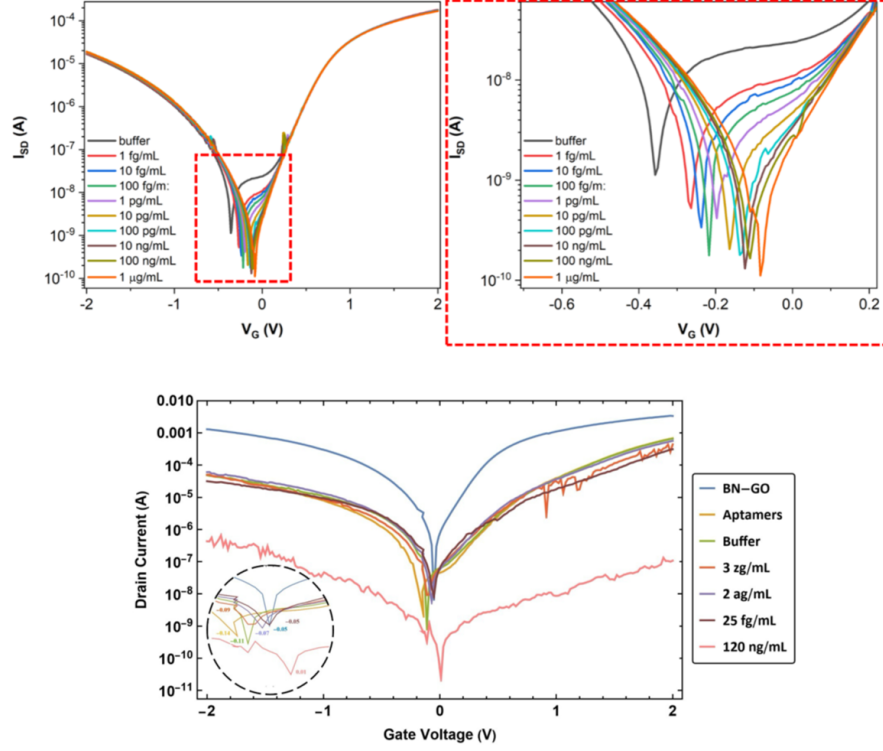


Figure 3.7: Transfer curves for detection of SARS-CoV-2 S and N proteins using antibodies (top) and aptamers (bottom). Adapted from [29]

The functionalization of BSA-targeting antibodies presented a unique and challenging case. Although initial immobilization appeared successful, the devices consistently exhibited electrical instability upon exposure to buffer. Numerous buffer formulations were tested, including PBS and low-salt buffers, yet stable transfer curves were difficult to achieve. This suggests that while the antibodies effectively bound to the channel surface, their integration was chemically or structurally incompatible with the device environment. Potential explanations include conformational fragility of the BSA antibody under gate bias, strong non-specific adsorption to the GO surface, or interference with the electrical double layer required for field-effect behavior [103]. While the high immobilization rate of BSA antibodies was promising, the persistent signal instability indicates that further engineering of the surface chemistry, buffer environment, or immobilization steps will be required to achieve reliable electrical signals for biosensing using this platform.

Collectively, these findings emphasize the need for tailored functionalization workflows

depending on the chosen bioreceptor. Each biomolecular probe brings its unique binding characteristics, buffer sensitivities, and surface interaction profiles, necessitating careful protocol adjustment for each new sensing target. Future applications of this platform must therefore treat bioreceptor functionalization not as a fixed procedure, but as a flexible, chemistry-specific process requiring empirical tuning and validation for each diagnostic use case.

3.4.2 Yield Summary and Batch Comparison

Wafer Type	Masking Method	Dicing Method	Channel Wire	Yield (%)
300 nm SiO ₂ (Graphene Supermarket)	Tape Shadow Mask	Dicing Saw	Hair	40.78
300 nm SiO ₂ (Graphene Supermarket)	Metal Shadow Mask	Dicing Saw	Wire	42.41
280 nm SiO ₂ (University Wafers)	Metal Shadow Mask	Manual Cleaving	Hair	62.67
280 nm SiO ₂ (University Wafers) (New Batch)	Metal Shadow Mask	Manual Cleaving	Hair	25.26
240 nm SiO ₂ (University Wafers)	Metal Shadow Mask	Manual Cleaving	Hair	17.78

Table 3.1: Summary of fabrication strategies, substrate types, and electric signal outcomes across different batches

To contextualize the impact of fabrication strategies on device performance, a summary of yield and design choices across all major batches is presented in Table 3.1. This table captures key variables, including wafer type, masking and dicing methods, and channel definition material, providing a holistic view of how each parameter affects device yield and biosensing readiness. The overall yield percentage reflects the proportion of devices in each batch that passed electrical screening and exhibited suitable transfer curves for further bioreceptor functionalization. By organizing the table to progress from fabrication decisions through to final application, patterns can be identified between specific process

choices and resulting biosensor performance, supporting the rationale for the iterative optimizations described in previous sections.

The second batch saw a modest increase in yield compared to the first due to improved electrode consistency after replacing the tape masking with a metal shadow mask. A more substantial jump in yield was observed in the third batch, which introduced two key changes: a switch to lower-cost university provided 280 nm SiO₂ wafers (\$31.61 CAD) from the wafers from Graphene Supermarket (\$67.40 USD) and using hair as the channel definition material. These adjustments were prompted by both price and the unavailability of commercial wafers due to the closure of Graphene Supermarket. Notably, the fourth used identical fabrication specifications but exhibited a significantly lower yield, emphasizing the batch-to-batch variability in the commercial wafers. This decline suggests that subtle, unreported differences in wafer doping, oxide quality, or defect density can drastically affect biosensor performance.

3.4.3 Proposed Optimized Parameters and Future Considerations

One potential avenue for improving device yield and reliability lies in gaining control over substrate quality by thermally growing silicon dioxide layers during the fabrication process. The current reliance on commercially sourced wafers introduces batch-to-batch variability, as demonstrated in previous sections, where identical substrates produced markedly different yields. Thermally growing oxide layers on site would enable tighter control over oxide thickness, doping concentration, and interfacial cleanliness, which are factors that directly influence gate capacitance, leakage behaviour, and biosensor stability. Although growing your own SiO₂ layers introduces additional costs in terms of equipment, time, and labour, the ability to fine-tune dielectric properties to match the device’s specific requirements could significantly improve yield and reduce failed devices [104]. When scaled across multiple batches, the improved reproducibility may ultimately offset the initial investment.

A second area of optimization involves replacing human hair with a more standardized carbon-based material for channel masking during metal deposition. While hair offered an accessible, residue-free solution compatible with e-beam evaporation, its natural variability in diameter introduces uncertainty into channel dimensions. Purchasing or engineering carbon-based fibers that maintain structural integrity at deposition temperatures while offering uniform geometry could provide more consistent channel formation and reduce the need for frequent manual intervention [105].

Process improvements could also be made at the gel synthesis step, particularly in automating the laser irradiation workflow. The current procedure requires manual cleaning of the protective glass slide every 30 seconds to 5 minutes during irradiation to maintain focus and beam clarity, which is an essential but highly labour-intensive step. Incorporating a motorized or microfluidic cleaning mechanism could maintain optical transparency without interrupting the laser process, significantly reducing operator interference and improving reproducibility across batches.

Collectively, these proposed optimizations highlight a broader shift toward market readiness. While the current platform offers proof-of-concept functionality and strong analytical performance, transitioning to commercially viable biosensor production requires improved consistency, scalability, and cost-efficiency. The following section explores this transition in greater depth by benchmarking the developed platform against existing COVID-19 diagnostic technologies, identifying the areas where this biosensor excels, and outlining what remains to be improved for successful translation beyond the research environment.

3.5 Conclusion

This chapter details the batch-wise fabrication and performance analysis of our lab's [BN-rGO FET](#) biosensor, with a focus on identifying failure modes and fabrication sensitivities that impact reproducibility. Rather than demonstrating consistent performance improvement across batches, the work emphasized the practical insights gained from iterative troubleshooting of gel synthesis, electrode definition, spin-coating conditions, and bioreceptor functionalization. Key takeaways included the importance of stable laser focusing during gel preparation, the impact of gel viscosity on channel coverage, and the critical role of substrate quality in determining yield. Even with improved masking and channel definition strategies, device yield remained highly sensitive to substrate variability, particularly across different commercial batches. Several batches with identical fabrication protocols yielded dramatically different results, pointing to unreported differences in doping levels or oxide quality as a dominant source of variability. These findings collectively underscore the necessity of treating each fabrication step as interdependent processes that require careful control and documentation. The analysis provided in this chapter offers a foundation of process knowledge to guide future efforts in scaling the biosensor for [POC](#) applications.

Chapter 4

Comparative Analysis of FET Biosensors with Current COVID-19 Diagnostic Tools

4.1 Introduction

While public perception has largely shifted away from COVID-19 as an urgent health threat, the need for accurate and ongoing detection remains critical. With reduced testing infrastructure and fading public attention, many assume the virus has run its course. However, this assumption overlooks key scientific, medical, and public health realities that continue to demand reliable diagnostic solutions, particularly those that are accessible, cost-effective, and suitable for decentralized use. COVID-19 diagnostics are central to understanding its evolving impact, protecting vulnerable populations, and preparing for future mutations or disease variants [106].

First, COVID-19 is still a relatively new disease, and its full long-term effects are only beginning to surface. Five years out from the initial outbreak, researchers are now documenting persistent neurological consequences, including hippocampal impairment, cognitive decline, and increased risk of neurodegenerative disorders [107, 108, 109]. Beyond the nervous system, more evidence links SARS-CoV-2 infection to elevated risks of cancer progression, cardiovascular complications, and developmental issues in newborns and toddlers [110, 111, 112, 113]. Long COVID, in particular, remains a poorly understood condition with a long and unpredictable list of symptoms: chronic fatigue, shortness of breath, chest pain, cognitive fog, and more. Critically, even individuals who experienced

only mild or asymptomatic infections remain at risk of developing these post-viral sequelae [114, 115]. Continued diagnostic testing is essential not just for acute management but also for enabling long-term studies that reveal the true biological and societal cost of the pandemic.

Second, ongoing detection is essential for safeguarding high-risk and immunocompromised populations. For individuals undergoing cancer treatment, organ transplant recipients, and those with autoimmune conditions, COVID-19 remains a serious and potentially life-threatening illness. Many in these groups exhibit reduced immune responses to vaccines and may be unable to rely on herd immunity. Yet access to consistent, sensitive testing is still unreliable. Centralized methods like RT-PCR are often inaccessible or too slow, while many rapid tests suffer from insufficient sensitivity. This underscores the need for reliable, POC diagnostic tools that can be deployed in clinics, long-term care facilities, or even at home to enable early intervention and tailored treatment for those who remain most vulnerable [116, 117].

Finally, with the help of reduced public concern, COVID-19 has become endemic, and that makes ongoing variant monitoring especially important. Similar to viruses such as influenza and HIV, SARS-CoV-2 continues to mutate, giving rise to variants with enhanced transmissibility and immunity to current vaccines. These changes increase reinfection rates and challenge existing therapeutic protocols [118]. Timely and widespread diagnostic testing allows health systems to track variant spread in real time, respond to surges with data-informed policies, and adapt public health recommendations. Without sustained surveillance, early warning signs may be missed, and society risks falling behind the curve in future waves [119]. In this context, the development of reliable and adaptable biosensor-based diagnostics is crucial in the timely and accurate sensing of COVID-19.

4.2 Defining Market Biosensor Performance Metrics

A standardized framework is essential for evaluating the utility of COVID-19 diagnostic tools across clinical and public health contexts. While analytical accuracy remains critical, other parameters, such as speed, cost-effectiveness, stability, and accessibility, are equally important for real-world implementation. These factors are especially relevant as testing shifts from centralized laboratory settings to decentralized, routine use in communities, workplaces, and travel environments. Reliable testing solutions must therefore balance diagnostic precision with practical considerations that influence usability, scalability, and adoption. Before conducting a comparative analysis of available diagnostic platforms, it is necessary to define the key performance metrics that determine a test’s effectiveness

in diverse operational scenarios. The following parameters, ranging from detection time to stability, form the foundation for evaluating both currently deployed and emerging technologies.

4.2.1 Limit of Detection

The **LOD** is defined as the lowest concentration of viral material that can be reliably detected, typically with a confidence level of at least 95%. This metric is crucial for detecting COVID-19 during early-stage or asymptomatic cases and directly influences a test's ability to identify infected individuals. A higher **LOD** is generally associated with an increased risk of false negatives, which can hinder efforts to control transmission by delaying effective isolation and appropriate treatment strategies [120]. **LOD** can vary not only by platform but also by the target gene, sample type, and testing protocol [121, 122]. **LOD** is most commonly expressed in viral copies per millilitre (copies/mL). However, many commercial tests report **LOD** using other units, such as the 50% Tissue Culture Infectious Dose (TCID₅₀)/mL or viral copies per swab. Inconsistent reporting units make direct comparisons across platforms challenging without standardized conversions. For comparison to **RT-PCR** benchmarks, all **LOD** values in this review will be converted to viral copies/mL. Based on an estimation by Lelie et al., one TCID₅₀/mL corresponds to approximately 1,000 viral copies/mL for SARS-CoV-2 [123]. To convert viral copies per swab to viral copies/mL, we divide the reported value by the volume of buffer used for elution. Buffer elution volumes typically range from 0.3 to 3 mL, depending on the test type. These conversion approaches and the resulting standardized values will be presented in a summary table in a later section.

4.2.2 PPA and NPA

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) are statistical measures used to evaluate the sensitivity and specificity of a new diagnostic test against an established reference method, typically **RT-PCR**, with a confidence level of at least 95% [124]. A lower PPA reflects a higher risk of false negatives, which can lead to missed diagnoses and delayed isolation or treatment. A lower NPA indicates a higher risk of false positives, which may lead to individuals mistakenly labeled as infected and unnecessary use of healthcare resources. PPA is mathematically defined as the proportion of individuals who test positive by both the new test and the reference method. NPA is mathematically defined as the proportion of individuals who test negative by both the new test and the reference method [125].

$$\text{PPA} = \frac{\text{True Positives}}{\text{True Positives} + \text{False Negatives}} \times 100\% \quad (4.1)$$

$$\text{NPA} = \frac{\text{True Negatives}}{\text{True Negatives} + \text{False Positives}} \times 100\% \quad (4.2)$$

PPA and NPA values cited in this review are either sourced from manufacturers or derived from independent third-party evaluations. For EUA, antigen tests are generally required to demonstrate a PPA of at least 80% and NPA of at least 95%, although specific PPA and NPA thresholds may vary based on test type, use setting, and population [126]. However, PPA and NPA values observed in clinical trials may not reflect real-world performance, which is influenced by user technique, timing of sample collection, and characteristics of the tested population. For example, self-administered tests in asymptomatic individuals may yield lower PPA than those conducted in controlled clinical settings [127].

4.2.3 Detection Time

As noted in the Introduction, rapid detection is particularly crucial in high-risk congregate settings where delayed results can lead to increased transmission and poorer outcomes. In these contexts, rapid diagnostics significantly enhance containment measures, especially regarding the risk of asymptomatic cases, and facilitate timely individual decision-making. Short detection times enable high efficiency and optimal throughput in large-scale screening and mass-testing scenarios. Detection time is typically influenced by test complexity and methodology; highly sensitive tests generally require longer processing times [128]. Numerous innovations have emerged since the start of the pandemic to reduce the detection times of RT-PCR, such as rapid antigen tests, isothermal amplification techniques, and novel biosensor technologies [125, 129]. These advancements will be further discussed in the comparative analysis section of this paper.

4.2.4 Cost and Cost Effectiveness

Cost and cost-effectiveness remain key considerations in the deployment of COVID-19 diagnostic tools as the virus transitions to an endemic threat [129, 130]. The price of a test to individuals, whether subsidized or paid out-of-pocket, continues to influence access to timely diagnosis and the feasibility of maintaining routine surveillance programs [131]. The appropriate balance between affordability and diagnostic accuracy depends on the intended

application, whether for symptomatic testing, outbreak response, or screening in high-risk environments. In an endemic context, strategies must consider not only the absolute cost of diagnostic tools but also their cost-effectiveness in supporting early detection, mitigating transmission, and minimizing healthcare burdens. Ensuring that COVID-19 testing remains both affordable and demonstrates reliable diagnostic performance is essential to sustaining long-term public health resilience and preparedness.

4.2.5 Shelf Life and Storage Requirements

Shelf life and storage requirements are vital parameters influencing the deployment and reliability of COVID-19 diagnostic tools. Shelf life refers to the period during which a test maintains its performance characteristics, while storage conditions describe the environmental requirements needed to preserve its integrity. These factors are central to supply chain stability, stockpiling capacity, and consistent performance across decentralized and clinical settings. Shelf life is often limited by the stability of biological reagents, such as antibodies, enzymes, and nucleic acids, which are sensitive to temperature fluctuations, humidity, and oxidation [132]. Therefore, many tests require cold chain logistics or climate-controlled storage, posing logistical challenges, particularly in remote or resource-limited settings [130]. Ensuring long shelf life and robust storage tolerance is particularly important for expanding diagnostic access in rural and underdeveloped areas, where infrastructure and supply chains may be less reliable [131]. Regulatory authorizations mandate validated stability data to establish expiration dates and storage recommendations. Manufacturers have introduced innovations such as lyophilization, stabilizing additives, and room-temperature-stable formulations to address these constraints [133]. As COVID-19 shifts to an endemic threat, long shelf life and simplified storage requirements will be essential for maintaining resilient testing infrastructure and ensuring continued diagnostic access.

4.2.6 Ease of Use

Ease of use remains a defining attribute of COVID-19 over-the-counter diagnostic kits, influencing their effectiveness in unsupervised testing environments. As these tests are administered outside clinical settings, a user-friendly design is essential to ensure accurate execution and interpretation [131]. Key elements include straightforward sample collection, clear instructions, and streamlined operational steps. Kits with complex procedures risk higher rates of invalid results and misinterpretation, which can compromise individual

health decisions and public health surveillance [134]. To mitigate these risks, manufacturers have incorporated intuitive features such as premeasured reagents, simplified workflows, and app-based support tools [133, 135]. As COVID-19 persists as an endemic threat, maintaining accessible and easy-to-use diagnostics will be pivotal for sustaining public engagement in self-testing and supporting ongoing surveillance efforts.

4.3 Benchmarking Against Commercial COVID-19 Diagnostic Tools

Biosensor Technology	Target Gene	Detection Time	LOD (viral copies/mL)	PPA (%)	NPA (%)	Cost (\$USD)	Shelf Life
RT-PCR [136, 137, 138]	N, E, S, RdRp Gene	90 min	32	N/A	N/A	\$50–100 per test	N/A
Pluslife MiniDock [139, 140]	N Protein Gene	15 min	400	98.3	99.3	\$300 + 8 per test	24 months
Cue Health COVID-19 Test Kit [141, 142]	N Protein Gene	20 min	1300	97.4	99.1	\$249 + 65 per test	12 months
LUCIRA COVID-19 & Flu Home Test [143]	N Protein Gene	30 min	183	97.3	99.7	\$84 per test	18 months
3EO Health COVID-19 Starter Kit [144]	Not Specified	30 min	110	95.0	100.0	\$195 + 24 per test	4 months
Lateral Flow Assays [145, 146, 147, 148, 149]	N Protein	10–15 min	10^5 to 10^7	80.0–90.0	97.0–99.0	\$8 per test	16–22 months
BN-GO gel FET Biosensor [29]	N Protein	4 min	0.131	N/A	N/A	N/A	N/A

Table 4.1: List of current market COVID-19 diagnostic tools, including RT-PCR and our BN-rGO FET biosensor

This section presents a comparative analysis of the currently available COVID-19 diagnostic technologies with the BN-rGO FET biosensor. Table 4.1 provides a summary of key performance metrics for COVID-19 diagnostic technologies. The values reported in the table were obtained from a combination of publicly available sources, including FDA EUA documentation and independent third-party validation studies published in peer-reviewed journals. To provide a streamlined overview, individual lateral flow antigen tests were consolidated into a single representative entry, reflecting the general consistency of

performance metrics across these assays, particularly in terms of sensitivity, specificity, limit of detection, and detection time. The molecular [POC](#) devices, a representative [RT-PCR](#) platform, and the [BN-rGO FET](#) biosensor were presented as distinct entries due to greater variability in analytical and operational parameters across these categories. Where multiple data sources were available for a single assay, priority was given to independent evaluation studies to minimize bias from manufacturer-reported performance.

It is important to note that the table does not include every authorized diagnostic test currently available. A selection of representative assays was included based on the availability of complete performance data, widespread market use, and differences in operational procedures relevant to practical deployment. The table is intended to serve as a reference point for standardized comparison of core diagnostic characteristics, providing a consolidated overview of detection times, analytical sensitivities, specificities, and other key parameters. While metrics were aligned for comparability, differences in study design, testing populations, and reference standards may contribute to variability across reported values. As such, the table provides an initial framework for interpretation, to be contextualized further in the comparative analysis that follows.

The diagnostic platforms summarized in Table 4.1 reveal critical trade-offs between sensitivity, specificity, speed, and cost. [RT-PCR](#) achieves an [LOD](#) of 32 viral copies/mL, setting the benchmark for sensitivity. However, this performance is offset by a processing time of four to six hours, higher costs ranging from \$50 to \$100 per test, and dependence on centralized laboratory infrastructure. In contrast, molecular [POC](#) devices such as the Plus-life MiniDock, Cue Health COVID-19 Test Kit, LUCIRA COVID-19 & Flu Home Test, and 3EO Health COVID-19 Starter Kit demonstrate a balance between speed and sensitivity. These devices achieve detection times between 15 to 30 minutes, with PPA values ranging from 95% to 98.3% and NPA values between 98.4% and 99.3%, while reporting [LODs](#) from 110 to 1300 viral copies/mL. Rapid antigen tests, while offering faster detection times of 15 to 30 minutes and substantially lower costs, exhibit significantly reduced sensitivity, with [LODs](#) between 10^5 to 10^7 viral copies/mL and PPAs generally ranging from 80% to 90%, alongside NPAs above 97%. Consistent with earlier discussion on cost-effectiveness, comparative analysis shows that faster, cheaper tests generally sacrifice sensitivity and specificity in their diagnostic performance.

Although the calculated [LOD](#) for the [FET](#) biosensor targeting the SARS-CoV-2 N protein is numerically more sensitive than [RT-PCR](#), this comparison requires more context. The value was derived using the molecular weight of the N protein (46.6 kDa) from the manufacturer, an assumption of 1000 N proteins per virion, and the previously reported [LOD](#) for the biosensor [150]. While this sensitivity appears to surpass that of [RT-PCR](#), it is important to note that these platforms detect fundamentally different targets. [RT-PCR](#)

amplifies viral RNA while the FET biosensor detects viral proteins. The presence of viral RNA is a direct indicator of viral presence and replication, whereas protein abundance can vary significantly and may not always correlate with the number of virions in a sample, especially in early or late infection phases when protein production is minimal or has degraded [13, 151]. Moreover, the current biosensor operates at TRL 4, meaning it has been validated only in controlled buffer environments, not in complex clinical samples. Based on performance losses observed in other researched FET biosensors, real-world LOD is expected to be reduced by at least an order of magnitude of 10 [152, 153, 154]. Additional challenges, such as the need for virion lysis to expose internal N proteins, increased non-specific interactions in clinical samples, the incorporation of passivation layers to enhance selectivity, and the integration of these devices into ready-to-use systems, are likely to further diminish analytical sensitivity. Thus, while the comparison to RT-PCR illustrates the platform’s impressive theoretical capabilities, it should be interpreted as a demonstration of future potential rather than direct equivalency at this stage. More appropriate benchmarking would be against other POC devices such as nucleic acid amplification devices and lateral flow assays, where protein-level sensing and similar use contexts allow for fairer comparisons.

Despite the caveats around analytical sensitivity, the biosensor offers several compelling advantages that make it well-suited for future POC development. Most notably, the detection speed is among the fastest reported across current diagnostic platforms, with real-time current monitoring enabling response within minutes of sample application. This is a significant improvement over both RT-PCR and many molecular POC devices, which often require longer processing times. Additionally, while market metrics like PPA, NPA, per-test cost, and shelf-life remain unavailable due to the device’s pre-commercial status, its lab-tested LOD and rapid results present a strong case for early infection detection and screening in low-resource or high-throughput settings.

The ease of using a diagnostic test is strongly influenced by the method of sample collection, which plays a critical role in test reliability outside laboratory settings. Most molecular POC devices and rapid antigen tests rely on anterior nasal swabs, but self-collection introduces significant variability. Improper technique, shallow sampling, and inconsistent user performance can compromise sample quality and increase false-negative rates, particularly in asymptomatic or early-stage infections. In contrast, saliva offers a non-invasive, more user-friendly sampling method with higher viral RNA yields and lower variability during certain infection phases [155]. The BN-rGO FET biosensor’s potential compatibility with saliva collection could provide an operational advantage by reducing dependence on invasive swabs while maintaining analytical sensitivity, potentially improving diagnostic accuracy in decentralized settings.

Building on the earlier analysis of cost-effectiveness, molecular [POC](#) devices improve sensitivity but remain cost-prohibitive for mass screening in low-resource settings, with per-test costs ranging from \$84 to \$300 plus consumables. In addition to higher per-test costs, molecular [POC](#) devices often have more stringent storage requirements, with some requiring refrigeration or controlled environments, and shelf lives ranging from 6 to 18 months. In contrast, rapid antigen tests generally maintain stability at room temperature and have longer shelf lives of 18 to 24 months, supporting easier distribution and stockpiling. While more expensive tests may achieve better analytical performance, their added cost and storage constraints may not be justified in every context. For example, in symptomatic individuals or high-prevalence settings, rapid antigen tests may suffice despite lower sensitivity, offering immediate, low-cost results. However, for early detection, asymptomatic screening, or testing high-risk groups, the higher cost of molecular [POC](#) devices or [RT-PCR](#) may be warranted. Moreover, although rapid antigen tests are inexpensive upfront, their poorer [LOD](#) and reduced sensitivity often require repeat testing to achieve diagnostic certainty, increasing cumulative costs over time. By contrast, higher-sensitivity platforms like [RT-PCR](#) or the [BN-rGO FET](#) biosensor reduce the need for repeat testing, potentially lowering downstream costs associated with missed cases and further transmission.

Together, these comparative insights highlight the multidimensional considerations required when selecting a diagnostic platform. Each technology offers a distinct balance of sensitivity, specificity, speed, cost, and user-friendliness, with trade-offs that must be contextualized to the intended testing environment and public health goals.

4.4 Technology Readiness and Future Considerations

The next critical milestone in advancing this biosensor platform to TRL 5 is validation using real-world biological samples, such as human saliva or serum. Unlike buffered test environments, these matrices introduce a range of interfering molecules, including proteins, enzymes, and salts, that can compromise signal accuracy through non-specific adsorption or dielectric screening. As such, additional surface passivation strategies may be necessary to preserve bioreceptor functionality and improve target selectivity [154]. Functional coatings or antifouling chemistries could be explored to minimize noise and enhance reproducibility across devices. This stage of testing is not merely a formality, as it serves as the true litmus test for the biosensor’s clinical viability. [FETs](#), while highly sensitive due to their signal transduction, are equally vulnerable to environmental and biochemical noise. Without effective engineering of both surface chemistry and electrical architecture, their advantages

can be quickly undermined in complex biological systems [100]. Success in real-world matrices will demonstrate not only the device’s sensitivity in detecting target analytes but also its specificity amid a sea of physiologically relevant interfering biomolecules and its ability to produce consistent outputs across large batches of sensors.

Beyond performance validation, a critical step toward commercialization lies in translating the biosensor into a usable POC device. The current design relies on external probe stations and electronic equipment to apply gate and drain voltages and record transfer curves, which is equipment that must be miniaturized and embedded into a compact, user-friendly interface. This requires thoughtful electrical engineering to balance sensitivity with portability, including low-power circuitry, disposable sensor cartridges, and possibly on-chip amplification or analog-to-digital conversion [35, 156]. As outlined in the previous benchmarking analysis, this biosensor holds strategic advantages in POC contexts, particularly where rapid, ultra-sensitive detection is needed and lateral flow assays fall short. However, it is unlikely to displace centralized RT-PCR testing in clinical settings due to differences in throughput, quantification capacity, and gene-targeting capability. Furthermore, practical considerations such as long-term stability of bioreceptors, environmental compatibility, and ease of sample collection must be addressed. These include decisions about lyophilization or dry storage methods for bioreceptors, the design of pre-loaded sample buffers, and user-safe biohazard handling. Ultimately, making the device deployable outside the lab means designing for reliability, affordability, and intuitive usability, which are criteria that define success in any real-world diagnostic tool [133].

4.5 Conclusion

The chapter contextualized the biosensor within the broader landscape of COVID-19 diagnostics. While its reported limit of detection was numerically lower than that of RT-PCR, the comparison served to demonstrate the platform’s analytical potential rather than establish direct clinical equivalency. Real-world deployment will require validation in biological fluids, miniaturization of electronic components, and storage and sample-handling innovations suitable for decentralized settings. Nevertheless, the device’s rapid detection time, high sensitivity, and adaptable bioreceptor compatibility position it as a strong candidate for POC testing applications. With further engineering, particularly around substrate quality control and integration into portable systems, the BN-rGO FET biosensor could serve as a next-generation diagnostic tool capable of addressing both pandemic response and future biosensing challenges.

Chapter 5

Conclusion

This thesis explored two graphene oxide-based biosensor platforms developed for the detection of disease-related biomarkers with applications in infectious disease diagnostics. Chapter 2 presented the development and characterization of a [SERS](#)-based biosensing platform using a [rGO@AuNP](#) nanocomposite for label-free [DNA](#) detection. The sensor demonstrated reliable detection of cDNA at concentrations as low as 10^{-7} M, with an enhancement factor of approximately 10^4 compared to the non-plasmonic baseline. These results validated the effectiveness of the [rGO@AuNP](#) nanostructure in amplifying Raman signals at low analyte concentrations. While these findings confirm the platform's analytical potential, future work is required to improve signal consistency and fabrication scalability. Specifically, further investigation into the influence of gold nanoparticle morphology, more uniform substrate coating protocols, and the incorporation of alternative plasmonic materials such as silver could help enhance sensitivity and reproducibility. These refinements will be key in elevating the system's performance and aligning it with the demands of translational biosensing applications.

Chapter 3 expanded the scope of graphene biosensing by detailing the fabrication, optimization, and benchmarking of a [BN-rGO FET](#) biosensor capable of detecting proteins from COVID-19, Lyme disease, and E. coli. Through adaptive improvements to gel synthesis, electrode layout, wafer sourcing, and bioreceptor immobilization, the device achieved moderate yield and strong signal transduction across various analytes. Yield comparisons across fabrication batches informed best practices for channel masking and material selection, while functionalization studies revealed key differences between aptamers and antibodies, underscoring the importance of bioreceptor-specific design. Market benchmarking against existing COVID-19 diagnostic technologies highlighted the biosensor's potential

unique position in the market. Still, challenges remain in scaling the platform, including real-world sample validation, electronics miniaturization, and reagent stability.

Moving forward, both biosensing technologies presented in this thesis hold strong promise for real-world deployment. For the [SERS](#) platform, future work should focus on stabilizing the nanocomposite surface, reducing background interference, and integrating analyte enrichment or concentration strategies. For the [FET](#) biosensor, next steps include real-world testing in human serum or saliva, improving surface passivation, and engineering a compact, user-friendly diagnostic interface. In both cases, the transition from proof-of-concept to product will require interdisciplinary collaboration, regulatory navigation, and iterative engineering. If successful, these graphene-based sensors could contribute to a new generation of diagnostic tools, providing fast, sensitive, and deployable solutions across a wide range of biomedical and public health applications.

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