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Advanced tools and technologies for liquid biopsy

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Original Citation:

Advanced tools and technologies for liquid biopsy / Colapietro, Paola. - ELETTRONICO. - (2026).

Availability:

This version is available at <http://hdl.handle.net/11589/295520> since: 2026-01-13

Published version

DOI:

Publisher: Politecnico di Bari

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Advanced tools and technologies for liquid biopsy

Thesis submitted for the degree of Philosophiae Doctor

Interuniversity Ph.D. Program in Industry 4.0
Politecnico di Bari

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2025



Politecnico
di Bari

Department of Electrical and Information Engineering
INDUSTRY 4.0

Ph.D. Program

SSD: IINF-01/A – ELECTRONICS

Final Dissertation

Advanced tools and technologies for liquid biopsy

by

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Course n°38, 01/11/2022 – 31/10/2025



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Abstract

Nanotechnology holds immense promise in revolutionising healthcare, offering opportunities in diagnostics, drug delivery, cancer therapy, and combating infectious diseases. The capacity to manipulate light-matter interactions at the nanoscale has opened new avenues for the development of integrated biomedical platforms, addressing pressing clinical needs for rapid, minimally invasive and patient-tailored solutions. Within this framework, this thesis explores two clinically relevant frontiers where nanophotonic technologies can exert transformative impact: the development of integrable photonic biosensors for precision oncology applications and the optical manipulation of viral bio-objects for infectious-disease management.

In the field of oncology, Liquid Biopsy (LB) has emerged as a promising alternative to conventional tissue biopsy, driven by the need for diagnostic methods that are less invasive, faster, and capable of providing real-time molecular insights. By enabling the molecular profiling of circulating biomarkers in body fluids, LB overcomes key limitations of traditional approaches, including invasiveness, sampling bias, and the inability to capture tumour heterogeneity in real time. Nevertheless, its widespread clinical adoption critically depends on the development of highly sensitive, reproducible, and integrable platforms capable of meeting the stringent requirements of precision medicine and Point-of-Care (PoC) diagnostics. Translating LB into routine practice requires not only highly sensitive and reproducible assays but also their seamless integration into miniaturised and automated platforms. Lab-On-Chip (LoC) systems provide this framework by combining precise microfluidic handling with high selective sensing platforms. In this framework, photonic biosensors emerge as a particularly attractive solution, enabling label-free and real-time detection with high sensitivity while remaining fully compatible with LoC system and PoC diagnostics. Accordingly, this thesis focuses on the design and assessment of two nanophotonic sensing devices tailored for the detection of tumour-associated biomarkers, with particular emphasis on Immunoglobulin G (IgG) proteins at diagnostically relevant concentrations. This first solution is a 1D photonic crystal waveguide incorporating engineered defects to generate a box-like resonance profile, thereby enhancing spectral interrogation, improving linearity, and mitigating noise in refractometric measurements. The second is a slot-assisted dielectric metasurface that provides strong electromagnetic field confinement and high Q-factor within a compact, wafer-scalable footprint, ensuring compatibility with LoC integration and large-scale fabrication. Together, these proposed platforms highlight complementary strategies for advancing liquid biopsy towards robust, label-free, and clinically deployable photonic diagnostics.

Complementing the focus on cancer research, the thesis further explores infectious diseases, where conventional diagnostic approaches, including culture-based assays, molecular analyses, and imaging techniques, are often limited by long turnaround times, operational complexity, and insufficient single-particle resolution, which in turn hampers timely outbreak management and effective antimicrobial resistance surveillance. To address these challenges, this doctoral thesis introduces a hybrid dielectric-plasmonic nanobowtie cavity as a multifunctional nanophotonic platform for viral-scale trapping and manipulation. Numerical analyses combining electromagnetic and thermal modelling demonstrate the stable confinement of ~ 100 nm particles under physiological conditions, enabled by enhanced near-field localisation through the integration of a thin metallic layer within a dielectric cavity. Moreover, controlled modulation of the optical power enables localised thermal inactivation, thereby merging trapping and selective deactivation within a single device and paving the way for compact nanophotonic tools for pathogen and rapid viral phenotyping.

Acknowledgments

Desidero innanzitutto ringraziare la Prof.ssa Caterina Ciminelli, per avermi offerto l'opportunità di intraprendere questo percorso di dottorato e per avermi trasmesso l'entusiasmo, la determinazione e la fiducia necessari per raggiungere gli obiettivi prefissati. La sua guida costante, la sua disponibilità e la sua visione scientifica sono state per me un punto di riferimento imprescindibile. Le sue parole, soprattutto nei momenti più difficili, sono state di grande incoraggiamento e rappresentano insegnamenti che porterò con me nel mio futuro professionale e personale. Desidero ringraziarla anche per l'affetto e la generosità con cui ha condiviso non solo le sue competenze e la sua esperienza accademica, ma anche i preziosi consigli di vita e momenti di umanità che hanno reso questo percorso più autentico e formativo. Si tratta di valori inestimabili che non si trovano in alcun libro di testo e che conserverò con profonda riconoscenza.

Ringrazio anche l'Ing. Giuseppe Brunetti, per aver rappresentato una guida preziosa in questo dottorato. Oltre alle competenze tecniche che mi ha trasmesso con grande chiarezza, gli sono grata per i consigli sinceri e concreti su come affrontare le sfide del lavoro ed i rapporti personali. La sua capacità di unire rigore e umanità ha reso questa esperienza formativa sotto ogni punto di vista ed i suoi insegnamenti continueranno ad accompagnarmi anche oltre il dottorato.

Desidero inoltre ringraziare tutti i miei colleghi dell'OptoLab, per il clima di collaborazione, amicizia e confronto che ha accompagnato questi anni di lavoro. Gli scambi di idee, il supporto reciproco e i momenti di condivisione quotidiana hanno reso questo percorso più stimolante e umano. Ognuno di loro, con la propria competenza e personalità, mi ha insegnato qualcosa di importante che porterò sempre con me.

Il mio percorso di dottorato è stato svolto in collaborazione con il CNR-Nanotec di Lecce, ambiente di ricerca multidisciplinare mi ha permesso di ampliare le mie prospettive scientifiche e di confrontarmi con approcci e competenze differenti.

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Chapter 1

Introduction

1.1 Background and motivation of the research

In the past few years, advances in science and technology have profoundly reshaped the way diseases are detected, monitored, and treated (Fig. 1.1). Among the most transformative drivers of this progress is nanotechnology, which integrates principles from multiple scientific disciplines and engineering to manipulate matter at the nanometre scale. At this level, materials and devices exhibit distinctive physical, chemical, and biological properties that can be harnessed for innovative medical applications, forming the basis of the rapidly expanding field of nanomedicine [1]. Nanomedicine can be broadly defined as the application of nanoscale tools and structures to monitor, control, repair and enhance human biological systems, with the aim of improving health and well-being. Operating at the molecular level, it enables precise modulation of physiological processes and targeted intervention in pathological mechanisms. Within this framework, nanotechnology has become one of the fastest-growing scientific domains of the 21st century, with applications ranging from regenerative medicine and drug delivery to medical imaging and advanced diagnostics [2].

In clinical practice, nanotechnology has profoundly transformed healthcare paradigms. More than one hundred nanomedicine products are commercially available, including anticancer drugs, anaesthetics, and treatments for cardiovascular, hepatic, and degenerative disorders, while nearly 150 additional products are undergoing clinical evaluation [2, 3]. Geographically, research and clinical adoption of nanomedicine have so far been led by North America and Europe, while the Asia-Pacific region, particularly China and India, is emerging as a major contributor due to growing investments in nanotechnology and healthcare innovations.

Nanotechnology also provides significant benefits to cell and gene therapies for the treatment of cancer and other life-threatening diseases, including infectious disorders. Both fields demand accurate, cost-effective, and rapid diagnostic tools to overcome current limitations in early detection and prognostic assessment. For instance, nanotechnology enables the development of miniaturized surgical tools and robotic systems capable of performing microsurgical interventions with high precision.

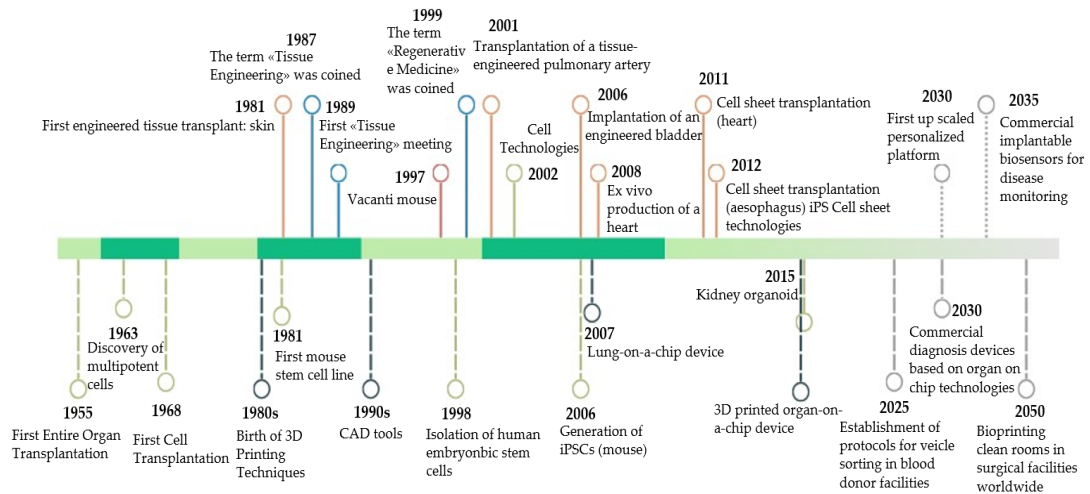


Fig. 1. 1 Timeline of scientific and technological milestones shaping medical diagnosis and treatment (1955-2050). The figure outlines key developments ranging from the first organ transplants and stem cell discoveries to the advent of tissue engineering, organ-on-chip platforms, and 3D printing techniques. Future perspectives include commercial implantable biosensors, large-scale personalized medicine platforms, and bioprinting for surgical applications, highlighting how nanotechnology and related fields are progressively transforming healthcare.

Unlike conventional approaches that may damage surrounding healthy tissue, these devices are designed to act exclusively on the target area, thereby improving surgical accuracy and patient outcomes. At the diagnostic level, current in vitro assays remain costly and time-consuming; however, next-generation Lab-On-Chip platforms incorporating nanostructured components are expected to overcome these limitations by offering reduced costs, enhanced portability and significantly shorter analysis times.

Infectious diseases remain one of the most pressing global health threats, as demonstrated by recurrent pandemics ranging from the Spanish flu to COVID-19, which have exposed structural weakness in healthcare systems and underscored the need for rapid and reliable diagnostics. Conventional methods such as culture assays, ELISA and PCR are constrained by long turnaround times, technical complexity, and limited sensitivity, challenges further intensified by the rise of antimicrobial resistance, projected to cause over 10 million deaths annually by 2050 [4, 5]. In this context, nanotechnology and photonic nanodevices offer innovative solutions, enabling highly sensitive, rapid, and scalable diagnostic platforms capable of addressing these current critical clinical needs.

Additionally, the incidence of cancer has risen sharply, establishing it as the leading cause of mortality in developed countries and one of the most critical challenges to global health. In response, the biomedical research community is actively pursuing innovative, non-invasive diagnostic approaches for the early detection of cancerous diseases. These efforts have led to the development of advanced technologies aimed at improving early cancer screening and diagnosis, with the goal of significantly

reducing the costs, time requirements, and failure rates associated with conventional clinical trials, as well as minimizing adverse drug reactions. Moreover, such innovations seek to enhance the early evaluation of individual responses to therapy, thereby optimizing personalized treatment strategies [6]. From a multidisciplinary standpoint, it is important to highlight that these advances have impacted nearly all aspects of cancer care, including surgery, radiotherapy, and supportive therapies. Medical oncology has undergone significant changes in the pharmacological management of most solid tumours, necessitating ongoing and comprehensive revisions of diagnostic and treatment guidelines. This evolving landscape has engaged hundreds of healthcare professionals and has been largely driven by breakthroughs in diagnostic methodologies and technologies, which have fundamentally reshaped oncologic disease management [7].

Building upon these technological and clinical advances, it is now well established that tumours exhibit considerable biological diversity, a phenomenon referred to as tumour heterogeneity. This concept extends beyond the basic distinction between malignancies arising in different organs (e.g., breast carcinoma versus lung or gastric cancer) and encompasses the substantial inter- and intratumorally variability observed even within a single tumour type. As a result, the molecular and biological characterization of tumours has become a central focus in oncology, informing prognosis, diagnosis, and disease monitoring [8]. The complexity introduced by tumour heterogeneity has thus created an urgent need for approaches that move beyond conventional, standardized treatments and instead focus on individualized strategies capable of capturing the unique molecular and clinical identity of each patient [8].

Rather than adopting a “*one-size-fits-all*” approach, precision medicine represents a paradigm shift toward tailored strategies for disease prevention, diagnosis, and treatment, based on the unique characteristics of each individual patient. This approach integrates diagnostic and therapeutic processes to define patient-specific profiles from genetic, molecular, and metabolic perspectives, thereby enabling clinicians to select treatments that target the underlying cause of disease with high specificity and efficacy. Precision medicine builds on both patient-specific clinical characteristics, such as the need for less invasive therapies in elderly or fragile individuals, and the unique biological features of each tumour [9]. A critical evaluation of current tools for tumour characterization and monitoring is therefore essential. Among these, tissue biopsy continues to represent the global gold standard for cancer diagnosis. This procedure, which entails extracting cellular material from the body by inserting a fine needle into the target organ, has also benefited from significant technological advancements. The integration of increasingly sophisticated analytical platforms now allows for highly precise characterization of genetic mutations within tumour samples, thereby supporting the development of personalized therapeutic strategies. However, while tissue biopsy provides direct access to the tumour mass and serves as the primary source

of information for diagnosis, prognosis, therapeutic decision-making, and the distinction between malignant and benign lesions, it is not without inherent limitations. Chief among these is its invasive nature, associated procedural risks, patient discomfort, and critically its limited capacity to fully capture the spatial and temporal heterogeneity of tumours, particularly in the case of anatomically inaccessible or metastatic lesions [9]. Within this framework, liquid biopsy (LB) has emerged as a powerful non-invasive alternative with broad clinical applicability and is increasingly adopted in oncology to enhance diagnostic accuracy and guide personalized therapies [10]. LB relies on the molecular analysis of tumour-derived biomarkers in body fluids, including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), cell-free DNA (cfDNA), microRNAs (miRNAs), and extracellular vesicles (EVs), which provide valuable information on tumour dynamics, treatment response, and disease progression (Fig. 1.2).

Despite its promise, LB still faces challenges, particularly the need for precision, sensitivity, cost-effectiveness, and methodological standardization [12]. To overcome these barriers, detection platforms must be rapid, minimally invasive, user-friendly, and scalable. Micro-engineered systems such as microfluidics [13], Lab-On-Chip (LoC) systems, and micro total analysis systems (μ TAS) [10, 12, 14, 15] have been developed to integrate sample preparation, biomarker capture, detection, and analysis into miniaturized chips. By manipulating picolitre fluid volumes within micrometre-scale channels, LoC platforms improve sensitivity, reduce reagent use and cross-contamination, and accelerate assays, making them highly suitable for Point-of-Care (PoC) diagnostics, personalized medicine, environmental monitoring, and therapeutic follow-up.

In parallel, biosensor-based technologies have gained traction for monitoring biomarkers in chronic, infectious, and oncological diseases [16]. Their most transformative sensitive, real-time diagnostic tools that reduce reliance on centralized laboratories [17]. In the LB field, the converge of micro- and nanophotonics is driving innovative PoC platforms for non-invasive cancer diagnostics [18].

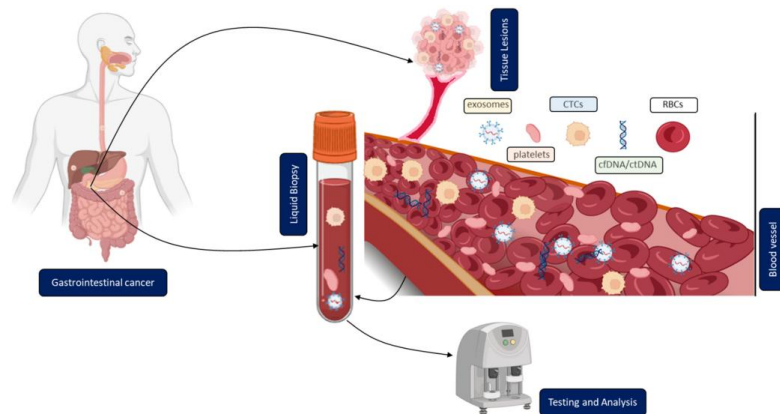


Fig. 1. 2 The tumour releases various components into the blood, which can be analysed through a simple blood draw, avoiding invasive procedures such as tissue biopsy [11].

Despite to operate outside traditional laboratories, these systems offer scalable solutions for clinics and even home-based monitoring. According to the World Health Organization (WHO), ideal PoC diagnostics must be affordable, specific, easy to use, rapid, robust, and independent to external instrumentation. In this regard, photonic biosensors stand out as leading candidates. Their high optical resolution enables the detection of ultra-low biomarker concentrations, ensuring sensitivity and specificity while minimizing false positives. Their compact, noise-resistant, and low-power architecture facilitates integration into clinical workflows, mobile systems, and wearables [18, 19]. Collectively, these features support decentralized, accessible, and patient-centred healthcare.

1.2 Research objectives

Motivated by the urgent clinical need for minimally invasive and highly sensitive diagnostic tools, this research focuses on the. The overarching goal is to provide compact and reliable devices capable of detecting tumour-associated biomarkers, with particular emphasis on Immunoglobulin G (IgG) proteins, at concentrations relevant to clinical diagnostics. Although the integration with microfluidic handling subsystems remains a future perspective, one of the main objectives of this doctoral thesis is to design sensing architectures that can be seamlessly integrated within Lab-On-Chip systems. A key aspect of the work has been the selection and optimization of the sensing architecture, with a focus on achieving high sensitivity and specificity, seamless integration with the microfluidic environment, and scalability through wafer-level fabrication techniques.

To this end, two complementary photonic platforms have been conceived and investigated. The first is based on a 1D photonic crystal with engineered defects, tailored to achieve a resonance profile optimized for enhanced analyte recognition. By departing from conventional Lorentzian line shapes, this configuration enables robust spectral interrogation and improved accuracy in biomarker detection. The second platform relies on a dielectric metasurface featuring arrays of dimers supporting the optical slot effect. This architecture ensures strong field confinement, high quality factors, and compact footprints, making it highly suitable for sensitive and scalable refractometric sensing.

Both platforms have been specifically designed with the aim of ensuring high sensitivity and specificity while maintaining compatibility with wafer-level fabrication processes. The research objectives can therefore be summarized as the development and optimization of two distinct photonic sensing approaches, intended for future integration into LoC systems, ultimately contributing to the advancement of next-generation LB technologies for precision oncology.

In addition to the development of photonic biosensing platforms, a second objective of this doctoral thesis has been to address the challenge of manipulating viral bio-objects at the nanoscale. The focus has been on the design and numerical

investigation of an inverted hybrid dielectric-plasmonic nanobowtie cavity, conceived to achieve stable optical trapping of viral-scale nanoparticles under physiologically relevant conditions. The proposed hybrid nanobowtie integrates a metallic layer within a dielectric framework to enhance near-field confinement, enabling stable trapping of viral scale nanoparticles (~ 100 nm) at reduced optical power while maintaining thermal compatibility. In addition to optical manipulation, the device can also induce localized thermal deactivation of viruses through controlled power tuning. This multifunctional capability, combining nanoscale trapping with selective inactivation, positions the nanobowtie a strong candidate platform for the trapping and control of viruses and bacteria.

1.3 Thesis outline

[Chapter 2](#) establishes the clinical context and outlines the principal diagnostic challenges. It first examines infectious diseases, with a focus on conventional diagnostic technologies and their inherent limitations, before turning to oncology, where cancer incidence, progression, and diagnostic practices are discussed. Particular attention is given to the role of tumour biomarkers, and the paradigm shift from traditional standards to liquid biopsy applications.

[Chapter 3](#) presents the concept and development of Lab-On-Chip systems for liquid biopsy applications. [Section 3.2.1](#) introduces the microfluidic platforms, reviewing both general strategies for cancer biomarker analysis and specific approaches for Immunoglobulin G (IgG) detection. [Section 3.2.2](#) addresses the photonic biosensor platform, beginning with an overview of photonic detection strategies for HER2, CEA, and PSA. The discussion then narrows to IgG detection and a comparative evaluation of state-of-the-art photonic biosensing platforms. Two proposed designs are subsequently introduced in [Section 3.2.2.6](#) and [Section 3.2.2.7](#): a dielectric grating with a tailored resonance profile and an optical slot-assisted metasurface, each described in terms of simulation methodology and performance assessment.

[Chapter 4](#) investigates photonic strategies for optical trapping and the inactivation of viral bio-objects. [Section 4.1](#) provides background and reviews the state of the art in virus trapping, while [Section 4.2](#) examines the fundamentals of optical trapping technologies. [Section 4.3](#) introduces the proposed dielectric-plasmonic hybrid nanobowtie dimer, detailing the simulation methodology, energy confinement design, and trapping performance. [Section 4.3.4](#) extends the study to viral inactivation, addressing both the underlying thermal mechanisms and the numerical results.

[Chapter 5](#) concludes the thesis by summarizing the main findings, highlighting the contributions of the work, and discussing future perspectives.

1.4 List of publications

Journal articles

- [1] A. di Toma, G. Brunetti, P. Colapietro, C. Ciminelli, "High-resolved near field sensing by means of dielectric grating with a box-like resonance shape," *IEEE Sensors Journal*, vol. 24, no. 5, 2023, pp. 6045-6053, doi: [10.1109/JSEN.2024.3349948](https://doi.org/10.1109/JSEN.2024.3349948)
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- [3] P. Colapietro, G. Brunetti, A. di Toma, F. Ferrara, M.S. Chiriaco, C. Ciminelli, "High Stability and Low Power Nanometric Bio-Objects Trapping through Dielectric-Plasmonic Hybrid Nanobowtie," *Biosensors*, vol. 14, no. 8, 2024, pp. 390, doi: <https://doi.org/10.3390/bios14080390>
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Conference proceedings

- [1] C. Ciminelli, P. Colapietro, G. Brunetti, M.N. Armenise, "Lab-On-Chip for Liquid Biopsy: A New Approach for the Detection of Biochemical Targets," in *23rd International Conference on Transparent Optical Networks (ICTON)*, Bucharest, Romania, July 2-6, 2023
- [2] P. Colapietro, G. Brunetti, A. Elicio, F. Ferrara, C. Ciminelli, "Trapping and destruction of virus through dielectric-plasmonic hybrid nanobowtie dimer," in *24th International Conference on Transparent Optical Networks (ICTON)*, Bari, Italy, July 14-16, 2024.

Posters

- [1] P. Colapietro, G. Brunetti, A. Di Toma, M. N. Armenise, C. Ciminelli, "Lab-On-Chip for Liquid Biopsy," poster for the *ePIXfab - 8th Silicon Photonics Summer School*, Ghent, Belgium, July 3-7, 2023
- [2] P. Colapietro, G. Brunetti, F. Ferrara, C. Ciminelli, "Lab-On-Chip for Liquid Biopsy," poster for *BIOCUBE- 2RD EDITION – Workshop and winter school in Biophotonics, Bioelectronics and Biomechanics*, Sestriere, Piemonte, Dec. 10-16, 2023

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23rd International Conference on Transparent Optical Networks (ICTON), Bucharest, Romania, July 2-6, 2023

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Chapter 2

Clinical context and diagnostic challenges

The growing demand for efficient, accessible, and personalized healthcare has become one of the defining challenges of modern medicine. Health systems across the globe are under increasing pressure to respond to complex medical conditions, demographic shifts, and healthcare inequalities, while striving to deliver timely and accurate diagnoses. The current evolution of healthcare systems is increasingly oriented towards personalized, preventive, and data-driven models of care [1]. This transformation is driven by the growing need to address complex and chronic diseases in a manner that is both patient-centric and responsive to molecular heterogeneity. Diagnostic testing, as a fundamental component of clinical decision-making, plays a pivotal role in this transition. However, traditional diagnostic paradigms often fall short in enabling truly individualized medicine, primarily due to intrinsic limitations regarding their ability to provide real-time, comprehensive, and context-specific information. However, conventional diagnostic techniques remain central to clinical workflows, yet they tend to capture static snapshots of disease at a single point in time. These methods may fail to reflect the dynamic, spatially heterogeneous, and systemic nature of many pathologies, particularly in oncology and infectious diseases [2, 3]. Moreover, the interpretation of diagnostic tests is challenged by methodological issues, such as limited evidence regarding their impact on clinical outcomes, insufficient validation across diverse populations, and a lack of integration with broader healthcare data infrastructures. These shortcomings contribute to delays in diagnosis, suboptimal treatment selection, and disparities in access to care. In parallel with these challenges, the healthcare landscape is undergoing a profound transformation driven by technological innovation [4, 5]. Emerging diagnostic platforms increasingly incorporate micro and nanoscale technologies, enabling high resolution and sensitivity, minimally invasive sampling, and high-throughput analysis of biological samples. Within this framework, photonic technologies are emerging as foundational tools in biomedical diagnostics. At present, photonic technologies constitute an enabling platform for next-generation biomedical devices and systems, particularly in

oncology and infectious diseases, offering unparalleled sensitivity, speed, and seamless integration into miniaturized and portable platforms [6]. By serving as a technological backbone for innovative biosensing and analytical systems, photonic approaches contribute to reshaping diagnostic paradigms and advancing the transition toward a more predictive, personalized, and timely healthcare model [4, 7]. Building on these foundations, a wide range of photonics-based strategies is now being explored for clinical translation. On one hand, photonic trapping devices enable the non-invasive capture and analysis of viruses and bacteria, with applications ranging from the diagnosis and monitoring of infections to the rapid assessment of antimicrobial resistance. On the other hand, photonic biosensors allow for label-free, ultrasensitive, and rapid detection of molecular biomarkers associated with cancer and metastasis, even at extremely low concentrations and across diverse biofluids. Thanks to their inherent compatibility with Lab-on-Chip systems and other compact platforms, these technologies open the way to decentralized and Point-Of-Care diagnostic solutions [8].

This chapter offers a critical overview of the clinical and diagnostic framework in which the present research is situated. It first addresses the limitations of conventional diagnostic modalities and the urgent need for novel technologies capable of supporting the transition toward precision medicine. Emphasis is placed on infectious diseases, where early detection, continuous monitoring, and the rapid assessment of antimicrobial resistance are essential to contain transmission and optimize clinical management. At the same time, understanding and addressing cancer as a global health priority requires an integrated perspective that considers not only its epidemiological burden and biological complexity but also the shortcomings of traditional diagnostic approaches such as imaging and tissue biopsy. Although central to current practice, these methods fail to fully capture the dynamic and heterogeneous nature of tumours, underscoring the need for more refined strategies. In this regard, the emergence of liquid biopsy represents a transformative step toward personalized treatment planning and real-time monitoring, laying the foundation for precision oncology. In line with this, the present doctoral work focuses on the design of photonic devices for the optical trapping of viruses and for the sensing of tumour biomarkers, aiming to contribute to next-generation diagnostic frameworks.

2.1 Diagnosis and monitoring of infectious diseases: clinical relevance and challenges

Infectious diseases remain one of the most pressing threats to global health, with current outbreaks continuing to endanger millions of lives and impose significant socioeconomic burdens. Over the past century, humanity has faced multiple viral pandemics, including the Russian flu (1889-1890), the Spanish flu

(1918-1920), the Asian flu (1957-1960), the HIV/AIDS epidemic since the early 1980s, and more recent outbreaks such as SARS-CoV-1 in 2003, H1N1 in 2009, MERS-CoV in 2012, and Zika virus in 2016. Most notably, the COVID-19 pandemic, caused by SARS-CoV-2 and declared in 2020, has highlighted both the devastating consequences of viral pandemics, as well as the structural weaknesses of healthcare systems worldwide [9]. To date, it has resulted in hundreds of millions of confirmed cases and millions of deaths globally, underscoring the urgent need for timely detection and effective monitoring strategies. The scale of the COVID-19 pandemic can be further appreciated by examining global epidemiological data. Fig. 2.1 shows the daily number of newly confirmed COVID-19 cases and deaths worldwide, expressed as a 7-day rolling average. While the absolute values may be underestimated due to limited testing capacity and uncertainties in attributing the cause of death, the trends clearly illustrate the dramatic impact of successive infection waves on mortality. These dynamics emphasize not only the rapidity with which viral outbreaks can spread across interconnected societies but also the crucial role of timely diagnostic and monitoring strategies in mitigating their effects.

Viruses are highly diverse in morphology and size, typically ranging from 20 to 900 nm, and are characterized by simple structures consisting of nucleic acids, DNA or RNA, either single-or double-stranded, encapsulated by a protein, glycoprotein, or lipid-based shell [11]. Their replication strictly depends on host cells, where viral genetic material integrates into the cellular machinery to produce new viral particles. This dependency, combined with rapid mutation rates and global spread of viruses, makes them particularly challenging to control. While viruses also play essential roles in ecosystems, contributing to evolution and biodiversity, their ability to cause widespread outbreaks underscores the critical importance of early diagnosis and continuous monitoring to contain transmission and optimize clinical management [12]. Early and accurate diagnosis plays a pivotal role in infectious disease management, as it enables timely initiation of treatment, interruption of transmission chains, and implementation of effective public health measures. Delays in detection not only compromise individual patient outcomes but also amplify the risk of uncontrolled spread, particularly for highly transmissible pathogens.

Beyond the acute response to outbreaks, long-term monitoring is equally essential to track viral evolution, the emergence of variants, and the development of antimicrobial resistance, all of which have direct implications for therapeutic efficacy and vaccine development [13]. The increasing interconnectedness of modern societies facilitates the rapid cross-border spread of pathogens, highlighting the urgent demand for diagnostic solutions that are not only rapid and scalable but also suitable for deployment in resource-limited settings.

Daily new confirmed COVID-19 cases and deaths, World

7-day rolling average. Limited testing and challenges in the attribution of cause of death mean the cases and deaths counts may not be accurate.

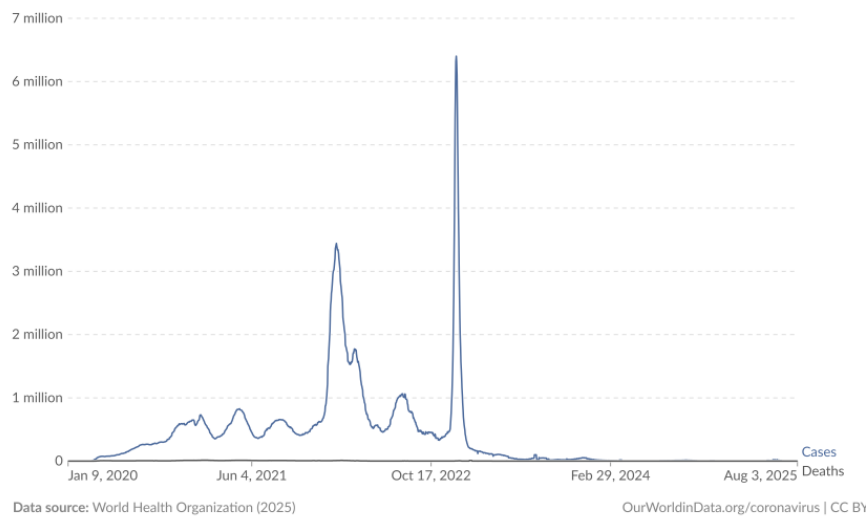


Fig. 2. 1 Daily new confirmed cases and deaths of COVID-19 worldwide (7 day rolling average). Data are subject to underreporting due to limited testing and challenges in attributing cause of death. Data source: World Health Organization (2025) [10].

Reliable diagnostic tools integrated within robust surveillance systems are essential for strengthening epidemic preparedness and enabling healthcare systems to adopt proactive, rather than reactive, strategies [14]. In this context, diagnostics extend beyond their clinical role, becoming a pillar of global health security and a critical element of pandemic resilience. These challenges underscore the pressing need to assess the strengths and limitations of conventional diagnostic technologies, which will be examined in the following sections.

2.1.1 Conventional diagnostic technologies: current approaches and limitations

Traditional diagnostic approaches for viral infections include viral culture, enzyme-linked immunosorbent assay (ELISA), immunofluorescence methods, loop-mediated isothermal amplification (LAMP), and reverse transcription polymerase chain reaction (RT-PCR), which became the gold standard for COVID-19 diagnosis [15]. Despite their central role, these methods face significant limitations. For instance, RT-PCR requires specialized equipment, trained personnel and complex sample preparation, while also suffering from long processing times and the risk of false negatives or positives. ELISA offers scalability and reproducibility but depends on the availability of high-quality antibodies and can be prone to cross-reactivity [16]. Lateral flow assays (LFAs), widely adopted for Point-of-Care (PoC) testing, deliver rapid and low-cost results, but often lack the sensitivity and specificity required for reliable clinical

use. Advanced imaging techniques, such as X-ray or computed tomography (CT), can support the assessment of virus-induced pathologies (e.g., pneumonia), yet remain unsuitable for early viral detection because of their low specificity and high resource requirements. These shortcomings have exposed major vulnerabilities in diagnostic preparedness, particularly during epidemic and pandemic crises, where timely, accurate, and scalable detection is essential.

In this context, an additional global health concern is antimicrobial resistance (AMR), which refers to the ability of microorganisms, most prominently bacteria but also viruses (e.g. influenza type A, HIV), fungi, and parasites, to develop resistance to antimicrobial treatments. The increasing prevalence of resistant pathogens has emerged as a major driver of severe infectious diseases, largely resulting from the overuse and misuse of antibiotics [17]. The healthcare and economic impact of AMR is already substantial, with costs estimated at approximately €1.5 billion annually in the European Union alone. Alarmingly, projections suggest that AMR could become the leading cause of death worldwide by 2050, accounting for more than 10 million fatalities per year if no effective solutions are implemented [18].

Current diagnostic tests for AMR are still largely based on culture-dependent methods, which are inherently slow as they measure the average response of large bacterial and viral populations. The most widely adopted technique, the plate-count method, requires bacterial growth on agar plates in the presence of different antibiotics. While well established, this approach is time-consuming (24-72 hours), dependent on multiple cell division cycles, and requires skilled operators for both sample preparation and data interpretation [19, 20]. Such delays are particularly critical for life-threatening infections such as sepsis, where immediate therapeutic intervention is necessary [21]. Therefore, clinicians often prescribe antibiotics empirically, guided by experience rather than by precise diagnostic evidence, a practice that contributes to treatment errors and the further spread of resistance.

To overcome these limitations, research is increasingly focused on developing novel diagnostic approaches capable of assessing antimicrobial susceptibility at the single-cell level in real time. Several advanced techniques have been proposed, including surface-enhanced Raman spectroscopy (SERS) and fluorescence-based methods, which have shown promising sensitivity [22]. However, these strategies are not label-free and require species specific functionalization of bacterial cells, a process that is particularly challenging due to the high mutation rates of many pathogens, which can alter binding properties. Recent advances in photonic technologies, such as plasmonic cavities [23] and photonic crystal cavities (PhC) [24, 25], have demonstrated the ability to trap, detect, and characterize single viral and bacterial cells in a rapid, label-free manner, representing a substantial advance in AMR diagnostics.

Given the diversity of pathogenic bacteria and the wide range of antibiotic mechanisms, some inhibiting cell growth, others including cell lysis, a single interrogation method is rarely sufficient for comprehensive susceptibility testing. Multiparametric strategies are therefore required, among which electrical methods have shown considerable promise [26]. It has long been known that the impedance of a culture medium changes as a function of bacterial metabolic activity [27], and several biosensor configurations have been developed to exploit this principle [28]. For instance, impedance flow cytometry can distinguish between live and dead bacteria without the need for labelling [29]. Electrochemical impedance spectroscopy (EIS), particularly when implemented with interdigitated microelectrodes, has demonstrated limits of detection as low as 10 CFU/mL [30, 31]. Nevertheless, most electrical techniques operate at the community level and require bacterial concentrations on the order of 10^5 - 10^6 CFU/mL to achieve detectable signals [32].

Altogether, the persistent shortcomings of conventional diagnostics and the growing urgency of monitoring of infectious diseases and antimicrobial resistance emphasize the need for disruptive technologies that combine high sensitivity, rapidity, and label-free detection with affordability and scalability. Decentralize and PoC solutions are increasingly recognized as strategic tools for epidemic preparedness and long-term surveillance of infectious diseases. In this evolving scenario, photonic platforms are emerging as particularly promising, providing opportunities for the real-time monitoring of pathogens and the rapid assessment of antimicrobial susceptibility.

2.2 Global oncology: Incidence, metastasis, and diagnostic advances

Cancer remains one of the most critical global health challenges, primarily due to its considerable and growing impact on overall mortality. According to data from the World Health Organization (WHO), cancer accounted for approximately 8.8 million deaths globally in 2015 [33], rising to an estimated 10.3 million in 2020 [34]. In the same year, the GLOBOCAN database documented 19.3 million new cancer diagnoses across 185 countries [34]. This escalating burden is largely attributable to demographic transitions, notably population growth and aging, and is projected to intensify further in the coming decades. Epidemiological forecasts suggest that by 2050, the annual incidence of cancer could reach 29.5 million cases worldwide, with mortality trends continuing to highlight substantial disparities across geographical and socioeconomic contexts [35]. The global trends in cancer incidence and mortality are further illustrated in Fig. 2.2, which depicts the age-standardized mortality rate (ASR) versus incidence rate for all cancers across countries, as compiled in 2022 by the International Agency for Research on Cancer (IARC).

The scatterplot clearly highlights the geographical disparities in cancer outcomes, with countries from Africa and parts of Asia (red and green dots) tending to show higher mortality-to-incidence ratios, reflecting limitations in early detection, access to care, and therapeutic infrastructure.

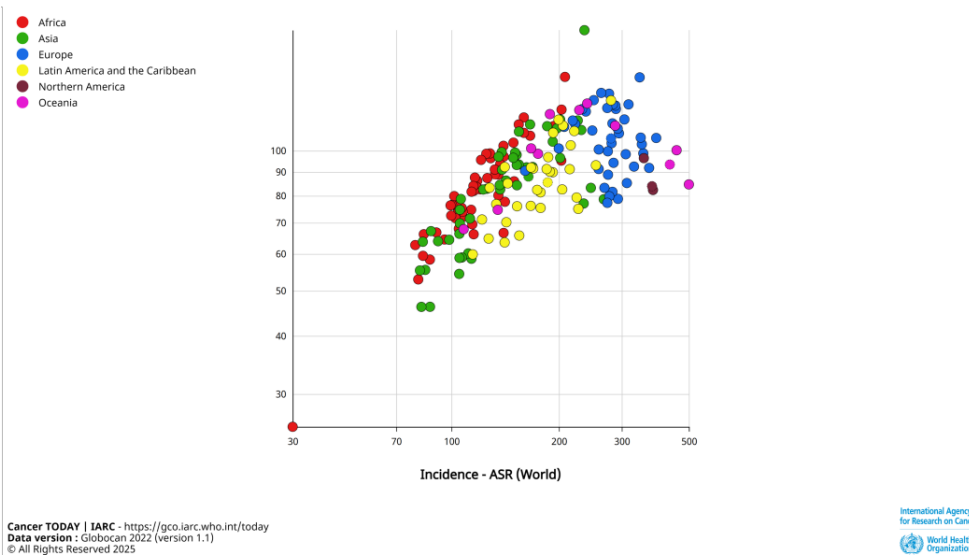


Fig. 2. 2 Scatterplot of age-standardized mortality rate (ASR) versus incidence rate for all cancers by country, both sexes, in 2022 (IARC GLOBOCAN 2022). The statistical data used for plotting both figures are collected from the Web sites, CANCER TODAY [36].

In contrast, high-income regions such as Europe, North America, and Oceania (blue, brown, and purple dots) present higher incidence rates but comparatively lower mortality, suggesting a more widespread adoption of screening programs, precision diagnostics, and effective treatments. This visualization reinforces the notion that, beyond biological determinants, socioeconomic and systemic healthcare factors play a fundamental role in shaping cancer outcomes globally [36].

In a broader historical and sociomedical context, cancer has frequently been characterized as the “*disease of the century*”, reflecting both its global ubiquity and its symbolic status as a disease deeply embedded in modernity. Researchers such as R. Porter and S. Mukherjee have respectively described cancer as the “*modern disease par excellence*” [37] and the “*quintessential product of modernity*” [38], conceptualizations that underscore the dramatic rise in incidence and mortality observed since the Industrial Revolution. Empirical data support this framing: between 2005 and 2015 alone, the number of newly diagnosed cancer cases increased by approximately 33%, primarily due to demographic expansion and the progressive increase in life expectancy [39].

Despite technological and therapeutic advances in oncology, overall cancer mortality rates have remained relatively stable in many countries, although reductions have been observed in specific high-income settings where early detection and targeted therapies have been more widely implemented. Longitudinal analyses of data from the U.S. National Cancer Institute (NCI) over the past four decades reveals a steady and sustained rise in cancer incidence across all major types [40]. While a general decline in cancer-related mortality has been noted, especially in the last two decades, this trend followed a modest increase observed earlier. The recent reduction in death rates has been largely

attributed to improvements in medical management, earlier diagnosis, and the introduction of more effective pharmacological agents and treatment regimens.

At the biological level, cancer arises from the progressive accumulation of somatic mutations, frequently affecting key genes involved in the regulation of cell division, differentiation, and apoptosis. These genetic alterations disrupt cellular homeostasis and initiate the multistep process of malignant transformation. Among the defining features of cancer, the capacity to metastasize is responsible for most cancer-related deaths, accounting for approximately 90% of fatalities [41]. Metastasis entails the dissemination of malignant cells from the primary site to distant tissues and organs, where they may establish secondary tumour colonies.

Early detection of both primary tumours and metastatic disseminations is therefore essential to reducing cancer mortality. Proactive diagnostic and preventive strategies should be prioritized to enable timely monitoring of tumour recurrence and metastatic progression, thus supporting the early implementation of effective therapeutic interventions. However, current clinical protocols still rely heavily on tissue biopsy, typically performed after the identification of a suspicious lesion through imaging techniques such as ultrasound, CT, or MRI. This conventional diagnostic paradigm, often triggered by symptoms or advanced-stage detection, inherently delays interventions. In contrast, a proactive framework emphasizing early screening and molecular surveillance offers the potential to detect malignancies during asymptomatic phases, thereby improving clinical outcomes through earlier and more effective treatment [34]. Compounding the difficulty of early diagnosis is the pronounced spatial and temporal heterogeneity of tumours, which complicates the identification of molecular mechanisms underlying disease progression and metastatic potential [41]. Recent advances in molecular profiling and high-resolution bioanalytical techniques have facilitated more refined tumour characterization, catalysing the emergence of precision oncology [42]. This approach seeks to individualize treatment based on the unique molecular and genetic signature of a patient's tumour. Somatic mutations not only initiate oncogenesis but also govern the invasive and metastatic capabilities of cancer cells by modulating their interactions with surrounding tissues, enabling intravasation, and promoting colonization at distal sites. Consequently, elucidating tumour biology and therapeutic responsiveness requires the identification of specific genomic alterations that serve as key biomarkers for diagnosis, prognosis, and treatment selection. Molecular diagnostics and genomic screening enable the detection of driver mutations and oncogenic pathways, supporting the personalization of therapeutic strategies, real-time monitoring of disease evolution, and early recognition of resistance mechanisms [43].

Within this evolving diagnostic landscape, Liquid Biopsy (LB) has emerged as a powerful, minimally invasive tool for real-time assessment of tumour dynamics. By analysing circulating tumour-derived biomarkers, including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), and extracellular

vesicles, directly from body fluids, LB provides dynamic molecular insights that complement traditional tissue-based methods. This approach enhances early detection, allows longitudinal tracking of tumour progression, and aligns with the principles of personalized medicine by facilitating the customization of therapeutic interventions. Despite its promise, broader clinical integration of LB is still evolving and will require standardized protocols, rigorous validation, and cost-effective implementation to achieve widespread adoption in routine oncology practice [41, 44, 45].

2.2.1 Traditional diagnostic methods for cancer

The historical progression of cancer diagnosis and treatment is marked by pivotal scientific milestones that reflect the co-evolution of medical knowledge and technological advancement. As illustrated in Figure 3, the management of cancer has undergone several paradigm shifts over the past millennia, transitioning from purely surgical interventions to the era of molecularly targeted and immunologically guided therapies [46]. The initial phase, spanning from approximately 3000 B.C. to the late 19th century, was dominated by surgical procedures and thermal cauterization, which represented the only therapeutic options available. During this period, tumour diagnosis was empirical and largely based on visible or palpable abnormalities, with limited understanding of the underlying pathophysiology [47]. A turning point emerged with the development of microscopy and the fundamental theories of cellular pathology in the 19th century.

These advancements laid the groundwork for a more scientific interpretation of neoplastic diseases. The true inception of modern oncological diagnostics and therapy, however, can be traced to the discovery of X-rays by Wilhelm Conrad Röntgen in 1895 [48]. Soon after, Marie and Pierre Curie's isolation of radium led to the pioneering use of ionizing radiation in both diagnostic imaging and cancer treatment [49]. Their early observations of radiation-induced cellular damage suggested its potential applications as a therapeutic agent. By the early 20th century, X-rays were already being employed in clinical settings for cancer treatment, although with rudimentary safety considerations [50]. The formalization of radiotherapy as a distinct therapeutic modality occurred in the 1920s, when Claudius Regaud demonstrated that fractionated radiation doses could improve therapeutic outcomes by minimizing damage to surrounding healthy tissue [51]. Despite these advances, the dominant therapeutic approach before the mid-20th century remained invasive surgical resection, which proved inadequate for metastatic or inoperable tumours. The mid-1900s witnessed a revolutionary shift with the advent of chemotherapy, particularly following the observation of cytotoxic effects of nitrogen mustards on rapidly dividing cells. This led to the development of the first generation of chemotherapeutic agents, initially aimed at haematological malignancies and later expanded to solid tumours. Nonetheless, the non-selective toxicity of these agents and the emergence of resistance mechanisms underscored the need for more targeted approaches. Subsequent decades saw the emergence of molecular oncology. The rise of genomic technologies enabled the identification of genetic mutations

driving oncogenesis. These discoveries catalysed the development of targeted therapies, including small-molecule inhibitors and monoclonal antibodies, which selectively inhibit specific oncogenic pathways. This era marked the transition from broadly cytotoxic agents to precision medicine strategies. More recently, since the early 2000s, the introduction of immune checkpoint inhibitors has further transformed the therapeutic landscape [46]. These biologics harness the patient's immune system to recognize and eliminate tumour cells, achieving durable responses in subsets of patients with advanced malignancies. Research efforts are currently focused on integrating biotechnological innovations, such as CAR-T cell therapy and gene-editing techniques, into comprehensive treatment protocols, alongside ongoing exploration of combination regimens to circumvent resistance and enhance therapeutic efficacy [52]. Fig. 2.3 encapsulates this evolution, highlighting key milestones in cancer treatment development and indirectly reflecting the corresponding advances in diagnostic capabilities. Each therapeutic breakthrough has not only expanded treatment options but also informed and refined diagnostic methodologies, setting the stage for the next generation of integrated, personalized oncological care.

Whitin this evolving framework, diagnostic strategies have also undergone significant transformation. Currently, imaging techniques remain the most widely utilized tools for the detection and anatomical localization of tumours. Despite their critical role in routine clinical practice, conventional imaging modalities, such as mammography, ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), often lack the sensitivity required to detect early-stage malignancies. Moreover, these methods exhibit inherent limitations that hinder their application in large-scale population screening programs. High operational costs, the necessity for specialized personnel, and patient exposure to ionizing radiation significantly constrain their scalability [53]. As a result, oncological screening programs are typically restricted to high-risk populations, with examples including mammography for breast cancer and low-dose CT for lung cancer.

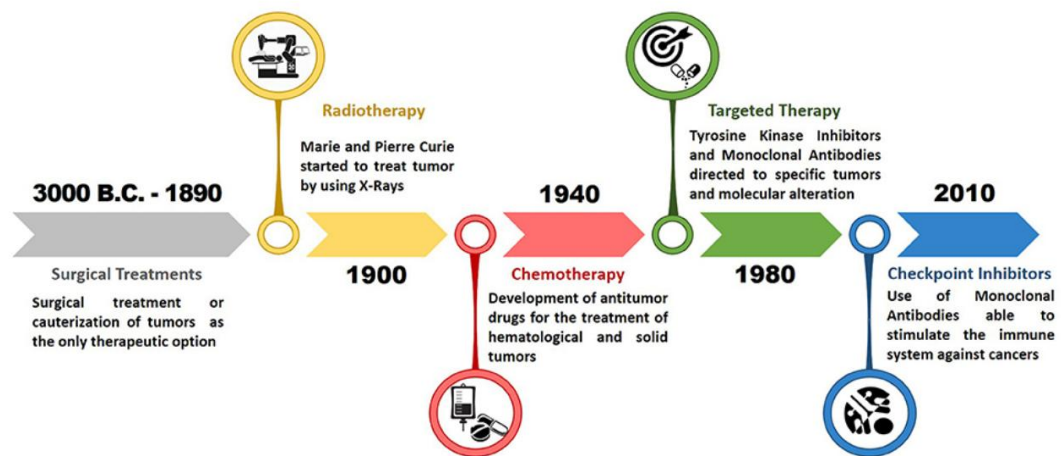


Fig. 2. 3 Timeline of epochal turning points in modern oncology [46].

However, the diagnostic performance of these approaches is not absolute, and the sensitivity and specificity of the method employed can leave a substantial fraction of tumours undetected. While imaging techniques offer high sensitivity in detecting abnormal masses, they are generally unable to reliably distinguish between benign and malignant lesions, resulting in low diagnostic specificity and a consequently elevated rate of false positives.

These diagnostic ambiguities often necessitate further confirmatory procedures, which are time-consuming, costly, and may delay definitive diagnosis, factors that increase healthcare burdens and patient anxiety [53].

Given the limitations of imaging techniques, biopsies play a crucial role in cancer diagnosis, providing direct tissue analysis for a more precise characterization of the disease.

Today, the existing categories of biopsies, such as surgical, core needle and fine needle [54], still involve the removal of suspicious tissue through invasive or minimally invasive procedures. Currently, the diagnosis of the most common and clinically relevant tumours, such as breast cancer in women and prostate cancer in men (Fig. 2.4), is based on an integrated approach that combines radiological assessments (such as mammography, ultrasound, magnetic resonance imaging, or computed tomography) with additional clinical investigations. These methodologies enable the identification of potential suspicious lesions, which are subsequently confirmed through histopathological analysis of tissue samples obtained.

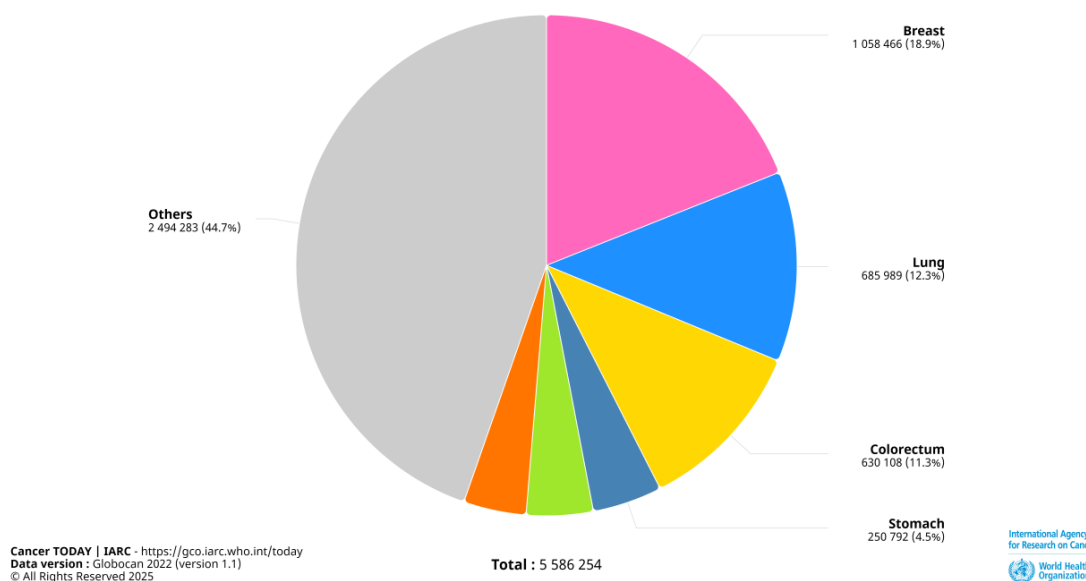
However, this diagnostic protocol has several limitations. First, the waiting times for confirmatory tests can be long, delaying the initiation of treatment and increasing patient anxiety.

Second, the techniques currently used for biopsy sampling are often invasive and may be associated with pain or complications, particularly in anatomically delicate areas. Finally, the conventional approach may not accurately detect certain molecular and structural characteristics of tumour lesions, posing a risk of underestimating their aggressiveness, heterogeneity, or the presence of infiltrative margins, which could ultimately impact the selection of the most appropriate therapeutic strategy. Currently, tissue biopsy is considered the “gold standard” for the diagnosis and treatment selection in oncology patients [55].

This procedure involves the extraction of a tumour tissue sample through surgical interventions or other invasive techniques, with the aim of analysing its morphological characteristics. Tumour tissue represents the primary source of information for diagnosis, prognosis, and therapeutic planning, enabling the differentiation between neoplastic and benign lesions.

The analysis of the collected tissue provides essential data on clinically relevant biomarkers and the biological characteristics of the tumour, encompassing not only molecular and biochemical aspect but also anatomical and physiological factors that are crucial for personalized oncological management.

Absolute numbers, Incidence, Females, age [60-85+], in 2022
Continents



Absolute numbers, Incidence, Males, age [60-85+], in 2022
Continents

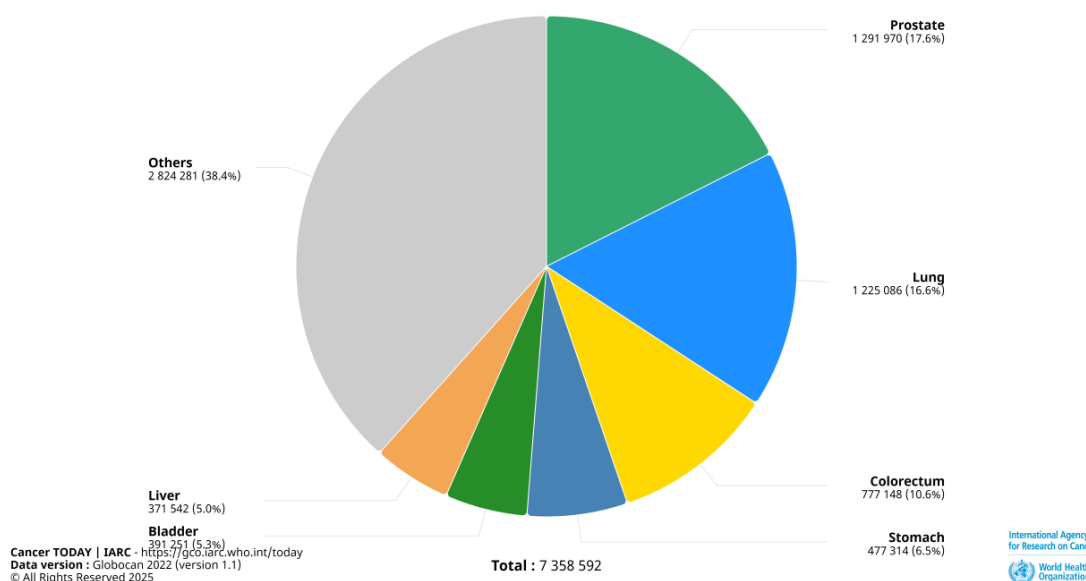


Fig. 2. 4 The pie charts illustrate the most common cancer types by incidence in women (*top*) and men (*bottom*). Among women, breast cancer shows the highest incidence at 18.9%, whereas in men, prostate cancer is the most prevalent, with an incidence of 17.6%. The statistical data used for both charts were obtained from the CANCER TODAY website [36].

Morphological and immunohistochemical analysis of tissue samples provides essential information for determining the type and characteristics of a tumour, as well as for assessing its grade and extent of dissemination. By identifying mutations in specific genes or detecting abnormal protein expression in tumour tissue, clinicians can predict a patient's responsiveness to targeted therapies.

This constitutes a fundamental basis for developing personalized treatment plans, which are pivotal for optimizing clinical outcomes. From a practical standpoint, tissue samples are typically chemically processed or cryopreserved. Because biopsy involves the direct sampling of tumour tissue, it enables the collection of a high concentration of malignant cells, which is crucial for obtaining sufficient biological information on the tumour's nature. Moreover, when matched normal tissue is available, comparing the genomic profiles of tumour and healthy cells through high-throughput sequencing can reduce inter-individual bias and enhance diagnostic specificity.

However, mutational analysis based on a single-site tissue biopsy presents several critical limitations [56]. First, the procedure may carry procedural risks and, in some cases, may be contraindicated due to the patient's clinical condition. Second, the amount of tissue obtained may be insufficient for comprehensive molecular analysis. Third, single-site biopsies inherently risk underestimating tumour heterogeneity, and ethical or logistical considerations often preclude the simultaneous sampling of multiple tumour regions. Finally, conventional biopsy is not suited for longitudinal monitoring of tumour dynamics over time. Compounding these challenges, traditional biopsy captures only a limited portion of the tumour mass, thereby reflecting only a fraction of its genetic and epigenetic heterogeneity. As a result, it often fails to provide a comprehensive overview of the tumour's biological complexity [56].

Considering these limitations, and in parallel with therapeutic innovation, current and future directions in oncology are increasingly focused on the identification and characterization of circulating biomarkers to enhance diagnostic accuracy, enable earlier cancer detection, and support the development of personalized therapeutic strategies [57]. While conventional diagnostic methods have historically relied on invasive sampling and static morphological evaluations, the focus is now shifting toward minimally invasive, molecular-level assessments capable of capturing the dynamic and heterogeneous nature of malignancies. The ability to detect and monitor tumour-specific molecular signatures directly from biological fluids marks a transformative shift in precision oncology, aligning with the broader paradigm of personalized diagnostics and real-time therapeutic decision-making. Within this evolving framework, the integration of liquid biopsy technologies and advanced biosensing platforms holds great promise in overcoming the intrinsic limitations of traditional diagnostic tools. The following section explores the principal methodologies for the detection of cancer biomarkers, tracing the transition from established clinical assays to next-generation liquid biopsy systems designed to meet the demands of modern, high-performance oncology.

2.2.2 Main approaches for detecting cancer biomarkers from gold clinical standard to Liquid Biopsy

Recent advancements in precision medicine are reshaping the oncological diagnostic landscape, promoting a transition toward patient-specific management strategies grounded in molecular profiling [58, 59]. Alongside the

development of innovative therapeutic strategies, such as cell-based interventions and nanotechnology, there is a growing need for diagnostic platforms that are fast, highly sensitive, cost-effective, and suitable for early-stage detection and longitudinal disease monitoring [60].

Although conventional diagnostic approaches remain fundamental to clinical practice, they exhibit notable limitations when evaluated within the framework of precision oncology. Established clinical methods such as histopathological analysis, immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR) continue to represent essential tools for the detection of cancer-related biomarkers. Despite offering excellent sensitivity and specificity, molecular techniques like PCR and immunoassays face inherent limitations in terms of scalability, processing time, and dependence on advanced laboratory infrastructure, which restrict their application in high-throughput screening and real-time patient monitoring. Before 2010, the predominant approaches for isolating and identifying biomarkers, particularly extracellular vesicles such as exosomes, included differential ultracentrifugation, magnetic bead-based immunoaffinity capture, and various commercially available isolation kits [61, 62]. Between 2006 and 2010, ultracentrifugation was widely regarded as the benchmark technique for exosome isolation, primarily due to its relatively low levels of protein contamination. Nonetheless, this method posed significant challenges for routine application in standard chemical and biological laboratories.

These challenges included the necessity for large, high-cost instrumentation and only moderate recovery rates as well as purity of exosomes, in part due to the co-isolation of non-exosomal protein aggregates. Both conventional ultracentrifugation and density gradient methods typically yield moderate levels of purity, although the latter tends to perform better in this regard. Density gradient ultracentrifugation can provide a sufficiently high yield of exosomes ($\sim 1.5 \times 10^9$ particles/mL from concentrated conditioned medium) and RNA ($\sim 0.2 \mu\text{g}$ per 10^8 particles), with a modest protein yield ($\sim 0.5 \mu\text{g}$ per 10^8 particles) [60]. To improve purity, size exclusion chromatography (SEC), often implemented via high-performance liquid chromatography (HPLC), is frequently employed.

However, this technique requires specialized instrumentation and incurs higher operational costs. While ultracentrifugation is less expensive per sample compared to chemical precipitation methods, it requires approximately 4 hours of processing, substantially shorter than other lab-scale protocols, that can extend up to 20 hours. To overcome the limitations inherent to ultracentrifugation and to support the development of more practical and clinically applicable protocols, several alternative exosome isolation methods have been introduced, including immunoaffinity-based magnetic bead capture and commercially available isolation kits [60-62]. Immunoaffinity techniques have demonstrated isolation efficiencies of approximately 75% for exosomes and up to 90% for associated microRNAs [63]. In addition, studies have shown that commercial kits yield superior recovery rates from clinical specimens and

are generally simpler and more efficient compared to traditional protocols [64]. Products such as ExoQuick and Exo-spin can complete the isolation process in about one hour.

However, performance metrics vary notably among different commercial solutions: some are engineered to maximize yield at the expense of purity, whereas others prioritize high-purity outputs with a trade-off in recovery efficiency. Certain high yield kits report up to a 150-fold increase in recovery than ultracentrifugation, though they tend to offer only moderate purity levels. Conversely, some products achieve isolation efficiencies around 70% while reaching exosome purities close to 95%, representing up to a tenfold improvement over conventional techniques [65, 66]. Despite these advancements, a common drawback across all three methods is extended processing times (typically 4–5 hours), reliance on costly and complex instrumentation, labour-intensive workflows, and substantial operational and maintenance costs.

Although widely adopted, these conventional methodologies still present significant technical and practical drawbacks that limit their integration into next-generation oncological diagnostics. Challenges include prolonged processing durations (typically exceeding 4–5 hours), dependence on sophisticated and costly instrumentation, procedures that require substantial manual effort, and high operational and maintenance expenses. Furthermore, most existing protocols rely on invasive tissue sampling, centralized laboratory infrastructure, and the expertise of highly trained personnel.

Given these considerable limitations, there is a growing impetus to develop innovative and patient-centric diagnostic strategies. Among these, liquid biopsy has emerged as a transformative alternative, offering a minimally invasive route for detecting and monitoring tumour-specific molecular signatures. LB devices are compact, integrated platforms engineered to perform critical operations, namely the isolation, identification, and analysis of circulating tumour biomarkers in biological fluids [68].

This approach encompasses the collection, preservation, and analysis of a wide range of biological fluids. Its primary objective is to detect circulating biomarkers associated with cancer, which may be directly released by tumour cells or derived from non-malignant cells responding to the presence of cancer. The bioanalytical workflow of LB follows a structured sequence of steps; each designed to ensure reliable detection and analysis of the biomarkers of interest. Moreover, the early detection of metastatic progression through this approach plays a crucial role in improving therapeutic interventions and supports the implementation of personalized medicine, enabling treatment strategies that are precisely tailored to the patient's clinical profile.

Liquid biopsy is considered a minimally invasive and highly informative tool, as it allows for tumour profiling through the collection of biological fluids such as blood, cerebrospinal fluid, urine, sputum, ascitic fluid, and potentially any other bodily fluid. Increasingly recognized as a valuable adjunct to tissue

biopsy, LB enables real-time cancer monitoring and biomarker assessment while addressing several limitations of conventional tissue-based diagnostics. It offers significant advantages over traditional methods (Table 2.1) and minimizes the need for invasive procedures, enabling repeated sampling and analysis with reduced patient discomfort. These features enhance the feasibility of frequent monitoring and accelerate the acquisition of clinically relevant results, both of which are critical for timely decision-making.

Moreover, LB is applicable to all patients, regardless of tumour location, whether a primary tumour or distant metastasis [68]. Additionally, as a non-invasive technique, it is well-suited for real-time monitoring of disease progression and treatment response, while also providing valuable insights into the emergence of resistance mechanisms against various therapeutic agents.

However, LB should not be considered a replacement for histopathological diagnosis, as it lacks the ability to fully capture and analyse the morphological details that tissue samples provide. Tissue biopsy remains the preferred method when the tumour's location is known and accessible for sampling. Conversely, LB is particularly well-suited for screening, detecting mutations in metastatic cancer, and monitoring molecular changes throughout treatments. Given their complementary strengths, both technologies should be utilized together to comprehensively track tumour evolution during therapy.

The initial capture phase exploits specific physical or chemical features of the target biomarkers to selectively extract them from complex biological matrices, using engineered systems that meet strict criteria of sensitivity, scalability, low contamination, and preservation of biomolecular integrity. For instance, the CellSearch platform employs immunomagnetic separation techniques and achieves recovery rates between 60% and 90%; however, its use is constrained by lengthy processing times and contamination with leukocytes [69]. To address these challenges, high-throughput, low-contamination microfluidic systems have been developed, such as the platform introduced by T.W. Lo et al. [70], capable of processing biological fluids at rates up to 10 mL per hour while maintaining the structural and functional integrity of extracellular vesicles for downstream analysis. Following the capture step, biomarker detection and quantification are typically performed using techniques as well as fluorescence labelling, optical, acoustic, or electrochemical sensing, achieving detection limits in the femtomolar (fM) range for specific nucleic acids and proteins [71]. The final analytical phase involves comprehensive characterization of the isolated biomarkers, including enumeration, molecular profiling, and bioinformatic analysis. These data support applications ranging from predictive screening and early diagnosis to disease monitoring and therapeutic stratification, all aligned with emerging precision medicine paradigms.

Table 2. 1 Comparison of key advantages and disadvantages of Liquid Biopsy and conventional tissue biopsy.

	Advantages	Disadvantages
Liquid Biopsy	• Less invasive procedure and easy to obtain. • Can be used for regular and repeated monitoring during treatment and disease progression, allowing for dynamic follow-up of tumour evolution. • Enables genomic analysis of ctDNA or CTCs, facilitating the repeated evaluation of clonal dynamics during treatment and early detection of factors driving therapeutic resistance. • Faster processing time compared to tissue biopsy.	• Risk of false negative results. • The variations in cfDNA levels among patients may compromise the accuracy and reliability of the tests. • Need for highly sensitive techniques. • Extensive validation required: Ultrasensitive detection and identification of mutations necessitate extensive validation to ensure that the results are clinically actionable and reliable.
Conventional Tissue Biopsy	• Allow for detailed histological analysis and accurate disease staging, providing critical information for diagnosis and treatment planning. • Tissue biopsies are irreplaceable in identifying the nature of tumours: type of cancer, gene expression of the tumour, and presence of treatment-resistant mutations. • Gold standard due to its high success rate in providing comprehensive and reliable diagnostic information. • It remains an accurate method for its thoroughness and accuracy in disease assessment.	• Invasive approach: source of discomfort for patients with several risks of clinical complications. • Requires local anaesthetic. • Requires image-guided surgery systems. • No real-time monitoring. • No possibility of dynamic follow-up of cancer molecular modifications. • Limited to localized tissue analysis, does not enable assessment of intra-or inter tumour heterogeneity (metastases).

2.2.3 Tumour Biomarkers as indicators in cancer diagnosis

Tumour biomarkers are essential components of modern oncology, playing critical roles in diagnosis, prognosis, risk stratification, and treatment monitoring [72]. These molecular indicators, including proteins, nucleic acids, and metabolites, can be expressed locally within the tumour microenvironment or released systemically,

offering valuable insights into cancer biology and disease progression. By detecting and analysing specific molecular alterations in a patient's genome, clinicians can determine the most appropriate therapeutic strategy, choosing among surgery, chemotherapy, radiotherapy, or a combination thereof. Thus, the identification and characterization of tumour biomarkers represent a cornerstone of clinical decision-making throughout [73]. Conceptually, the term “*biomarker*” refers to any objectively measurable biological parameter that indicates a physiological or pathological process, or responses to therapeutic interventions [74]. Based on their clinical utility, tumour biomarkers are generally classified as diagnostic, aiding in the early detection of malignancies; prognostic, providing information on disease outcomes independently of therapy (e.g., HER2 overexpression, KRAS mutation); and predictive, estimating the likelihood of response to specific treatments. This tripartite classification highlights their central role in enabling precision medicine and guiding therapeutic decisions.

To illustrate their clinical applicability, Table 2.2 provides an overview of the main tumour biomarkers linked to different cancer types, several of which are already validated and routinely employed for diagnostic or prognostic purposes. Despite their potential, the clinical utility of tumour biomarkers is often hindered by the intrinsic heterogeneity of cancer. This complexity limits the diagnostic reliability of single markers, particularly when evaluated in isolation. Some biomarkers, while highly sensitive, may lack specificity, thereby increasing the risk of false-positive results. Such inaccuracies can lead to unnecessary invasive procedures, psychological distress for patients, and additional healthcare costs.

Table 2. 2 The main biomarkers associated with cancer diagnosis and prognosis.

Type of cancerous disease	Biomarkers
Prostate	PSA, PAP
Breast	CA15-3, CA125, CA27.29, CEABRCA1, BRCA2, MUC-1, CEA, NY-BR-1, ING-1
Leukaemia	Chromosomal abnormalities
Ovarian	CA125, AFP, hCG, p53, CEA
Testicular	α -fetoprotein (AFP), β -human chorionic gonadotropin (β -hCG), CAGE-1, ESO-1
Colon and Pancreas	CEA, CA19-9, CA24-2, p53
Lung	NY-ESO-1, CEA, CA19-9, SCC, CYFRA21-1, NSE
Melanoma	Tyrosinase, NY-ESO-1
Liver	AFP, CEA
Gastric Carcinoma	CA72-4, CEA, CA19-9
Oesophageal	SCC
Carcinoma	SCC, hCG
Bladder	BAT, FDP, NMP22, HA-Hase, BLCA-4, CYFRA 21-1

Importantly, all human cells can release biological material into the bloodstream, including cell-free DNA and RNA (cfDNA, cfRNA), proteins and extracellular vesicles such as exosomes, as well as circulating immune and endothelial cells. Cancer cells, due to their high proliferative rate, undergo rapid turnover, resulting in the continuous release of tumour-derived nucleic acids and vesicles into circulation. Moreover, malignant cells may detach from the primary tumour and migrate into the bloodstream, contributing directly to metastatic spread [75]. The concentration of these biomarkers in circulation is often extremely low, making their detection technically challenging and necessitating enrichment strategies targeting tumour cells or extracellular vesicles.

The combined application of enrichment and lysis techniques enables the detection of biomarkers even in samples with scarce content, thereby advancing minimally invasive diagnostic strategies and supporting more personalized cancer care. In clinical practice, the relevance of circulating tumour biomarkers is reflected in their measurable thresholds in serum, which can distinguish benign from malignant conditions. Table 2.3 summarizes the typical ranges of selected serum biomarkers in benign and malignant effusions, highlighting their diagnostic value as well as the overlap that can complicate interpretation. Against this backdrop, conventional tissue-based methods, such as histopathological examination, remain indispensable, as they enable an integrated morphological and molecular assessment, providing a more comprehensive understanding of tumour architecture and variability. Furthermore, anatomical and functional imaging modalities (e.g., MRI, CT, PET) are routinely employed to complement biomarker-based approaches, particularly in distinguishing malignant from benign lesions [76].

To enhance diagnostic accuracy, the simultaneous evaluation of multiple biomarkers has proven more effective, with advanced platforms such as array-based and multiplexed biosensors improving specificity and robustness. This integrative approach reflects the broader shift toward precision oncology, where comprehensive molecular profiling serves as the foundation of personalized treatment regimens. The successful translation of biomarker research into clinical practice requires interdisciplinary collaboration, drawing on the expertise of chemists, biologists, physicists, engineers, and computational scientists. Building on these considerations, the clinical translation of tumour biomarkers has increasingly relied on liquid biopsy, a minimally invasive approach designed to overcome the limitations of conventional tissue-based diagnostics.

This strategy capitalizes on the analysis of circulating tumour-derived biomarkers, including circulating tumour cells (CTCs), extracellular vesicles (EVs), circulating tumour DNA (ctDNA), and microRNAs (miRNAs). These entities are widely recognized as the most informative indicators for minimally invasive cancer diagnostics and longitudinal disease monitoring [77].

Table 2. 3 Threshold for tumour markers present in patients' serum.

Cancer Biomarkers	Benign effusions	Malignant effusions
CEA (ng ml ⁻¹)	1 (0-6.2)	17.2 (0.2 – 12785)
CE 72-4 (U ml ⁻¹)	2 (1.6-4.5)	21.8 (1.6-20000)
CA 15-3 (U ml ⁻¹)	13.5 (2-50.8)	68.9 (0.5-3581)
CA 19-9 (U ml ⁻¹)	1.2 (0-550.3)	5-9 (0-4800000)
CYFRA (ng ml ⁻¹)	16.3 (0- 188-4)	107.4 (1.8-12796.5)
NSE (ng ml ⁻¹)	3.3 (0.1-375)	7.3(0-408)
SCC (ng ml ⁻¹)	1.6 (0-38.4)	2.2 (0-2420)

A schematic representation of their distribution within blood components is shown in Fig. 2.5, illustrating the complexity of tumour-derived material accessible through liquid biopsy [78]. To further delineate their clinical and analytical significance, Table 2.4 provides a structured overview of the five major biomarker classes (CTCs, EVs, exosomes, ctDNA, and miRNAs) and their associated cancer types. For each cancer-biomarker pair, the table reports the typical concentration ranges across different biofluids (e.g., blood, plasma, urine), while also acknowledging the variability introduced by the biological matrix.

In addition, conventional diagnostic technologies and their corresponding limits of detection are detailed. This comparative summary highlights both the heterogeneity in biomarker abundance and the performance constraints of existing methods, thereby reinforcing the rationale for integrating photonic biosensing techniques into next-generation diagnostic platforms.

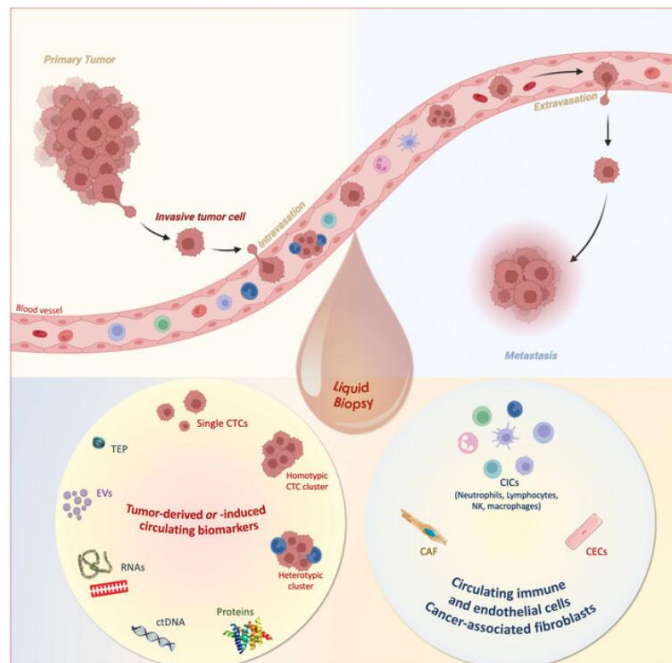


Fig. 2. 5 Schematic representation of circulating biomarkers in blood samples [78].

Table 2. 4 Summary of key liquid biopsy biomarkers across cancer types: associated biological fluids, typical concentration, conventional diagnostic methods, and limits of detection.

Biomarker	Associated Cancer Type	Biological Fluid	Typical Concentration	Diagnostic Technology	LoD	Ref.
CTC	• Metastatic lungs, prostate, pancreatic, and colon cancers	Peripheral whole blood	1–5 cells per 7.5 mL	CTC-chip	5–1281 CTCs/mL	[79]
	• Breast cancer	Peripheral whole blood	500 CTCs per 7.5 mL	NP-HBCTC-Chip	6–12 CTCs/mL	[80, 81]
	• Prostate, ovarian, breast, gastric, colorectal, bladder, and renal cancers	Peripheral whole blood	1–5 cells per 7.5 mL	CellSearch system	60 ± 693 CTCs per 7.5 mL	[82]
EVs	• Prostate cancer	Urine	~1010 EVs per mL	Ultracentrifugation	4.13 ± 3 × 10 ¹¹ particles/mL	[83, 84]
	• Breast cancer	Plasma	~1010 EVs per mL	DNA aptamer-based system	0.21–1.87 × 10 ⁹ particles/mL	[84, 85]
Exosome	• Melanoma	Plasma	~1010 EVs per mL	newExoChip	2.79 × 10 ⁸ particles/mL	[84, 85]
	• Lung cancer	Plasma	~1010 EVs per mL	newExoChip	2.89 × 10 ⁸ particles/mL	[84, 86]
ctDNA	• Breast, prostate, NSCLC, ovarian, and gastric cancers, lymphoma, and glioblastoma	Plasma	0.01–0.1% allele frequency	BEAMin TAm-Seq CAPP-Seq ddPCR	0.01% allele frequency (ddPCR)	[88]
miRNA	• Breast, lung, and colorectal cancers	Serum, plasma	~pg-ng/mL	Immunomagnetic Exosomal RNA (iMER) technology	~pg-ng/mL	[88]

2.2.3.1 CTCs

Circulating Tumour Cells (CTCs) are intact tumour cells originating from primary and metastatic lesions that are actively shed or displaced into the bloodstream by external forces such as tumour growth and microenvironmental pressure. These cells play a pivotal role in solid tumour progression and metastatic dissemination, as they have been detected even in the early stages of cancer. However, CTCs are extremely rare, with an estimated frequency of approximately 1 CTC per 10⁶ – 10⁷ leukocytes, varying according to tumour type and disease stage [89, 90]. Specifically, studies have reported CTC concentrations range from 1 to 5 cells per 7.5 mL of blood [91]. In patients with advanced metastatic cancer, their occurrence

is even lower, with approximately 1 CTC per 10^9 blood cells.

Given their extreme rarity, the isolation and characterization of CTCs represent a major challenge in oncological diagnostics. CTCs differ significantly from other blood cells in terms of size (typically 20-30 μm), plasticity, and dielectric mobility, which enables their capture using size-based or physical separation techniques. Various technologies have been developed for their detection and analysis, yet the only method currently approved by the U.S. Food and Drug Administration (FDA) for quantifying CTCs in metastatic breast, colorectal, lung and prostate cancers in the Cell Search System (Veridix LLC, currently Menarini Silicon Biosystems), which employs magnetic beads coated with anti-EpCAM antibodies [92].

Once isolated, CTCs can undergo extensive molecular characterization, including genomic, transcriptomic, proteomic, and secretomic analysis [93]. This enables comprehensive investigation of DNA, RNA, and protein profiles, as well as functional studies, such as the establishment of cell lines, xenograft models, and in vitro or in vivo drug testing. Furthermore, identifying specific CTC subpopulations with high metastatic potential is essential to understanding tumour dissemination and disease progression. Their molecular profiling provides valuable prognostic insights and may support the development of treatment strategies tailored to individual patients.

2.2.3.2 ctDNA

Circulating tumour DNA (ctDNA) is a subclass of cell-free DNA (cfDNA) that originates directly from apoptotic or necrotic tumour cells and is released into the bloodstream during cellular turnover. Since the majority of cfDNA derives from normal cells under physiological conditions, ctDNA constitutes only a small fraction ($< 0.01\%$) of the total cfDNA. The first experimental evidence of cfDNA in human blood was reported by Mandel and Metais in 1948 [94]. Its oncological significance, however, was not recognized until 1977, when Leon et al. [95] demonstrated that cfDNA levels were significantly elevated in patients with pancreatic cancer compared to healthy individuals, and that cfDNA concentrations declined following therapy [96].

In recent years, ctDNA has garnered significant interest for its potential to detect tumour-specific alterations, making it a valuable tool for cancer diagnosis, treatment monitoring, and prognosis, a concept now widely framed under liquid biopsy. This approach is particularly advantageous due to its minimally invasive nature, allowing the detection of genetic and epigenetic changes associated with both primary and metastatic tumours. By integrating ctDNA released from multiple tumour sites, liquid biopsy provides a comprehensive molecular snapshot, thereby enhancing its role in disease surveillance and therapeutic decision-making.

Despite its potential, ctDNA analysis presents several technical and diagnostic challenges. One major limitation is its high susceptibility to degradation, requiring careful sample processing and extraction protocols to maintain integrity. Furthermore, ctDNA concentrations in blood are inherently low, typically ranging from 5-10 ng/mL, with even lower levels observed in patients with non-metastatic

disease. Plasma is considered the most reliable biological matrix for ctDNA isolation, as serum samples are more prone to contamination by genomic DNA released from leukocytes during coagulation [97].

The detection and characterization of ctDNA rely on both targeted and non-targeted sequencing techniques. The most used targeted approaches include digital polymerase chain reaction (dPCR), bead-emulsion amplification-magnetics (BEAMing), and tagged amplicon deep sequencing (TamSeq) [98], which exhibit higher sensitivity and specificity compared to non-targeted sequencing methods, such as next-generation sequencing (NGS). However, one of the main limitations of large-scale genomic analysis of ctDNA is their lower sensitivity, requiring higher concentrations of ctDNA for accurate and precise detection. This drawback is particularly evident in early-stage or non-metastatic patients, where ctDNA levels may be insufficient for reliable profiling.

Given these challenges, ongoing research is focused on developing more sensitive methodologies for ctDNA detection, with the aim of improving its clinical application as a minimally invasive biomarker for early cancer detection, disease monitoring, and real-time assessment of tumour evolution.

2.2.3.3 Extracellular vesicles

Extracellular vesicles (EVs) are lipid-based nanoparticles secreted by tumour cells and detectable in various biological fluids, including blood, saliva, and urine. They exhibit substantial variability in both size and biogenesis mechanisms [99]. Their significance as biomarkers for liquid biopsy in oncology lies in the molecular cargo they carry, proteins, lipids, DNA, RNA and other cellular components, all well protected by a lipid bilayer [100]. Notably, the content of extracellular vesicles (EVs) reflects both the physiological and pathological state of their cell of origin, thereby providing a reliable representation of the tumour stage and its progression [101]. These biomarkers can promote tumour cell proliferation, migration, and invasion, while also modulating the tumour microenvironment by facilitating angiogenesis and immune evasion. Furthermore, they have the ability to establish a pre-metastatic niche in distant organs, preparing the microenvironment for the origin of tumour cells and enhancing their growth and proliferation [102].

Different types of extracellular vesicles (EVs) are released into biological fluids by normal and tumour cells. Studies have shown that cancer patients exhibit an increased concentration of EVs in the bloodstream, particularly following chemotherapy administration [103]. Based on their size and biological functions, extracellular vesicles (EVs) are classified into three main subtypes: microvesicles (MVs), exosomes, and apoptotic bodies. Among these, exosomes (30-160 nm in diameter) play a particularly important role in oncology. Secreted by various cell types, including tumour cells, into extracellular spaces and biological fluids, they often enable the identification of the tissue or cell type of origin [104].

Exosomes are involved in numerous physiological and pathological processes, including intercellular communication, proliferation, and tumour metastasis. Their role is crucial not only in tumour biology, affecting growth, progression, and drug

resistance, but also in mediating cell-to-cell communication. Through their cargo of proteins and nucleic acids (DNA, mRNA, and miRNA), they can transfer molecular information to target cells, modulating their activity. Moreover, tumour-derived extracellular vesicles (EVs) are utilized to estimate and generate the epithelial-to-mesenchymal transition (EMT) index, a parameter used to assess metastatic potential in patients.

The isolation of EVs is primarily based on their biophysical properties, such as size, morphology, and density. Additional methods rely on immunoaffinity capture or solubility-based modifications to enhance precipitation. Currently, differential ultracentrifugation remains the gold standard for EV isolation; however, this technique suffers from limitations, including contamination, particle loss, and long processing times [105]. Consequently, ongoing research is directed toward developing more efficient isolation protocols that improve yields and enable selective isolation of tumour-derived vesicles from those of non-tumour origin. Despite promising preclinical evidence, longitudinal investigations and large-scale clinical trials are still required to validate EVs as reliable tumour biomarkers.

2.2.3.4 circulating miRNA

MicroRNAs (miRNAs) are short RNA molecules, typically ranging from 14 to 20 nucleotides, that regulate gene expression by binding to specific target messenger RNAs (mRNAs). These small cell-free RNA (cfRNA) sequences are highly abundant in tissues and, importantly, in biological fluids, where they can be transported via exosomes, apoptotic bodies, or protein-miRNA complexes. A single miRNA molecule can regulate the expression of multiple genes by targeting hundreds of mRNAs, thereby influencing critical cancer-related processes such as cell proliferation, migration, and apoptosis. Owing to their stability and strong tissue specificity, miRNAs have emerged as promising biomarkers for the non-invasive detection of various cancers [102]. Distinct miRNA expression patterns not only indicate the presence of early-stage malignancies but also provide insights into tumour progression at more advanced stages, aiding in both prognostic assessment and therapeutic guidance. Nevertheless, their clinical implementation remains uncertain due to several challenges.

Firstly, there is a lack of clarity regarding the correlation between miRNA levels in serum and those found in tumour tissues. Secondly, the dynamics of circulating miRNAs, which are influenced by factors such as health status, age, and variations in serum miRNA levels, remain insufficiently characterized. These variables significantly impact the accuracy of miRNA-based detection methods. Consequently, future research should focus on a comprehensive analysis of circulating miRNA kinetics, including how individual variability and personalized treatment regimens influence changes in serum miRNA profiles. Additionally, the primary technologies used for miRNA detection, including quantitative real-time polymerase chain reaction (qRT-PCR), northern blotting, and oligonucleotide microarray, present limitations in terms of cost and processing time. Moreover, these methods often suffer from inadequate sensitivity, restricting their application in in vitro diagnostics (IVD) [106].

2.2.3.5 Proteins IgG: role and diagnostic relevance

Among the diverse classes of proteins investigated as cancer biomarkers, Immunoglobulin G (IgG) has garnered increasing attention due to its intrinsic stability, persistent presence in circulating fluids, and well-documented alterations in expression associated with oncogenic processes [107]. IgG is a glycoprotein antibody predominantly produced by B lymphocytes in response to the recognition of foreign antigens, such as pathogens or abnormal self-molecules. It represents the most abundant immunoglobulin in the human blood, accounting for approximately 75-80% of the total antibody pool, and it plays a critical role in the adaptive immune response through antigen recognition and neutralization.

In the oncological context, emerging evidence suggests that both the concentration and structural characteristics of circulating IgG can undergo significant alterations in the presence of malignancies. These changes may involve not only increased or decreased IgG concentrations, but also modifications in glycosylation patterns, an essential post-translation process influencing protein stability, activity, and cell-cell interactions [108, 109]. Aberrant glycosylation of IgG has been correlated with tumour initiation, immune evasion, angiogenesis, and metastatic dissemination, thereby positioning this protein as a potentially valuable biomarker for early cancer detection and monitoring.

The detection of IgG in LB samples can be achieved through various analytical techniques, including electrochemical sensors, immunoimaging, and label-free photonic platforms [110]. Among these, photonic biosensors have demonstrated particular promise due to their high sensitivity, compactness, real-time operation, and compatibility with PoC applications. These platforms enable the quantification of IgG at low concentrations, facilitating early diagnosis without the need for invasive tissue sampling. Importantly, while current sensing architectures have been optimized for IgG detection, its versatility allows for the interrogation of other protein biomarkers simply by functionalizing the sensor surface with alternative capture antibodies. This modularity underscores the potential of the proposed device to support multiplexed and customizable diagnostic workflows in precision oncology.

2.2.3.6 Conventional molecular biomarkers: HER2, CEA and PSA

Among the numerous tumour biomarkers investigated to date, prostate-specific antigen (PSA), carcinoembryonic antigen (CEA), and human epidermal growth factor receptor 2 (HER2) remain the most established molecular indicators in routine oncology. Their broad application in prostate, breast, and lung cancer highlights their lasting importance as reliable tools for screening, risk stratification, and disease monitoring, even in the era of next-generation biomarkers [111].

HER2, initially validated in breast cancer, is now also relevant in gastroesophageal, lung, and other solid tumours. Its alterations, mainly gene amplification and protein overexpression, are linked to aggressive disease but also predict responsiveness to

targeted therapies such as monoclonal antibodies, tyrosine kinase inhibitors, and antibody-drug conjugates. HER2 assessment relies primarily on immunohistochemistry (IHC) for protein overexpression and fluorescence in situ hybridization (FISH) for gene amplification, while next-generation sequencing (NGS) is increasingly employed to detect actionable mutations, particularly in non-small cell lung cancer. International guidelines provide tumour-specific scoring systems and have recently defined the “HER2-low” category, which expands therapeutic options but also adds interpretive complexity. Nevertheless, intertumoral heterogeneity, inter-cancer variability, and the limited predictive value of IHC in some tumours emphasize the need for standardized protocols and refined criteria to ensure accurate and clinically meaningful evaluation [112].

CEA, originally identified as a fetal glycoprotein, is minimally expressed in healthy adults but frequently elevated in colorectal, pancreatic, lung and breast cancers. Despite limitations in sensitivity and specificity, it remains one of the most widely used tumour biomarkers, mainly for prognosis, treatment monitoring, and recurrence detection. CEA is primarily measured in serum by immunoassays but can also be detected in other biofluids, where it shows prognostic value, particularly in colorectal and gastric cancers. Because of its restricted diagnostic accuracy, it is often incorporated into multi-marker panels and combined with imaging to improve specificity. Although methodological heterogeneity and the absence of standardized cut-offs remain challenges, CEA continues to serve as robust and clinically relevant biomarker with broad utility [113].

PSA is the most widely used biomarker for prostate cancer, routinely applied screening, treatment monitoring, and recurrence detection. It is a serine protease secreted by prostatic epithelial cells, and its serum concentration rises in malignancy but also in benign conditions such as prostatitis or hyperplasia, limiting its specificity. The conventional 4 ng/mL threshold is debated due to frequent false positives and unnecessary biopsies. To enhance accuracy, refinements such as PSA density, free-to-total PSA ratios (f/t PSA), isoforms (proPSA, intact PSA), and multivariate risk models have been introduced. More recently, polygenic risk-adjusted thresholds have shown promise in reducing overdiagnosis. Alongside immunoassays, emerging biosensing technologies, including electrochemical and photonic platforms, are enabling ultrasensitive and rapid PSA detection, paving the way for next-generation PoC diagnostics [114].

Given their clinical relevance and measurable expression in accessible biofluids, PSA, CEA, and HER2 are ideal candidates for integration into advanced biosensing technologies, where photonic innovations promise more sensitive, rapid, and minimally invasive detection to support precision oncology.

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Chapter 3

Lab-On-Chip System for Liquid Biopsy

Lab-On-Chip technologies have emerged as transformative tools in the field of LB, offering miniaturized integrated platforms capable of handling complex biofluids. By combining microfluidics with advanced sensing strategies, LoC systems provide efficient separation, enrichment, detection and analysis of circulating biomarkers, including CTCs, cfDNA, and IgG protein.

This chapter reviews the fundamental design principles and enabling technologies underlying LoC systems, addressing both microfluidic architectures for sample processing and photonic approaches for high-sensitivity biomarker detection. Emphasis is placed on strategies that improve throughput, specificity, and PoC applicability, while also considering challenges related to device integration, standardization, and clinical validation. Against this background, this doctoral thesis focuses on advanced photonic biosensors tailored for seamless LoC integration. Two different configurations are introduced to enable the development of photonic biosensors to enable highly sensitive and specific detection of IgG, exploited as tumour-associated biomarkers in liquid biopsy applications. The first is a box-like resonance sensor on SOI, engineered through sidewall dielectric gratings with embedded Fabry-Pèrot defects, capable of translating subtle refractive index perturbations into pronounced amplitude variations while preserving CMOS compatibility. The device combines $Q > 10^4$, $ER > 15$ dB, and $S_{\text{bulk}} = 490$ nm/RIU and when functionalized for IgG, it delivers > 4 dB transmission change over 1 pg mL^{-1} – 500 pg mL^{-1} , establishing its role as a high-performance photonic biosensor for LB applications. The second device is an optical slot-assisted all dielectric metasurface, engineered to support a high Q-factor, confined mode under normal incidence, attains $Q \approx 3.2 \times 10^3$ and a $S \approx 25$ nm/RIU. It is designed to excite anapole-supported resonances with strong near-field confinement, enabling highly sensitive IgG biomarkers under normal-incidence. Both devices are conceived to operate in synergy with microfluidic modules for sample delivery and enrichment, ensuring compatibility with the compact footprint, low power budget, and integration requirements of LoC diagnostic platforms. Taken together, these results highlight how photonic biosensors with engineered resonance profiles and field distributions can substantially enhance the analytical performance of LB chips, paving the way for reliable, scalable, and clinically applicable PoC solutions.

3.1 Lab-On-Chip technologies: Innovations, advantages and challenges

Lab-On-Chip (LoC) systems, also referred to as micro total analysis systems (μ TAS), are increasingly recognised in scientific and clinical contexts as innovative and high-performance platforms for liquid biopsy applications. Despite their growing importance, a universally accepted definition of LoC technology is still lacking, reflecting persistent challenges in standardisation and clinical translation [1]. In general, LoC devices are defined by the miniaturization, integration, and automation of complex laboratory procedures onto a single microfabricated platform [2]. These systems fall under the broader category of Biomedical MicroElectroMechanical Systems (BioMEMS), a specialised subset of MEMS technologies designed for biomedical applications, particularly in the context of early diagnosis, continuous disease monitoring, and preventive medicine.

LoC systems integrate multiple laboratory functions, including sample preparation, biochemical reactions, and signal transduction, into a single miniaturised chip. Advancements in microfabrication have enabled the design of compact and efficient devices, typically spanning a few millimetres to several square centimetres, and capable of handling fluid volumes at the picolitre scale. These systems provide significant advantages in terms of portability, operational efficiency, and cost-effectiveness, making them particularly suitable for PoC diagnostics and decentralised healthcare settings.

Compared to conventional diagnostic and preventive modalities, LoC platforms offer distinct advantages that directly address many of the limitations of traditional laboratory-based approaches, thereby accelerating their adoption in biomedical research and clinical development [3]. While conventional diagnostics often require large volumes of samples, centralized facilities, and time-consuming workflows, LoC systems exploit miniaturization and portability to enable frequent, decentralized, and consistent testing in both clinical and home-based settings, as well as in resource-limited environments, thereby broadening access to diagnostic services in underserved regions. By facilitating on-site analysis, these systems reduce reliance on centralized laboratories, eliminate the delays associated with sample transport, and substantially shorten turnaround times. Furthermore, their capacity for operation by a single user mitigates the risks of human error and contamination typically encountered in multi-step conventional protocols, ultimately enhancing both reliability and analytical accuracy.

The versatility of LoC systems has led to their application across a wide range of biomedical domains, including advanced models such as 3D cell cultures, organ-on-chip platforms, and high-throughput screening assays [4, 5]. These technologies are particularly well-suited for biological applications due to the use of biocompatible polymeric substrates such as polymethyl methacrylate (PMMA), polycarbonate and polystyrene, which enable safe and effective interfacing with living cells and tissues [6].

This degree of biocompatibility, unlike that typically achieved with conventional

silicon-based microfabrication, broadens the potential of LoC platforms in fields such as cell biology, drug discovery, and personalized medicine.

A particularly compelling direction in LoC research involves the development of fully integrated devices capable of performing multiple functions, such as sample manipulation and biomolecular analysis, on a single platform. This integration stands in sharp contrast to conventional laboratory workflows, which require separate instruments for each step and are thus more time-consuming and prone to sample loss or contamination. By consolidating these operations, LoC technologies pave the way for the so-called “*sample in/answer-out*” paradigm, a model that offers significant advantages for rapid and minimally invasive diagnostic applications, particularly in context of LB [7]. To place these technological advances within a broader translational framework, Table 3.1 summarises key trends in LoC adoption and development from 2014 to the present, highlighting their progressive integration into biomedical research and clinical practice [8].

Despite the substantial progress achieved in LoC technology since the pioneering work of Terry et al. in 1979 [9] and Marz et al. in 1990 [10], its large-scale commercialisation in advanced diagnostics and research applications remains limited, facing several persistent challenges. From a technological perspective, an ideal LoC platform should be sufficiently automated and robust to allow operation by non-specialist users, while also supporting direct data transmission to centralised monitoring systems. Yet fully autonomous devices remain scarce, reflecting the gap between proof-of-concept prototypes and deployable clinical tools.

From a critical standpoint, one of the primary barriers to widespread adoption lies in the difficulty of scaling up production while maintaining performance consistency and manufacturing quality. The integration of multiple analytical functions on a single chip, while conceptually attractive, often compromises sensitivity and diagnostic accuracy, particularly when handling heterogeneous or clinically complex biological samples. As a result, most commercially available LoC devices currently address only a narrow set of diagnostic applications, remaining far from the analytical versatility and reliability offered by conventional laboratory infrastructures. Equally important are challenges related to usability. Both clinical staff and patients require adequate training, as improper handling or deviations from standard protocols may lead to unreliable results with potentially serious consequences for clinical decision-making. Beyond technical limitations, the LoC sector is also constrained by the constant demand for innovation [11].

Advances in biotechnology, nanofabrication, and materials science compel LoC technology developers to maintain continuous investment in research and development. This innovation race, however, must be balanced against market expectations for cost-effective, reliable, and user-friendly solutions, a tension that often delays product maturation and hampers large-scale deployment, especially in resource-limited settings.

Table 3. 1 Evolution trends in application of LoC systems [8].

Year	Research and analysis	Clinical	Environmental	Miscellaneous
2014–2014	Analytical devices	-	-	-
2014–2015	Drug development	PoC	-	-
2015–2016	Genetic, biomarkers	Drug delivery	-	-
2016–2017	-	Patient monitoring	Agriculture	-
2017–2018	-	-	Water treatment	Forensic science
2018–2019	-	-	Food processing	Stem cells
2019–2020	-	-	-	Synthetic organs
2020-present	-	-	-	Synthetic organs

In parallel, the regulatory framework remains one of the most significant barriers to the commercialization of LoC technologies [12]. Clinical and diagnostic applications must comply with stringent regulatory requirements aimed at ensuring device safety, performance, and reproducibility. Authorities such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) require extensive preclinical testing, validation protocols, and clinical evaluations. While indispensable, these processes significantly extend the time to market and impose considerable financial and logistical burdens, particularly on small and medium-sized enterprises engaged in LoC development [13]–[14]. Against this backdrop, both the scientific and industrial communities developing LoC systems, especially for LB, must focus on overcoming persistent limitations related to functional integration, process standardization, and scalable manufacturing. At present, many devices are limited to simple planar microfluidic chips without integrated sensing modules and still rely on bulky external instrumentation for analysis after reactions are complete. This lack of integration undermines portability, limits automation, and hinders scalability, ultimately reducing their suitability for real-world clinical implementation.

To address these limitations, the integration of on-chip biosensing technologies represents a promising and increasingly essential direction of research. Embedding highly sensitive, label-free detection modules directly within the chip architecture not only enhances miniaturisation but also enables real-time data acquisition, reduces user intervention, and facilitates true “*sample-in/answer-out*” workflows [15, 16]. Equally important is the need to optimise device performance with respect to sensitivity and specificity, which must meet the stringent standards of clinical diagnostics. Achieving this level of robustness is fundamental to ensuring that LoC

systems deliver clinically actionable and reliable data capable of guiding cancer diagnosis, treatment planning, and prognosis assessment with high confidence.

Over the past five years, LoC technologies have expanded considerably across the biomedical landscape, demonstrating their utility in diverse applications such as integrated bioassays, cellular studies, metabolomics, drug screening and delivery, tissue and organ physiology modelling, disease pathogenesis research, and personalised medicine [15, 16]. These advances underscore the transformative potential of LoC platforms, not only as diagnostic tools but also as bridging technologies that connect fundamental research with translation and clinical translation and medicine [17].

3.2 Lab-On-Chip integration for Liquid Biopsy applications

One of the most technically demanding yet transformative aspects of LoC technology lies in its capacity to integrate not only multiple laboratory functions, but also diverse functional modules, including microfluidic actuation and sample-preparation units, thermal control and reaction chambers, on-chip biosensors, signal-conditioning electronics, power management, embedded data processing, and user interfaces. In the specific case of LB, where biological analytes such as CTCs, ctDNA, EVs and IgG proteins, are often present in extremely low concentrations, the integration of microfluidic and biosensing components is crucial to achieving adequate sensitivity, specificity, and sample preservation [2].

Conventional LoC architectures typically comprise three core functional modules: sample processing (including extraction and pre-treatment), biomolecular amplification, and target biomarker detection. These modules are implemented through the integration of two primary platforms: a microfluidic subsystem for precise fluid handling and transport, and a biosensing interface for signal detection and transduction. The level of integration can vary considerably, ranging from disposable chips requiring external analytical instrumentation to fully self-contained systems capable of performing all analytical stages on-chip [18].

Recent advances have intensified the focus on sensor integration within the LoC systems specifically designed for LB. Fully integrated LoC platforms enable the development of cost-effective, automated systems for early cancer diagnosis, treatment response prediction, and prognosis assessment through biomarker-based personalized tumour profiling [19]. By combining microfluidic and biosensing, LoC systems are recognized as one of the most promising approaches for miniaturizing laboratory-based chemical and biological techniques into low-cost, portable diagnostic devices, with applications extending beyond medicine to environmental monitoring. As illustrated in Fig. 3.1, a standard LoC system for liquid biopsy encompasses distinct operational modules: sample introduction (e.g., blood or biological fluid), microfluidic transport through functional components (e.g., valves, mixers, filters), controlled chemical reaction zones, separation and isolation chambers, and final detection units based on optical, electrical, or magnetic transduction. The system is typically supported by integrated electronics for control and signal processing, ensuring automated data collection and interpretation [20].

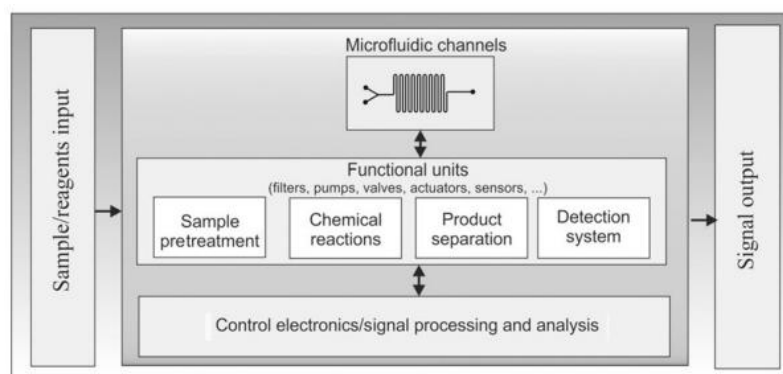


Fig. 3. 1 Schematic representation of a LoC system architecture designed for LB applications. The figure illustrates the design of an integrated LoC platform, highlighting the sequential flow of operations from sample introduction to signal detection. The architecture emphasises the spatial organisation and interconnectivity of microfluidic and sensing components, enabling efficient sample handling, reaction control, and real-time biomarker quantification within a compact diagnostic format [20].

Over nearly three decades of development, LoC systems have progressively reshaped the landscape of biological assays by offering robust alternatives to traditional methods, including enzymatic analysis, nucleic acid amplification, immunodetection, and drug-response profiling on cellular models [21]. To build upon these foundations, the following sections review the state-of-the-art in the most advanced and impactful microfluidic platforms and biosensing technologies, with particular attention to their principles, performance, and clinical relevance. This critical overview provides the framework for the core objective of this doctoral thesis: the design and development of photonic sensing devices conceived for future integration with microfluidic components, aimed at creating advanced LoC systems capable of detecting cancer biomarkers directly from biological fluids with enhanced sensitivity and reliability.

3.2.1 Microfluidic Platform: origin and state of the art for LB

In the development of Lab-On-Chip systems for biological fluid handling, microfluidic platforms play a pivotal role by enabling the precise manipulation of small volumes of samples and reagent, typically ranging from 100 μ L to 10 nL. These systems consist of interconnected microchannels and chambers and are often integrated with functional micro components, microvalves, microneedles, and mixers, which collectively allow for controlled fluid transport and the execution of complex analytical workflows [22]. Depending on the specific application and fluidic requirements, microfluidic elements can be engineered in a wide range of structural and material configurations. Fig. 3.2 illustrates representative examples of commonly employed architectures and flow control components [23].

Microfluidic technology offers several inherent advantages that make it particularly attractive for diagnostic applications: minimal sample and reagent consumption, faster processing times, streamlined workflows, and reduced risk of diagnostic errors due to contamination. The adoption of closed-channel designs further reduces sample loss and exposure to the external environment, while supporting miniaturisation, automation, and parallelisation of analytical tasks. In addition,

their low fabrication costs and scalability reinforce their suitability for both PoC diagnostics and high-throughput clinical settings.

Since its first introduction by Manz and co-workers in 1990, microfluidic technology has consolidated its role as a versatile analytical platform [24]. One of its most distinctive features lies in the flexibility of chip design, which allows customized architectures tailored to the physical and biochemical properties of the biomarkers under investigation. This versatility enables the integration of multiple processes, such as sampling, sorting, trapping, enrichment, and detection, within a single device. The use of minimal amounts of samples and reagents further enhances cost-efficiency, an aspect particularly relevant in LB applications, where biological material is often scarce. Moreover, microfluidic chips provide an effective environment for biomarker per-concentration and for performing cyclic enzymatic amplification reactions, thereby improving both sensitivity and specificity. Their miniaturised nature also facilitates seamless coupling with optical, electrochemical, and mass spectrometry detection modules, expanding their analytical capabilities and supporting multiplexed assays within compact devices [25].

In the context of LB, microfluidic module has been extensively applied to the isolation, capture, and characterisation of key biomarkers such as CTCs, cfDNA, and EVs. Recent advances in integrated microfluidic systems have enabled the on-chip implementation of analytical techniques that were previously confined to benchtop instruments, thus allowing for high-precision, multiplexed assays within compact and self-contained platforms. The integration of multifunctional modules into LoC systems represents a promising strategy for consolidating sample preparation, processing, and analysis, reducing workflow complexity, minimizing analyte loss, and mitigating cross-contamination risks associated with manual handling [22].

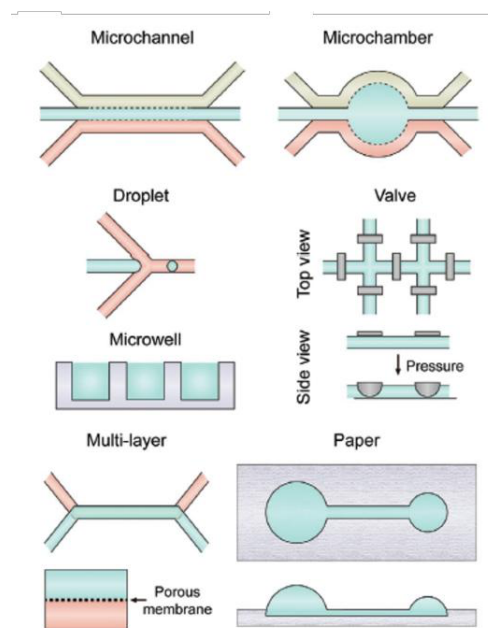


Fig. 3. 2 Representative structural designs used in microfluidic platform for LoC systems [23].

The development of microfluidic systems began in the early 1990s, primarily to optimize chemical and biological analyses by enhancing efficiency and reproducibility. The earliest devices attempted to integrate multiple functional elements within a single chip for specific applications, but the lack of standardized fabrication protocols and computational design tools made system-level integration highly challenging. Research at this stage focused mainly on the development of discrete fluidic elements, such as pumps, valves, sensors, and detectors, each tailored to individual research needs or application-driven constraints [26]. As a result, early microfluidic devices exhibited limited operational versatility, typically performing basic functions like fluid transport, separation, or detection, and were frequently designed for single use. Importantly, both pre-processing and post-processing steps still relied on external instruments and protocols, which limited the overall integration of the analytical workflow.

Initial fabrication methods primarily employed silicon and glass substrates due to their compatibility with established semiconductor processes. However, the field later transitioned toward polymeric materials, most notably polydimethylsiloxane (PDMS), owing to their biocompatibility, optical transparency, gas permeability, and ease of fabrication [27]. Since then, the scope of microfluidic technologies has expanded significantly, incorporating diverse materials and fabrication methods to accommodate a broad spectrum of biomedical applications. One notable example includes a PDMS-glass multilayer device functionalized with aptamer-coated microbeads, which enabled the selective extraction and thermal release of arginine vasopressin through integrated microheaters and temperature sensors [28]. The described device is shown in Fig. 3.3 [27, 28].

A critical milestone in the evolution of microfluidics was the introduction of non-mechanical fluid control strategies, such as electroosmotic flow and electrophoresis, which provided effective alternatives to mechanical components for directing and separating fluids within microchannels [29]. Additionally, the adoption of laser-induced fluorescence (LIF) facilitated the sensitive detection of trace biomolecular species, while advances in low-cost photolithography and mask printing technologies significantly accelerated the prototyping of soft lithography-based devices [30].

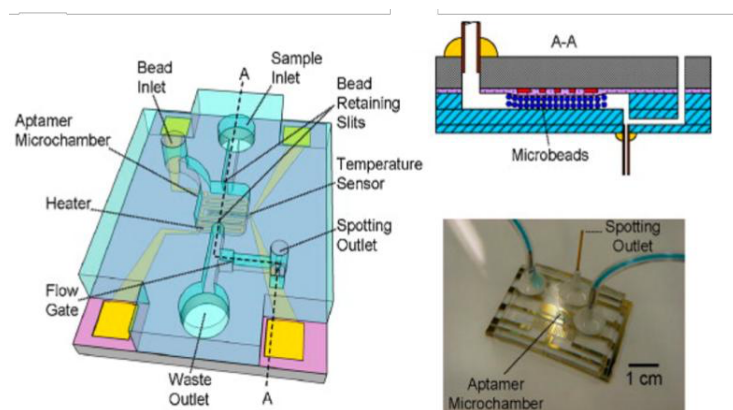


Fig. 3. 3 Schematic, cross-section view and photograph of microfluidic aptasensor [27],[28].

Structurally, microfluidic devices are composed of a network of well-defined void regions, such as reservoirs, chambers, and channels, that serve as the primary means for fluid handling. These are often complemented by embedded solid components, including membranes monolithic structures, pneumatic actuators, and micropillars, which enhance the chip's functionality. Microchannels, typically featuring volumes in the nanoliter to picoliter range, are characterized by their laminar flow regime and high surface to volume ratios. For instance, in microchip electrophoresis (μ CE), typical channel dimensions may measure approximately 50 μ m in width, 15 μ m in height, and 5 mm in length. Overall, LoC systems incorporating microfluidic networks have enabled miniaturised, efficient, and often automated analysis of diverse biological targets, including bacteria, mammalian cells, microalgae, amino acids, proteins, and nucleic acids, directly on-chip. Fluid motion in these systems can be actuated by various mechanisms, including applied pressure, electroosmotic flow, electrowetting, and capillary action. These fundamental capabilities have laid the foundation for more sophisticated microfluidic-assisted strategies, which will be discussed in the following sections with a particular focus on exosome, CTC and IgG analysis in the context of LB.

3.2.1.1. Microfluidic-Assisted strategies in cancer biomarkers Research

The implementation of microfluidic technology in circulating tumour cell research has significantly advanced the ability to isolate, enrich, and characterize these rare and heterogeneous biomarkers [31]. By leveraging the distinct biophysical and biochemical features of key cancer biomarkers, microfluidic devices provide a versatile and integrable platform to overcome challenges related to their low abundance in circulation and high phenotypic variability [32]. A broad range of analytical strategies have been developed, including size-based filtration, density gradient centrifugation, electrophoresis, immunomagnetic capture, and microfluidic chip systems [33]. Among these approaches, microfluidic platforms are increasingly favoured due to their adaptable architectures and precise control of fluids and cells at the micrometre scale [34]. Highly integrated platforms enable rapid separation and identification of key biomarkers, including CTCs and exosomes, while minimising sample requirements, simplifying workflows, and reducing risks of analyte loss or contamination.

A commonly employed principle in microfluidic CTC isolation is immunoaffinity capture through the epithelial cell adhesion molecule (EpCAM), a transmembrane glycoprotein frequently overexpressed on epithelial-derived tumour cells [35]. Microchannels functionalized with anti-EpCAM antibodies enable selective binding; however, planar geometries provide limited surface area for effective antigen – antibody interactions. To overcome this, innovative microfluidic designs such as herringbone mixers [36-38], micropost arrays [39, 40], and nanopillar configuration [41] have been introduced, generate secondary flow patterns and increasing contact efficiency between cells and functionalized channel walls (Fig. 3.4).

In addition to microchannel-based approaches, immunomagnetic bead-based strategies have been established, with CellSearch® currently representing the only FDA-approved clinical platform for CTC enrichment in metastatic breast, prostate, and colorectal cancers [42, 43]. An alternative in vivo approach is provided by the GILUPI CellCollector®, which directly exposes an EpCAM-coated probe to the bloodstream, thereby increasing the likelihood of capturing rare tumor cells otherwise missed in conventional assays [40].

Despite their high sensitivity, EpCAM-based methods are intrinsically limited by the heterogeneity of EpCAM expression. CTCs undergoing epithelial-to-mesenchymal transition frequently downregulate EpCAM, thereby reducing capture efficiency. To address this limitation, label-free strategies based on negative selection have been developed, whereby hematologic cells are selectively removed by functionalized surfaces, allowing unlabelled CTCs to be recovered at the outlet [44].

In parallel, several microfluidic platforms exploit intrinsic biophysical properties, such as size, density, deformability, compressibility, and magnetic susceptibility, to achieve label-free separation. Representative approaches include size-based filtration [45, 46], density gradient manipulation [47], deformability-based sorting [48], and magnetic levitation or deflection (Fig. 3.5).

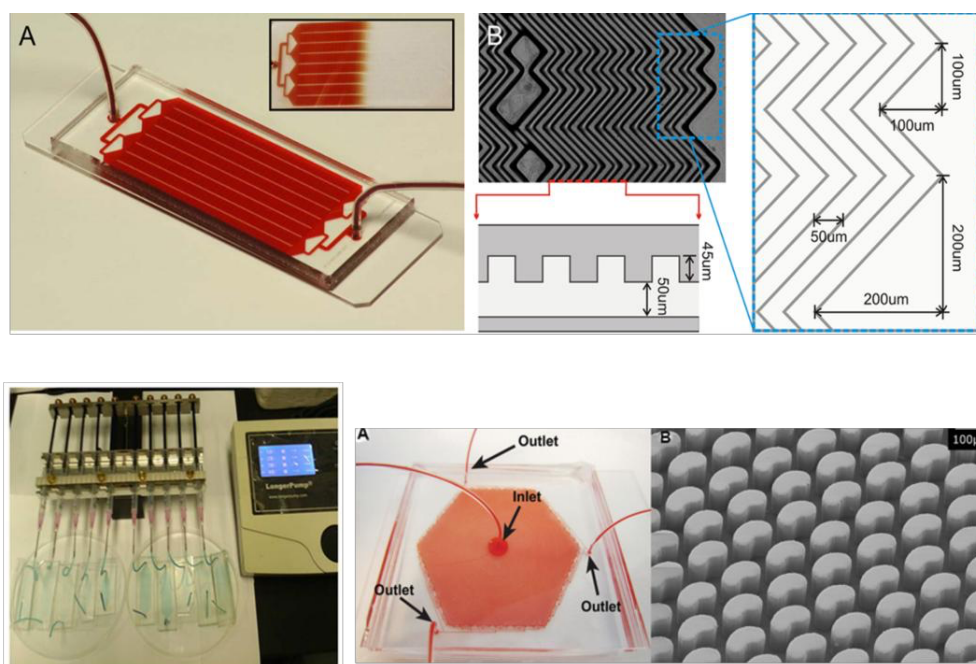


Fig. 3. 4 The HB-Chip features a microfluidic network composed of channels with a single entry and exit point. The inset highlights the consistent flow of blood across the device (*top left*) [36]; a micrograph of the patterned surface reveals the periodic and asymmetric structure of the herringbone-shaped grooves (*top right*) [36]; Photography of microfluidic system for ultra-sensitive capture of CTCs (*bottom left*) [39]; Schematic representation of a radial-flow microfluidic device designed for high-throughput, affinity-based capture of circulating tumour cells (CTCs). The radial channel layout enables uniform fluid distribution and efficient target isolation across the entire surface area (*bottom right*) [40].

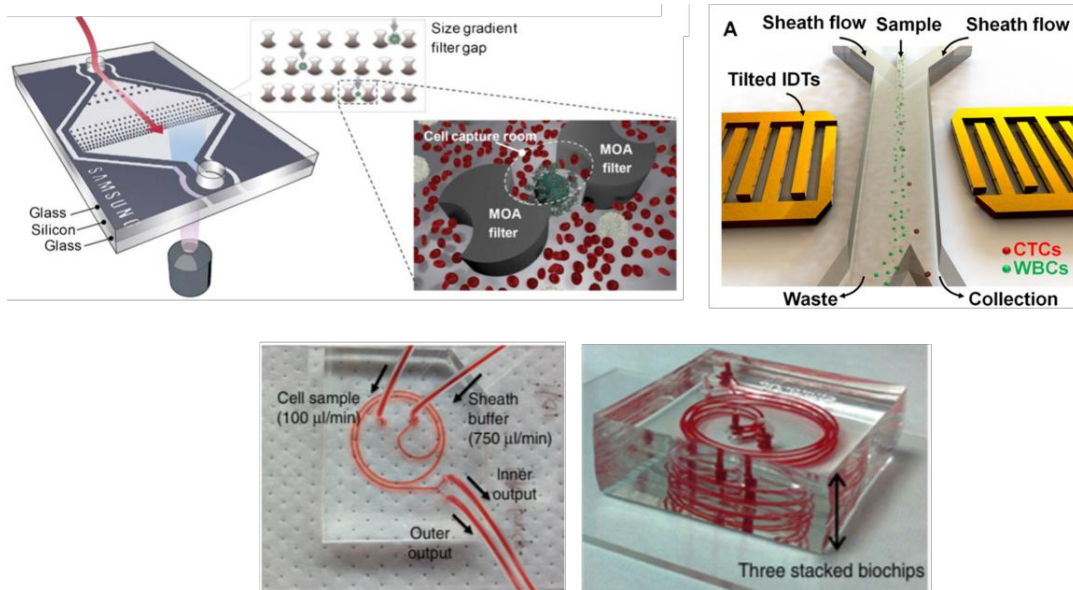


Fig. 3. 5 Size-based filtration chip with multi-obstacle architecture (MOA). The device features two sequential filtration gaps separated by an intermediate “cell retention chamber” designed to reduce clogging. The chip was manufactured using silicon-on-glass (SOG) microfabrication technology (*top left*) [46]; Schematic illustration and image of the high-throughput titled-angle standing subrace acoustic waves (taSSAW) device for cancer cell separation (*top right*) [48]; Diagram of a system based of two ring spirals with two inlets and two outlets, designed to separate cells based on their size by leveraging hydrodynamic forces (*bottom*)[49].

Building on these principles, size-based microfilters have been developed as advanced devices for CTC isolation. These systems exploit the typically larger size of CTCs compared to red and white blood cells, which can pass through defined pores, often assisted by buffer flushing to minimize residual contaminants [50]. Nevertheless, because the size ranges to tumour cells and certain blood cells partially overlap, separation purity is frequently compromised.

To address this, Liu et al. [51] introduced the FerroChip, a ferrohydrodynamic device capable of separating exosome-like nanoparticles depending on their hydrodynamic behaviour: larger EVs migrate toward the channel center, while smaller exosomes remain close to the walls, enabling size-dependent fractionation in a continuous and label-free manner. Deterministic lateral displacement (DLD) devices employ ordered micropost arrays to create asymmetric flow paths, enabling continuous particle separation with minimal shear stress though they are challenged by CTC clusters or EVs of similar size to contaminants [52-54]. To overcome these issues, nonequilibrium inertial separation arrays (NISA) have been designed, exploiting inertial lift forces in wider channels to achieve efficient label-free separation [55]. Complementary approaches, such as dielectrophoresis (DEP) integrated with electrothermal flows, enable fractionation based on dielectric properties, providing higher precision than size-based methods alone [56].

Exosome isolation has also benefited from microfluidic integration. While ultracentrifugation remains the reference method, its long processing times, high operational costs, and limited purity have accelerated the adoption of microfluidic

systems, which provide greater yield, faster operation, and improved compatibility with downstream assays. Surface-functionalized microchannels or immunomagnetic beads targeting canonical EV markers (CD9, CD63, CD83) or tumour-associated antigens (EpCAM, CD24) enable selective capture, although many commercial devices still rely on off-chip analysis. More integrated examples include the ExoChip, which combines exosome isolation and quantification in a single device, and the iMER chip, which couples immunomagnetic capture with on-chip RNA analysis via qPCR, enabling high-throughput diagnostics directly from patient samples [57, 58]. In parallel, viscoelastic microfluidic systems have been developed for label-free enrichment of small EVs directly from whole blood, achieving up to thirty-fold higher yield than ultracentrifugation in under one hour, without labelling or complex instrumentation, as shown in Fig. 3.6.

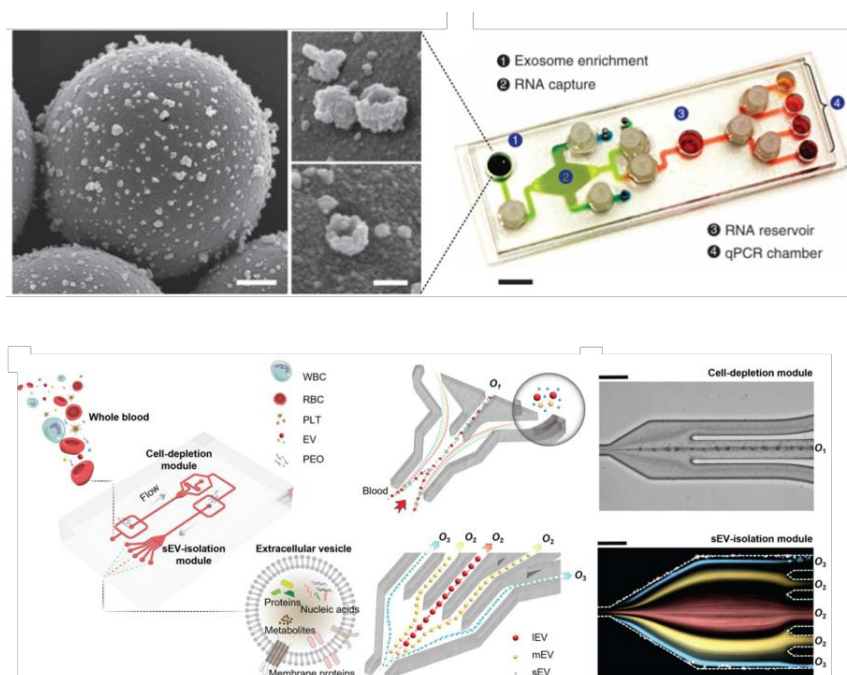


Fig. 3. 6 Scanning electron micrographs of magnetic microbeads after immunoaffinity capture (*top left*); Photograph of the microfluidic iMER prototype. The iMER device employs 3 μm magnetic microbeads functionalized with antibodies targeting the epidermal growth factor receptor (EGFR). Upon incubation with cell-free serum samples, tumor-specific exosomes were selectively captured using a magnetic field with a reported efficiency of 93% from a 100 μL sample. The entire process, from exosome capture to RNA analysis via qPCR, was completed in approximately 2 hours, highlighting the potential of the iMER platforms as a prognostic tool for monitoring disease progression and assessing drug resistance in GBM patients (*top right*) [58]; schematic and optical images of the viscoelastic microfluidic platform for sEV isolation. The device is composed of two sequential regions: a first module for blood cell removal (WBCs, RBCs, and platelets $>1\ \mu\text{m}$) and a second module for size-based separation of extracellular vesicles. In the initial section, larger components are directed toward outlet O1, while smaller particles continue downstream. In the sEV-isolation section, large and medium vesicles are diverted to outlet O2, whereas small extracellular vesicles ($\sim 100\ \text{nm}$) are collected at outlet O3. The bright-field image illustrates the focusing and removal of particles $\geq 3\ \mu\text{m}$, while the fluorescence image confirms trajectory separation among 1 μm (red), 500 nm (yellow), and 100 nm (blue) particles (*bottom*) [59].

The refinement of affinity-based techniques has also been supported by the introduction of aptamers, synthetic oligonucleotides generated via SELEX which offer greater reproducibility and chemical stability than antibodies [60-62]. Their smaller size enables higher-density immobilization and improved access to bind sites, thereby enhancing capture efficiency [63, 64]. Aptamer-functionalized microchips have been employed to detect rare lung cancer CTC subtypes [65], while hybrid systems combining aptamers with magnetic interfaces, such as the FluidmagFace platform, have enabled efficient capture of EpCAM-positive cells followed by direct downstream proteomic profiling [66].

Altogether, microfluidic-based strategies extend beyond simple physical or affinity-driven isolation of cancer biomarkers, positioning microfluidics as a core technology for integrated diagnostics. By interfacing with advanced sensing platforms, microfluidic devices enable the development of LoC architectures for liquid biopsy, where sample handling, enrichment, and molecular detection are seamlessly combined to achieve sensitive, rapid, and minimally invasive cancer diagnostics.

3.2.1.2 Microfluidic- Assisted strategies in protein IgG Research

The selective isolation and controlled release of IgG-bound biomolecules represent a critical step in protein analysis and targeted cell manipulation [67]. While traditional techniques such as magnetic bead separation and membrane filtration are widely employed, microfluidic systems provide superior precision, miniaturization, and compatibility with complex biological samples. In the broader context of silicon-based microfluidic technologies for selective cell enrichment, two main approaches can be identified. The first relies on size-based physical separation, employing microfabricated structures with well-defined pore sizes and flow channels, thereby enabling label-free isolation of target cells such as MCF-7, MDA-MB-231, IgG, and CTCs directly from whole blood samples without pretreatment. The second approach exploits IgG-functionalized surfaces for the specific capture of antigen-expressing cells through antibody-antigen interactions. While highly selective, conventional IgG-modified systems often depend on harsh chemical or mechanical means for cell release, which may compromise cell viability. To overcome these limitations, advanced microfluidic platforms have been developed that exploit stimuli-responsive chemistries, including linkers that are cleavable by light, temperature changes, or enzymatic activity, thus enabling non-destructive and selective release of captured targets.

Among recent innovations, a silicon-based microfluidic platform has been developed in which anti-HEL IgG is immobilized via a photocleavable ANP linker, enabling the efficient capture and light-triggered release of HEL-expressing cells under mild conditions, with preserved viability and reculturing potential [68]. Notably, efficient cell capture was observed even at low concentrations of anti-HEL IgG (2 µg/mL), with no significant increase in cell trapping when increasing the concentration up to 20 µg/mL. This finding indicates that surface-immobilized IgG via the ANP linker is sufficient for specific and effective capture. Moreover, the ability to photochemically cleave the linker allows the on-demand release of viable cells, a feature not attainable with conventional immobilization strategies.

In parallel with cell isolation strategies, several microfluidic solutions have been proposed to improve the analytical performance and operational efficiency of IgG detection and characterization. Conventional enzyme-linked immunosorbent assays (ELISA) remain the reference method for quantifying antibody levels, but are constrained by high reagent consumption, multiple manual steps, and long assay times. Microfluidic adaptations, such as centrifugal platforms based on compact disk (CD) formats, have significantly improved antibody detection workflows. These systems automate ELISA through controlled rotation speeds, enabling sequential reagent delivery via centrifugal and capillary forces [69]. For instance, Lai et al. [69] demonstrated IgG detection on a CD-based platform with a detection limit of $5 \text{ mg} \cdot \text{L}^{-1}$ (31 nM), using only 30 μL of reagents and reducing assay time to approximately one hour, compared to the 300 μL and several hours or days required by conventional 96-well microplate formats. Further improvements were reported by Espulgar et al. [70], who optimized fluid flow using rotational speed control, achieving a detection limit of $6.16 \text{ ng} \cdot \text{mL}^{-1}$ for IgA. Beyond ELISA miniaturization, alternative microscale strategies have emerged. Cohen et al. [71] proposed a microsphere-based immunoassay for dynamic monitoring of anti-TNF- α antibody concentrations, eliminating the need for conventional washing steps. Additionally, microfluidic chip electrophoresis has proven more efficient than SDS-PAGE for assessing IgG purity and molecular size in hybridoma cell cultures [72].

3.2.2 Photonics Biosensor Platform

A biosensor is an integrated analytical device designed to convert a biological or chemical interaction into a quantifiable signal, typically electrical, acoustic, or optical, proportional to the concentration of a target analyte. The signal arises from a specific biochemical reaction or binding event involving the analyte of interest. Positioned at the interface of biology, chemistry, medicine, and engineering, biosensors underpin a wide spectrum of applications, with diagnostics being the most prominent, accounting for nearly 70% of clinical decision-making [73]. These devices enable miniaturized, cost-effective execution of laboratory workflows, including sample preparation, analyte separation, and result analysis. The pre-analytical phase ensures proper biomarker handling, while isolation techniques such as chromatography, liquid-liquid extraction, or nanomaterial-based enrichment remove interfering substances. The subsequent transduction and processing steps generate data directly correlated with analyte concentration. In their canonical architecture, biosensors comprise three fundamental components: (i) a biorecognition element, such as enzymes, antibodies, nucleic acids, aptamers, or whole cells, responsible for the selective interaction with the analyte; (ii) a transducer, which converts the recognition event into a measurable signal; and (iii) a processing unit for signal conditioning and data analysis (Fig. 3.7). Biosensors can be broadly classified according to either their biorecognition mechanisms or their transduction strategy. Based on the recognition element, they include enzymatic biosensors, immunosensors, nucleic acid/aptamer-based sensors, and whole-cell biosensors.

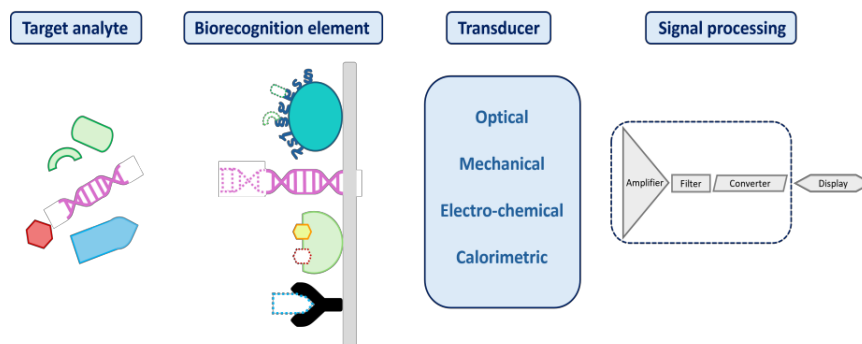


Fig. 3. 7 Illustration of a biosensor structure, with its three key components: a biorecognition element specific to the target analyte; a transducer to transform the biorecognition interaction into a detectable signal; a processor responsible for amplifying, processing, and presenting the signal [7].

Alternatively, classification by transducer type distinguishes electrochemical (potentiometric, amperometric, and conductometric) [74], optical [75], electronic [76], acoustic [77], and mechanical [78] platforms. For Lab-On-Chip applications, the most relevant categories, electrochemical, optical, and mechanical biosensing strategies, are comparatively summarized in Table 3.2.

Among the different categories, photonic biosensors are gaining increasing prominence, particularly in the field of liquid biopsy. Their ability to operate label-free, support multiplex detection, and achieve ultra-low detection thresholds in the femtomolar range makes them highly effective for non-invasive cancer diagnostics. These platforms transduce microscale and nanoscale variations in optical properties, including phase, amplitude, polarization, or frequency, into quantifiable signals. Their inherent strengths include real-time monitoring, immunity to electromagnetic interference, and compatibility with minimally invasive sampling, features that make them especially attractive for early disease detection [93, 94].

Photonic biosensors can be broadly categorized as either label-free or label-based. In label-free configurations, biomolecular interactions at the sensor surface induce local refractive index variations, which are directly monitored without the need for external markers. This enables high-fidelity, real-time monitoring with minimal assay complexity. By contrast, label-based approaches employ exogenous optical probes, such as fluorophores, quantum dots, or luminescent nanoparticles, to generate detectable signals through fluorescence, luminescence, or related mechanisms [95, 96]. Although label-based systems offer precise molecular targeting, they are limited by steric hindrance and restricted capability for kinetic measurements. Consequently, label-free strategies are increasingly preferred due to their operational simplicity, reduced sample preparation, and capacity to capture dynamic biomolecular interactions in real time. From a physical perspective, most label-free photonic biosensors rely on evanescent field sensing, where the exponentially decaying optical field interacts with surrounding analytes. Sensitivity is therefore dictated by the degree of overlap between the evanescent field and the biomolecules at the sensor surface.

Table 3. 2 Overview of biosensor types for liquid biopsy: key characteristics, benefits, and limitations [7].

Transducer Type	Key Characteristics	Strengths	Limitations	Performance
Electrochemical	- Enables real-time monitoring, typically within several hundred seconds - Common in PoC systems. - Short operational lifespan - Cost-effective microelectrode production	- Straightforward operation - Suitable for continuous analysis across multiple targets - Economic	- Devices tend to be bulky - Susceptible to environmental fluctuations (e.g., temperature)	- LoD = 70 pg/mL [79] - LoD = 1.55 pM [80] - LoD = 0.26 pM [81] - LoD = 0.13 pM [82] - LoD = 0.46 ± 0.006 fM [83]
	- Operates label-free - Requires ~30 min for detection	- Integrates well with PoC formats - Easy to use	- Highly sensitive to mechanical and thermal disturbances - Complex fabrication processes	- LoD = 7.6 pg/mL [84] - LoD = 300 pM [85] - LoD = 100 pM–DR = 6 ng/mL–60 µg/mL [86] - LoD = 0.3 pM; Δf = 50 Hz [87]
Optical/photonic	- Requires little sample preparation - Capable of real-time analysis - Generally, it is more costly	- Capable of real-time analysis - Delivers high temporal resolution and S - Provides consistent and reliable results. - Suitable for low-cost integration - High compatibility with miniaturized systems	- Systems are typically bulky - Performance may vary with environmental conditions	- S = 4034 dB/RIU; LoD = 3.7 pg/mL [88] - S~97, 51%, and 96.29% [89] - LoD = 0.6 nM; $\Delta\lambda$~pm [90] - LoD = 3 nM [91] - LoD = 6.5 pM; $\Delta\lambda$ = 15 pm [92]

Within this paradigm, four major categories of photonic biosensors are relevant for biomedical applications: surface plasmon resonance (SPR), optical waveguides and fiber platforms, optical resonators, and metasurfaces.

SPR remains the most mature and widely deployed technology, offering real-time, label-free detection of biomolecular interactions with high sensitivity and temporal resolution [97].

SPR sensors exploit the excitation of surface plasmon waves, coherent electron oscillations, at a metal-dielectric interface under specific optical conditions (Fig. 3.8) [98].

Advances in nanofabrications and material engineering have improved their sensitivity, enabled detection of small molecules ($< 10\text{kDa}$), and facilitated multiplexing through hybrid LSPR and extraordinary optical transmission (EOT) architectures. Despite challenges such as optical losses and thermal instability associated with metallic layers, SPR platforms are commercially established and extensively employed in biopharmaceutical characterization and drug discovery. Optical waveguides and fiber-based sensors provide complementary benefits, including low optical losses, structural versatility, and seamless integration into LoC systems [99, 100].

Recent developments in polymer-based waveguides have demonstrated enhanced refractive index contrast (up to 10^{-2} RIU), improving sensitivity while maintaining biocompatibility [101].

Optical resonators, such as Whispering Gallery Mode resonators, photonic crystals, and Fabry-Perot interferometers, are recognized for their high analytical sensitivity and real-time readout, with compatibility for large-scale microfabrication [102, 103]. A notable example is the FDA- approved MaverickTM system, which leverages silicon photonics and microring resonators for multiplexed detection of SARS-CoV-2 antibodies and viral proteins, highlighting the clinical translation of this class of devices [104].

Metasurface-based biosensors employ subwavelength nanoarrays engineered to manipulate amplitude, phase, and polarization of light, achieving ultrathin sensing formats with high spatial resolution (Fig. 3.8) [105-107]. Their advantages include CMOS compatibility, low optical losses, and tunable spectral responses, positioning them as promising candidates for next generation biosensing.

Nonetheless, fabrication complexity and limited clinical validation remain significant barriers. Current research is exploring dynamic and reconfigurable metasurfaces capable of adaptive sensing through structural modulation [108].

To synthesize the distinctive features, advantages, and limitations of these platforms, a comparative overview is reported in Table 3.3. Recent technological progress has accelerated the development of highly sensitive label-free photonic platforms for liquid biopsy applications, enabling the detection of circulating disease biomarkers with ever-lower detection limits.

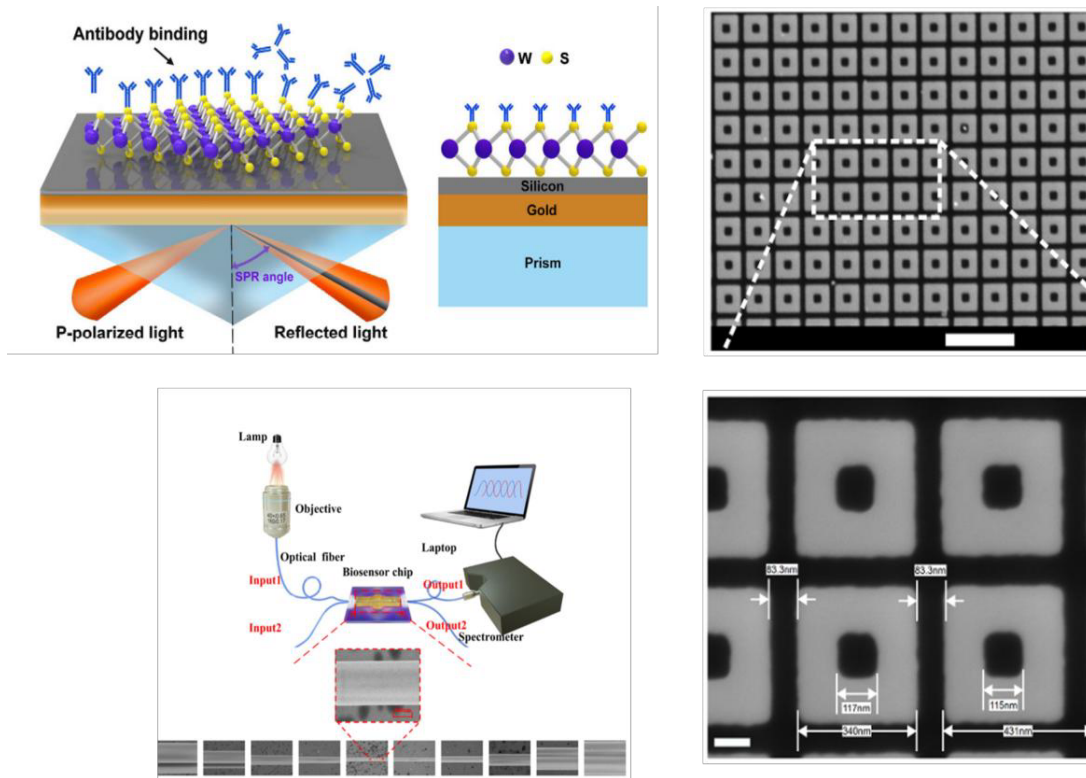


Fig. 3. 8 Schematic of an SPR biosensor (*top left*) [98]; Optical fiber-based biosensor setup and SEM views (*bottom left*) [109]; SEM micrographs of nanocuboid metasurfaces (*top and bottom right*) [105], [7].

The current landscape of this field is characterized by the refinement of existing technologies and the introduction of revolutionary approaches. Microring resonator architectures have attracted particular interest due to their compactness and multiplexing capability. For example, Liu et al. [110] reported a sub-wavelength grating microring resonator integrated with a Vernier effect configuration, achieving a sensitivity of 7061 nm/RIU and a limit of detection (LoD) of 1.74×10^{-5} RIU (Fig. 3.9). Similarly, Chen et al. [111] demonstrated microring resonators for detecting human serum albumin (HSA) achieving a sensitivity of 168 nm/RIU and an LoD of 6354 ng/mL (Fig. 3.9). Other optical approaches have exploited plasmonic effects.

Fiber-optic sensors based on localized surface plasmon resonance (LSPR) have shown remarkable performance; for example, Kim et al. [112] detected thyroglobulin, a thyroid cancer biomarker, in clinical samples with an LoD of 93.11 fg/mL (Fig. 3.9). Despite such achievements, the reproducibility of LSPR sensors remains a limiting factor for routine clinical implementation, although recent progress has begun to address this issue.

Table 3. 3 Comparative overview of photonic biosensing platforms relevant to liquid biopsy applications [7].

Platform	Principle of operation	Strengths	Limitations	Clinical translation
Surface Plasmon Resonance (SPR)	Excitation of surface plasmon waves at a metal–dielectric interface under specific optical conditions.	• Real-time, label-free detection; • High sensitivity, particularly for protein–ligand interactions; • Established commercial platforms.	• Optical losses in metallic layers; • Thermal drift and limited miniaturization; • Performance depends on surface chemistry.	Widely used in drug discovery and biopharmaceutical characterization; some FDA-approved instruments.
Optical Waveguides / Fiber Sensors	Detection of refractive index changes via evanescent field interactions in optical waveguides or fibers.	• Low optical losses; • Structural versatility and ease of integration into LoC systems; • Biocompatible polymer designs with improved index contrast.	• Reproducibility issues in complex biofluids; • Sensitivity limited by field overlap; • Susceptible to fouling and signal drift.	Growing interest in PoC applications; clinical adoption still limited.
Optical Resonators	Resonant structures (microrings, photonic crystals, WGM resonators) detect refractive index shifts with ultrahigh sensitivity.	• Very high analytical sensitivity; • Real-time readout; • Compatible with large-scale microfabrication.	• Sensitive to fabrication imperfections; • Narrow dynamic range; • Limited robustness in heterogeneous samples.	Clinical translation emerging; FDA-approved Maverick™ system for SARS-CoV-2 detection demonstrates feasibility.
Metasurfaces	Subwavelength nanoarrays engineered to manipulate amplitude, phase, and polarization of light for ultrathin sensing.	• CMOS compatibility and integration potential; • High spatial resolution; • Tunable spectral responses; • Ultraflat, miniaturized formats.	• Complex fabrication; • Limited clinical validation; • Functionalization and stability remain challenges.	Still largely at research stage; promising for next-generation adaptive and reconfigurable biosensors.

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Beyond protein biomarkers, photonic biosensing has recently expanded toward multi-analyte detection. Favaretto et al. [113] developed a microring resonator (MRR)-based platform capable of simultaneously detecting C-reactive protein (CRP) and creatine kinase-MB (CK-MB) in undiluted saliva, achieving LoDs of 103 pM and 140 pM, respectively (Figure 13). This illustrates the promise of PoC diagnostics but also highlights the challenge of accurate multiplexing without cross-analyte interference.

To mitigate this, Kabir et al. [114] proposed a novel silicon-insulator-silicon (SIS) LoC-chip based on a demultiplexer configuration, enabling dual-analyte detection (Ca^{2+} and hemoglobin) with a sensitivity of 1273 nm/RIU and a LoD of 7.8×10^{-7} RIU. The introduction of the sensitivity-to-volume ratio (SVR) as a performance metric underscores the trend toward compact yet highly efficient multi-analyte biosensors, although validation in complex biofluids remains limited. Further advancing nanophotonic sensing, Hamza et al. [115] introduced a terahertz nano-photonic perfect absorber (NPA) optimized for label-free detection of circulating cancer exosomes. Operating across 0.1-50 THz with > 97.5% absorption efficiency, the device exploits ultra-broadband absorption and strong field localization to discriminate cancer-derived from normal exosomes. Compared with plasmonic and microring platforms, NPAs offer unparalleled bandwidth and sensitivity, but challenges remain in functionalization specificity and scalable fabrication. Table 3.4 summarizes their analytical performance in LB applications.

In conclusion, the current photonic biosensing landscape is best interpreted through complementarity rather than competition. Each platform contributes unique strengths tailored to specific biomarkers and clinical contexts, but none provides a universal solution.

Future progress is expected to come from hybrid systems that integrate the maturity of SPR, the versatility of waveguides, the sensitivity of resonators, and the integration potential of metasurfaces. Such convergence could pave the way for robust, scalable, and clinically validated platforms, ultimately enabling photonic biosensors to play a central role in next-generation liquid biopsy.

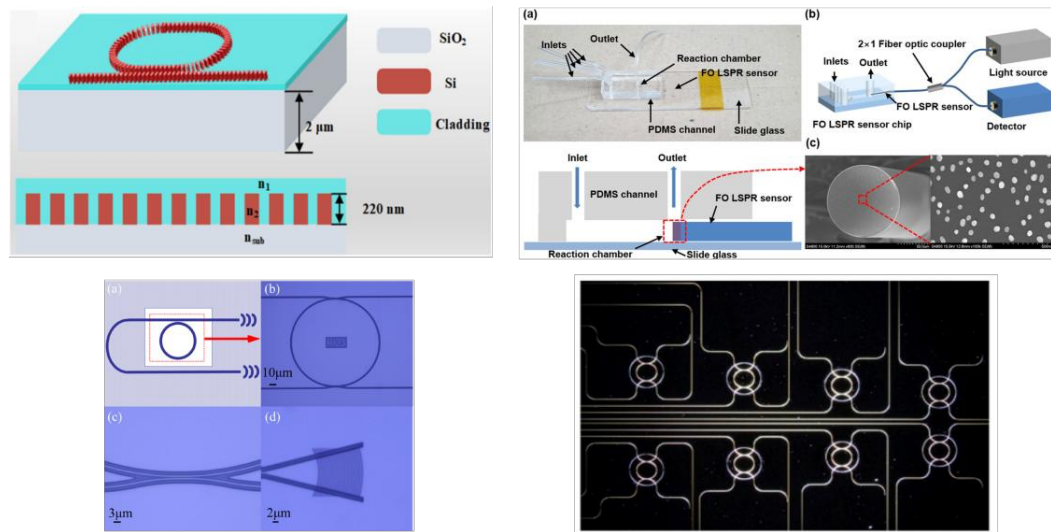


Fig. 3. 9 Sub-wavelength grating-based sensor (top left) [110]; MRR platform for HAS (top right) [111]; Fiber-optic LSPR sensor for Tg (bottom left) [112]; MRR array for CRP and CK-MB (bottom right) [113],[7].

After outlining the fundamental features of photonic biosensing platforms for the detection of circulating biomarkers, the following subsections concentrate on clinically significant tumour biomarkers, namely HER2, CEA, PSA and IgG. As introduced in Sections 2.2.3.5 and 2.2.3.6, these analytes were selected because of their strong clinical association with breast, lung, and prostate cancers, which represent some of the most prevalent malignancies worldwide and stand to benefit from early detection through these markers.

For each biomarker, a critical examination of photonic biosensing strategies for liquid biopsy is provided, with particular emphasis on analytical performance, technological feasibility, and potential clinical translation.

Table 3. 4 Summary of recent label-free photonic biosensor for the detection of circulating biomarkers in LB applications, including their performance metrics [7].

Photonic Biosensor Technologies	LB Biomarkers	Performance	Ref.
1D defective ternary photonic crystal	CTCs (glioblastoma)	Bulk sensitivity = 4170.34 nm/RIU	[116]
SERS sensors	CTCs	LoD < 1 cell mL ⁻¹	[117]
SPR sensors	miRNA	LoD = 1 × 10 ⁻¹⁸ M	[118]
SPR sensors	Exosomes	LoD ranging from 5000 to 10,000 exosomes per μL	[119, 120]
LSPR	Exosome	Single exosome detection	[121]
WGM/PhC optical resonators	cfRNA	LoD = 10 × 10 ⁻¹² M	[122]
LSPR	cfRNA	LoD~10 × 10 ⁻¹⁵ M	[123]

3.2.2.1 Photonic Biosensors for detection of HER2

HER2 is a transmembrane tyrosine kinase receptor that is overexpressed in approximately 15% of breast cancer cases and is associated with aggressive disease progression and adverse clinical outcomes [124]. Although not currently recommended for guiding adjuvant therapy, circulating HER2 has emerged as a promising biomarker for disease monitoring, relapse detection, and evaluation of therapeutic response in advanced breast cancer. This clinical relevance has stimulated the development of photonic biosensing platforms specifically tailored for HER2 detection in biological fluids within the framework of liquid biopsy [125].

In this context, I. Petrova et al. [126] introduced EVA 2.0, a planar one-dimensional photonic crystal biosensor exploiting photonic crystal surface modes (PCSMs) for multiplexed, label-free detection of HER2 (Fig. 3.10). The device, fabricated on a BK-7 glass substrate with alternating SiO₂ (233.1 nm) and Ta₂O₅ (72.5 nm) layers deposited by magnetron sputtering, exhibited outstanding analytical performance, with a LoD of 630 fg/mL and a linear dynamic range up to 50 pg/mL. These results considerably outperformed conventional gold nanoparticle-enhanced SPR sensors. The PCSM architecture not only provided high sensitivity but also supported multiplexing on a single chip, establishing EVA 2.0 as a highly promising candidate for real-time clinical diagnostics.

While EVA 2.0 represents a significant advance in PCSM-based biosensing, recent innovations in SPR are also noteworthy. L. Rahmidar et al. [127] reported a label-free SPR biosensor functionalized with zirconium-based metal-organic frameworks (UiO-66 and UiO-66-NH₂) to enhance sensitivity and binding affinity toward HER2. The incorporation of MOFs improved surface functionalization and enabled an LoD of 0.457 ng/mL with UiO-66-NH₂, a value well below clinically relevant HER2 concentrations. Although this platform does not reach the ultra-low detection levels of EVA 2.0, it provides a favourable compromise between sensitivity, chemical stability and ease of integration, supporting its use in early breast cancer diagnostics where multiplexing is not essential.

Beyond PCSM and SPR platforms, optical fiber and metasurface-based approaches have also demonstrated considerable potential. W. Guo et al. [128] developed an immunosensor based on a S-tapered optical fiber (STF) coated with gold nanoparticles and graphene oxide for label-free HER2 quantification (Fig. 3.10). A distinctive feature of this design was the dual functionalization with monoclonal antibodies (mAbs) and nanobodies (Nbs), enabling comparative performance evaluation. The Nb-functionalized version achieved an LoD of 0.001 nM, markedly surpassing its mAb-based counterpart. These findings highlight the potential of nanobody-modified optical fibers, which combine high sensitivity with miniaturization and flexibility, making them highly suitable for PoC diagnostics.

Photonic crystal biosensors have also been explored for HER2 and CEA detection through refractive index modulation. A. Molaei- Yeznabad et al. [129] proposed a photonic crystal device exhibiting a sensitivity of 692.8 nm/RIU, a Figure of Merit (FOM) of 2561.5 RIU⁻¹, and a Q-factor exceeding 5870. This architecture provided remarkable spectral resolution and signal-to-noise performance. Compared with fiber-optic sensors, photonic crystal designs offer improved potential for chip-scale integration and cost-effectiveness, although their versatility in surface functionalization remains more limited than that of nanobody-based platforms.

In parallel, metasurface technologies operating in the terahertz (THz) regime have gained increasing attention. Q. Zeng et al. [130] reported an aptamer-modified THz metasurface biosensor composed of split-ring resonators (SRRs) with a 500 nm SiO₂ spacer layer to enhance the Q-factor (Fig. 3.10). This device achieved an LoD of 0.1 ng/mL for HER2, outperforming conventional silicon-based SRRs. Importantly, it also enabled the detection of additional biomarkers, such as CEA and alpha-fetoprotein (AFP), underscoring its multiplexing potential. Similarly, graphene-based metasurfaces have demonstrated high sensitivity in detecting circulating biomolecules and have proven particularly effective in early cancer detection and prognosis [131].

Building on these advances, S.K. Patel et al. [132] introduced a gold nano-octagonal metastructure integrated with graphene, designed to enhance optical absorption and biomolecular sensitivity. This biosensor could identify circulating cancer-related markers in human serum with high sensitivity to small concentration differences, reaching a maximum sensitivity of 800 GHz/RIU and an LoD of 0.328 RIU⁻¹. While this platform may not match the absolute LoD achieved by some nanobody-or aptamer-based sensors, its ability to detect even the smallest change in the refractive index makes it particularly suitable for diagnostic contexts where early deviations from physiological values are critical.

Overall, photonic strategies for HER2 detection span a wide range of architectures, each leveraging distinct physical principles. Rather than offering a universal solution, optimal sensor design requires a careful balance among sensitivity, specificity, multiplexing capacity, and integration potential. This convergence of approaches fosters both technological innovation and clinical applicability, reinforcing the role of photonic biosensing in precision oncology.

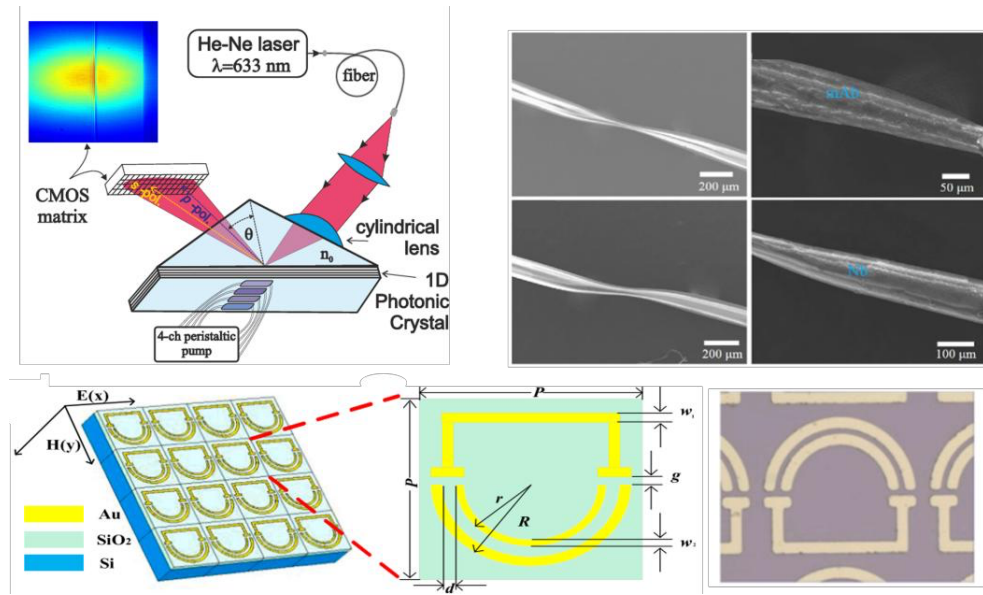


Fig. 3. 10 Schematic diagram of the EVA 2.0 sensor system (*top left*) [126]; SEM image sequence illustrating the evolution of the surface thin film (STF) morphology in mAb- and Nb-based immunosensors. Shown are the STF structures before and after antibody or nanobody functionalization in both sensor types (*top right*) [7].

3.2.2.2 Photonic Biosensors for detection of CEA

CEA is a glycoprotein widely recognized as a tumor-associated biomarker, with elevated serum concentrations frequently observed in several malignancies, including lung cancer [133]. Owing to its relevance in prognosis and therapeutic monitoring, CEA has become a primary target for the development of label-free, high sensitivity photonic biosensing platforms. Among these, surface plasmon resonance (SPR) and localized surface plasmon resonance (LSPR) architectures have been most extensively investigated for CEA quantification. Z. Ma et al. [134] developed a microfluidic-integrated LSPR biosensor featuring multiple sensing units functionalized with gold nanoparticles, achieving a detection range of 0.5-100 ng/mL and LoD below 1 ng/mL. Optimization of the system yielded an 85-fold increase in SNR, yielding ELISA-comparable performance and highlighting the suitability of LSPR for early diagnostic applications. Building on this, R. Li et al. [135] reported a streptavidin-modified SPR biosensor that incorporated gold nanoparticles in a sandwich configuration. This design enhanced both specificity and sensitivity, achieving an LoD of 1.0 ng/mL and a 13.8-fold improvement in sensitivity compared with nanoparticle-free configurations, while maintaining high selectivity against potential interfering biomarkers such as AFP and PSA.

Progress in nanomaterials has enabled novel SPR configurations with improved performance. A. Kumar et al. [136] proposed a graphene-enhanced multilayer SPR biosensor incorporating MXene and MoS₂ films between silver and graphene layers, which increased electromagnetic field confinement and yielded a sensitivity of 144.72 deg/RIU. This strategy illustrates how two-dimensional materials can be

exploited to boost the performance of conventional SPR systems. Waveguide-based biosensors also provide powerful alternatives. H. Mukundan et al. [137] developed a SiON_x planar waveguide biosensor capable of detecting sub-picomolar concentrations of CEA (< 0.5pM) in serum and nipple aspirate fluid (NAF). The device required minimal sample volumes (10 µL) and achieved high sensitivity through avidin-biotin biofunctionalization and SiO₂ surface coating, demonstrating the advantages of combining photonic integration with optimized surface chemistry for multiplexed and PoC applications.

Optical fiber platforms have further advanced CEA detection. Y. Wei et al. [138] reported an optical microfiber coupler (OMC)-based immunosensor integrated into a microfluidic chip, achieving an exceptionally low LoD of 34.6 fg/mL (0.475 fM). The system relied on a halogen lamp, microscope optics, and a 0.3 nm resolution spectrometer, ensuring precise spectral measurements across clinically relevant ranges. Similarly, W. Guo et al. [139] investigated graphene oxide (GO)- and gold nanoparticle (GNP)-functionalized optical fiber microstructures fabricated via a layer-by-layer assembly process (Fig. 3.11). These sensors achieved sensitivities of 15.1 nm/nM (GO) and 20.2 nm/nM (GNPs), with LoDs of 0.05 nM and 0.02 nM, respectively. The use of nanobodies instead of conventional antibodies improved selectivity and reduced response times, reinforcing the potential of miniaturized fiber-based sensors for rapid CEA detection.

Interferometric and metamaterial-based approaches have also been explored. L. Chen et al. [140] reported an open-cavity Fabry-Pérot interferometer (FPI) enhanced by the Vernier effect and functionalized with anti-CEA antibodies (Fig. 3.11). The device achieved an LoD of 36.14 fg/mL, with high stability, reproducibility, and specificity, and response times under 30 minutes. Complementarily, Q. Niu et al. [141] introduced a terahertz (THz) metamaterial biosensor integrating gold nanoparticles to exploit toroidal electromagnetic modes (Fig. 3.11). This sensor achieved a sensitivity of 287.8 GHz/RIU, a Q-factor of 15.04, and an LoD of 0.17 ng, establishing a robust platform for CEA analysis. Expanding on this direction, S. Geng et al. [142] proposed a photonic metamaterial platform integrating near-field enhancement and wavelength selectivity for minimally invasive detection of CEA, CA125, and CYFRA21-1 in interstitial fluid (Fig. 3.11). Although fabrication complexity remains a limitation, the system outperformed conventional sensors in both sensitivity and resolution, offering a scalable route for early cancer diagnostics.

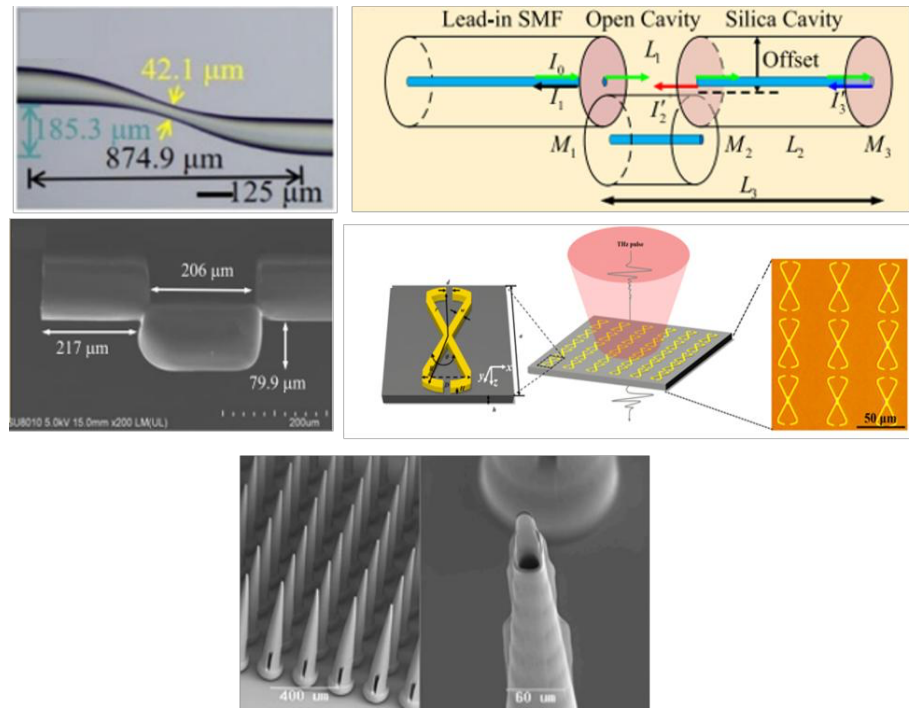


Fig. 3.11 Optical microscope image of fiber immunosensor (top left); schematic representation of the toroidal metasurface and a microscope image of the fabricated toroidal unit cells (top right); SEM images of microneedle array (bottom centre) [7].

Collectively, these advances underscore the versatility of photonic biosensors for CEA detection. While LSPR and SPR platforms remain the most established, the integration of nanomaterials, waveguides, interferometric methods, and metamaterials provides complementary pathways. Each approach is strategically optimized to balance sensitivity, specificity, response time, and integration potential, reflecting the clinical and technological diversity required for effective cancer diagnostics.

3.2.2.3 Photonic Biosensors for detection of PSA

PSA remains the most widely adopted molecular biomarker for prostate cancer (PCa) screening; however, its limited specificity often results in false positives, unnecessary biopsies, and overdiagnosis [143]. To overcome these limitations, a variety of photonic biosensing platforms have been developed to improve the analytical performance of PSA detection within the framework of LB.

Among plasmonic approaches, M. Mahani et al. [144] demonstrated a gold nanoparticle (GNP)-based biosensor capable of detecting ultralow PSA concentrations in serum by exploiting a colorimetric shift caused by nanoparticle aggregation. The sensor, with a sensitivity (S) of 43.75 nm/ng·mL⁻¹, relied on LSPR redshift phenomena arising from refractive index variations upon antigen-antibody binding. Although effective, this method may be constrained in terms of multiplexing and real-time applicability. A more advanced SPR-based strategy was

introduced by R. Li et al. [135], who incorporated streptavidin-modified GNPs (SA-GNPs) in a sandwich configuration. While their study focused on CEA, the system achieved a 13.8-fold improvement in sensitivity compared to conventional formats and exhibited no cross-reactivity with PSA, indicating potential adaptability for PSA assays.

Resonance-enhanced photonic sensors have also emerged as competitive alternatives. X. Dai et al. [145] developed a dual-resonance lossy mode resonance (LMR) immunoprobe by integrating MgF_2 and indium tin oxide (ITO) layers to optimize phase matching and resonance quality. The device achieved a sensitivity of 206.457 nm/RIU and a LoD of 52 pg/mL, combined with a 90% diagnostic recognition rate in clinical samples. These results highlight the utility of dual-resonance schemes for high-precision and portable PCa screening. Fiber-optic biosensors have likewise demonstrated strong potential. A. Z. M. Zamri et al. [146] reported a D-shaped, gold-coated fiber laser biosensor with a sensitivity of 0.2171 nm/($\mu\text{g/mL}$) for PSA. The platform, integrated with a 980 nm laser diode and a WDM unit monitored through an Optical Spectrum Analyzer (OSA), illustrated the feasibility of compact, real-time diagnostic systems (Fig. 3.12).

Complementarily, M. Shokorlou et al. [147] proposed a metal-insulator-metal (MIM) nanoring sensor, achieving a sensitivity of 567.23 nm/RIU and a FOM of 3.72. Its miniaturized and responsive format underscored the suitability of nanophotonic platforms for integrated biosensing. Similarly, T. Zhang et al. [148] developed an array nanostructured FPI microchip for label-free free PSA (f-PSA) detection, reaching an LoD of 10 pg/mL. By tuning antibody immobilization, the sensor offered a customizable dynamic range, allowing tailored analytical performance across clinical scenarios.

In addition to plasmonic and interferometric systems, dielectric metasurfaces have recently gained traction for PSA detection due to their high spectral resolution and compatibility with LoC integration. O. Yavas et al. [149] proposed two silicon metasurface platforms that combined nanophotonic precision with microfluidic compatibility. The first design, fabricated via EBL, employed silicon nanoantenna arrays operating in the near-infrared and integrated into an LoC system with eight independent sensing channels (Fig. 3.12). This platform achieved a bulk sensitivity of 227 nm/RIU, and FOM of 5.4 RIU⁻¹, and an LoD of 0.69 ng/mL, within clinically relevant ranges for PSA detection in serum. Despite its high performance, reliance on EBL limits large-scale manufacturability.

To address this, the authors also developed a cost-effective metasurface fabricated by colloidal lithography, consisting of Si nanocylinders exhibiting Mie resonances at 900 nm in aqueous environments (Fig. 3.12). This system achieved a sensitivity of 86 nm/RIU and relied on both resonance redshift and extinction reduction for signal transduction [150].

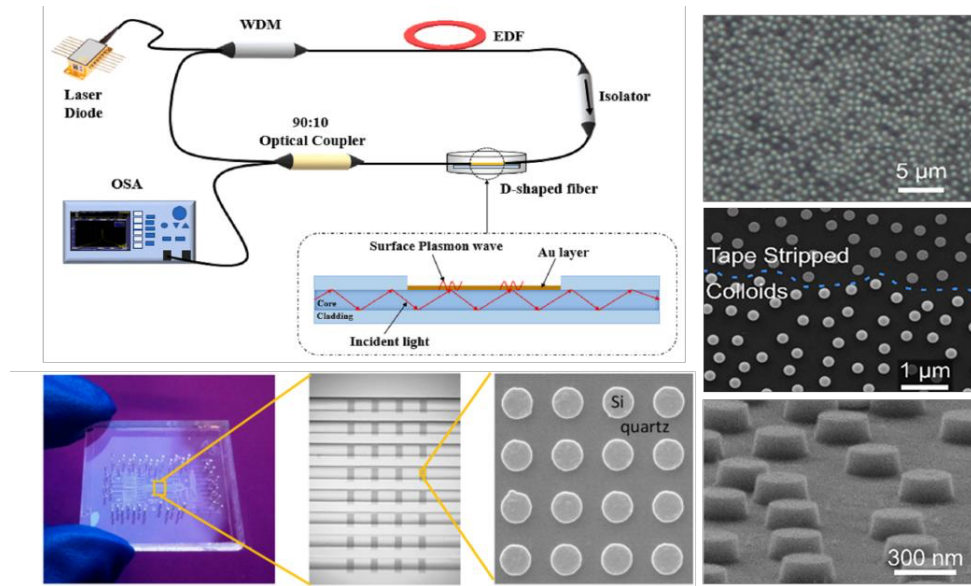


Fig. 3.12 Schematic diagram of fiber laser-based biosensor system [138] (top left); biosensing system based on refractometric method that presents eight flow channels integrated with silicon nanodisk array (bottom left); SEM microscope image of nanocylinders before and after substrate cleaning with piranha solution (bottom centre) [149]; SEM images of the silicon nanodisk (bottom right); SEM images of the arrays of silicon nanocylinders (right) [150] [7].

Although it offered a lower Q-factor compared to the EBL-fabricated platform, it demonstrated competitive LoD values and suitability for scalable production. Collectively, these studies illustrate the breadth of photonic strategies for PSA detection, ranging from plasmonic and interferometric devices to waveguide-based and metasurface architectures. Each platform presents specific trade-offs in sensitivity, specificity, multiplexing capability, and integrability. By strategically balancing these factors, photonic biosensors provide a means of addressing the long-standing challenges of PSA testing, ultimately improving diagnostic precision and reducing overdiagnosis and unnecessary interventions in prostate cancer screening.

3.2.2.4 Photonics biosensor platform for detection of IgG

The early detection of cancer through liquid biopsy can be facilitated by targeting specific protein biomarkers such as immunoglobulin G (IgG) [151]. A wide range of biosensing strategies has been explored for this purpose, including electrochemical methods, imaging-based approaches, and photonics technologies. Among these, photonic biosensors offer distinct advantages, notably label-free detection, real-time monitoring, high sensitivity, and potential for device miniaturization and multiplexing. These systems rely on the modulation of an optical mode by changes in the refractive index near the sensor surface, thereby enabling direct and quantitative interaction analysis. To enhance sensitivity and specificity, several optical configurations have been proposed, including surface plasmon resonance (SPR), optical fibres, integrated waveguides, and metasurface-based platforms.

Metasurfaces have gained momentum in recent years owing to their strong field confinement, ease of mode excitation, and adaptability to various transduction schemes. However, despite their high refractive index sensitivity, their effective implementation in practical biosensing scenarios often suffers from fabrication complexity and the need for precise nanostructuring. Integrated photonic waveguide-based devices, such as ring resonators, represent another well-established architecture [152]. These devices confine the optical mode either in the core or the cladding, depending on the polarization (TE or TM), and can exhibit strong light-matter interaction in compact geometries. For instance, Whispering Gallery Mode (WGM) resonators have also been applied to IgG detection using protein G-coated microbeads, which enable oriented antibody immobilization and real-time monitoring of binding events. This configuration was validated with therapeutic antibodies (Cetuximab, Atezolizumab) and their Fc-fusion ligands (EGFR-Fc, PD-L1-Fc), achieving selective detection with equilibrium dissociation constants (KD) in the low nanomolar range and kinetic parameters in good agreement with reference SPR measurements [153]. Nevertheless, the sensitivity of these devices remains limited by the modal overlap with the analyte, prompting further developments in nanophotonics architectures. Photonic crystal (PhC) waveguide-based sensors represent a significant advancement in this regard, offering enhanced field-analyte overlap and subwavelength confinement. A notable example includes a PhC nanocavity biosensor specifically engineered for label-free, error-corrected detection of IgG [154]. The system operates by monitoring resonant wavelength shifts in nanocavities side-coupled to a silicon PhC waveguide. Finite difference time domain (FDTD) simulations were employed for design optimization, and standard nanolithographic techniques were used for fabrication. Experimental results demonstrated a refractive index sensitivity of 10^{-2} RIU and a LoD down to 1.5 femtograms, with an IgG sensitivity of $2.3 \pm 0.24 \times 10^5$ nm/M, among the highest reported for PhC-based platforms [154]. Nonetheless, these systems still require complex alignment and cleanroom fabrication protocols, which can limit their scalability and robustness for PoC use. Alternative strategies have investigated the integration of SPR with fiber optic platforms, as exemplified by a compact SPR biosensor based on a Tilted Fiber Bragg Grating (TFBG) [155]. The device is co-functionalized with graphene oxide and staphylococcal protein A to enable oriented IgG immobilization, thereby enhancing binding efficiency. It achieves a sensitivity of 0.096 dB ($\mu\text{g/mL}$) and a LoD of 0.6 $\mu\text{g/mL}$. While offering the advantage of easy integration into flexible probe geometries, fiber-based configurations typically exhibit lower surface sensitivity compared to planar photonic counterparts and are often affected by mechanical instability during operation. More recently, photonics biosensors based on 1D PhC supporting Bloch Surface Waves (BSWs) have gained attention due to their high surface sensitivity, easy functionalization processes, and multiplexing capabilities [156]. A recent implementation enabled real-time, parallel monitoring of antibody interactions with a LoD of 0.19 nM for anti-human IgG and the extraction of kinetic constants.

Another notable contribution to fiber optic SPR biosensing exploits a gold-coated TFBG functionalized with a composite of 2H-MoS₂ nanosheets and L-cysteine [157]. The use of 2D materials increases surface area and enhances the stability of antibody immobilization, enabling higher capture efficiency of anti-human IgG. The device reports a sensitivity of 0.11 dB (ng/mL), a response time of 220 s and a LoD of 0.87 ng/mL, surpassing prior fiber-based SPR designs. In addition to plasmonic and waveguide-based architectures, dielectric metasurfaces have recently emerged as a promising class of photonic platforms for biosensing, offering a compelling trade-off between spectral resolution and field confinement. These structures exploit high-Q Fano resonances and tailored spatial confinement to enhance both sensitivity and spatial precision. A representative implementation features a nanohole array patterned in amorphous silicon, designed to support dual optical modes with sharp resonances. This metasurface demonstrated a LoD as low as 1 pg/mL for IgG, along with sub-micrometric spatial resolution capable of resolving individual *E. Coli* bacteria [158].

In summary, while photonic biosensors offer significant advantages for IgG detection, each configuration presents specific strengths and limitations. Planar photonic crystal platforms and WGM resonators ensure high sensitivity and compactness but are constrained by demanding fabrication and alignment protocols. Fiber-optic SPR devices, though flexible and integration-friendly, generally exhibit lower surface sensitivity and reduced robustness. Metasurfaces, particularly dielectric configurations, stand out for their high Q-factors, tunable near-field properties, and compatibility with complex optical functions such as imaging and multiplexing. However, their implementation still requires advanced nanofabrication and remains sensitive to structural deviations.

In this landscape, the two photonic biosensors developed and analysed in the following sections (3.2.2.6 and 3.2.2.7) have been designed for label-free detection of IgG, strategically addressing the challenges.

3.2.2.5 Comparative analysis of state-of-the-art photonics biosensing platforms

Table 3.5 provides a comparative summary of the photonic biosensing technologies discussed, highlighting their principal features and analytical performance metrics.

Such a comparison facilitates the evaluation of both diagnostic potential and technological suitability for clinical implementation. It is important to note that no single biosensing approach demonstrates superiority across all performance indicators. Instead, each platform presents distinct strengths and limitations that must be carefully balanced in relation to the clinical scenario and the biomarker of interest. Optical fiber- and waveguide-based biosensors stand out for their ultralow limits of detection, rapid response times, and high analytical accuracy, features that position them as particularly attractive candidates for PoC applications and early-stage cancer diagnostics.

Table 3. 5 Comparative summary of photonic biosensing technologies for cancer biomarker (HER2, CEA, PSA, and IgG protein).

Biomarker	Technology	Biological Sample	Performance	Notes	Ref.
HER	SPR	Blood serum	LoD = 630 fg/mL	Multiplexing, real-time detection	[126]
	SPR	Blood serum	S = ng/mL LoD = 0.457 ng/mL	Multiplexing	[127]
	Optical fiber/WG	Blood serum	LoD = 0.001 nM	Extremely high S	[128]
	Optical resonator (photonic crystal)	Suspension of normal and cancerous cells	S = 692.8 nm/RIU	Q-factor = 5870 FOM = 2561.5 RIU ⁻¹ Robust and cost-effective platform	[129]
	Metasurface	HER standard solution	LoD = 0.1 ng/mL	Higher S compared to conventional SRRs, high versatility	[130]
CEA	LSPR	Human serum	S = 85 × SNR LoD < 1 ng/mL	Integrated with microfluidics, with the use of GNPs	[134]
	SPR	Human serum	S = 13.8× with respect to std configuration LoD = 1.0 ng/mL	Good selectivity (no interference from other tumor biomarkers)	[135]
	SPR	CEA standard solution (PBS solution)	S = 144.72 deg./RIU	Integrates MXenes and MoS2 between graphene and silver layers	[136]
	Optical fiber/WG	Human serum/NAF	LoD < 0.5 pM	High S, reaction time < 15 min, requiring 20 µL of NAF	[137]
	Optical fiber/WG	Human serum	LoD = 34.6 fg/mL (0.475 fM)	High S and wide dynamic range	[123]
	Optical fiber/WG	Human serum	S = 20.2 nm/nM (GNPs) S = 15.1 nm/nM (GO) LoD = 0.02 nM (GNPs) LoD = 0.05 nM (GO)	High specificity and rapid response	[139]
	Optical resonator (Fabry-Perot)	No real fluid (purified CEA)	LoD = 36.14 fg/mL	Excellent stability, reproducibility, specificity, S, and time response < 30 min	[140]
	Metasurface	No real fluid (purified CEA)	S = 287.8 GHz/RIU LoD = 0.17 ng	Q-factor = 15.04 Excellent specificity	[141]
	LSPR	Human serum	S = 43.75 nm/ng·mL ⁻¹ LoD = ng/mL	With the use of GNPs	[144]
PSA	Optical fiber/WG	Human serum	S = 206.657 nm/RIU LoD = 52 pg/mL	High precision, 90% diagnostic rate	[145]
	Optical fiber/WG	Human serum	S = 0.2171 nm/(µg/mL)	Good sensibility	[146]
	Optical resonator (WGM)	Standard solutions in water	S = 567.23 nm/RIU	FOM = 3.72; compact platform	[147]
	Metasurface	Human serum	S = 227 nm/RIU LoD = 0.69 ng/mL	High integrability FOM = 5.4 RIU ⁻¹	[149]
	Metasurface	Human serum	S = 86 nm/RIU	Excellent S and a favorable LoD	[150]

IgG	nanocavity PhC	Human serum	S = 10-2 RIU LoD = 1,5 fg	Among the highest values reported for PhC-based platforms.	[154]
	SPR	Human serum	S = 0.096 dB (μg/mL) LoD = 0,6 μg/mL	Compact SPR biosensor based on Tilted Fiber Bragg Grating (TFBG) with high sensitivity.	[155]
	1D PhC	Human serum	LoD = 0,19 nM	High sensitivity photonic biosensors based on one-dimensional photonic crystals supporting Bloch Surface Waves (BSWs).	[146]
	Optical resonator (WGM)	Standard solutions in water	S = 0,11 dB (ng/mL)	Resonance time of 220 s	[157]

3.2.2.6 High resolved near-field sensing by means of dielectric grating with a box-like resonance shape

In the context of evanescent-field biosensing, significant advances have been reported with interferometric platforms such as BiModal Waveguide (BiMW) devices and Guided-Mode Resonance (GMR) sensors. BiMW interferometers exploit the interference between TE and TM modes to enhance the analyte-mode interaction, achieving sensitivity values up to 1350 nm/RIU and LoD as low as 2×10^{-5} RIU, with good robustness and compactness [159, 160]. Their performance has been further improved through the integration of subwavelength gratings or photonic crystal structures, although at the expense of increased footprint and more complex excitation conditions, which can reduce modulation depth and complicate peak detection [161]. GMR sensors, based on the simultaneous excitation of orthogonally polarized modes, have also demonstrated promising sensitivities (289 π/RIU) and ultralow LoD ($\approx 1.8 \times 10^{-6}$ RIU), though their operation is highly susceptible to environmental noise [162, 163]. Despite these achievements, the adoption of such resonant devices in LoC applications remains constrained by their Lorentzian spectral response. Under single-wavelength interrogation, commonly employed in integrated platforms, small analyte-induced shifts in resonance wavelength often translate into negligible transmission variations, increasing the likelihood of crosstalk and misdetection [164]. This limitation arises from the inherently slow roll-off of Lorentzian resonances, which reduces their ability to resolve subtle concentration changes.

To overcome this fundamental constraint, a novel biosensor has been developed on an SOI platform, exploiting an engineered sidewall dielectric grating to generate a box-like resonance profile [165]. By introducing carefully designed defects that form coupled Fabry-Perot cavities, the device reshapes the resonance into a flat-top

response with steep roll-off, thereby ensuring a pronounced amplitude change even for small refractive index perturbations. Numerical simulations demonstrate a high Q-factor exceeding 10^4 , an extinction ratio ER above 15 dB, and a bulk sensitivity of 490 nm/RIU, among the highest values reported for dielectric resonant sensors. When functionalized with anti-IgG antibodies, the biosensor exhibits measurable red shifts upon IgG binding, accompanied by transmission changes greater than 4 dB for concentrations ranging from 1 pg/mL to 500 pg/mL. These features highlight the potential of the box-shaped resonance to overcome the intrinsic limitations of Lorentzian devices, enabling highly resolvable and reliable protein quantification in compact LoC diagnostic systems for liquid biopsy applications.

3.2.2.6.1 Proposed device and simulation methodology

The device was designed on an SOI platform and relies on a rib waveguide incorporating a sidewall grating (Fig. 3.13). The structure is implemented by periodically modulating the waveguide width, alternating between a maximum ($w_{\max}$) and a minimum ($w_{\min}$) value, and is characterized by a period (Λ), duty cycle (DC), and a total number of semi-periods (N_t).

The unmodified rib waveguide has a thickness of 220 nm and a width of 450 nm, dimensions that provide low propagation losses (<1 dB/cm at 1550 nm) and guarantee single-mode operation, thereby avoiding issues related to mode overlap [166]. In the biofunctionalized device configuration, the sensing region is coated with a thin layer of chemical and organic compounds, including immobilized antibodies, covering the entire surface of the grating. This approach has been widely adopted and shown to yield strong agreement between theoretical simulations and experimental validation [167,168-170]. To evaluate the sensing performance and investigate the device behaviour, numerical simulations were carried out using COMSOL Multiphysics®, a software platform based on the Finite Element Method (FEM). Details of the implementation and main software features are provided in [Appendix A](#).

The choice of $w_{\max}$ and $w_{\min}$ was optimized to preserve single-mode behaviour while preventing an increase in optical losses. Specifically, $w_{\max} = 500$ nm was selected to avoid the excitation of higher-order modes, whereas $w_{\min} = 400$ nm ensures structural symmetry with respect to the unperturbed waveguide ($w_{\max} - w_{\text{wg}} = w_{\text{wg}} - w_{\min}$), thereby reducing the additional propagation losses introduced by the grating. Within a modulation depth in the range 10-80 nm, the propagation losses remain essentially independent of the grating width ($w_{\max} - w_{\text{wg}} / w_{\text{wg}} - w_{\min}$) [172].

Electromagnetic field simulations further indicate that the TM_0 mode is less confined within the silicon core compared to the TE_0 mode (Fig. 3.14). As a result, the TM_0 field exhibits stronger interaction at the solution-silicon interface, while its propagation losses are lower, since the field predominantly couples with the vertical surfaces rather than with the sidewalls [166].

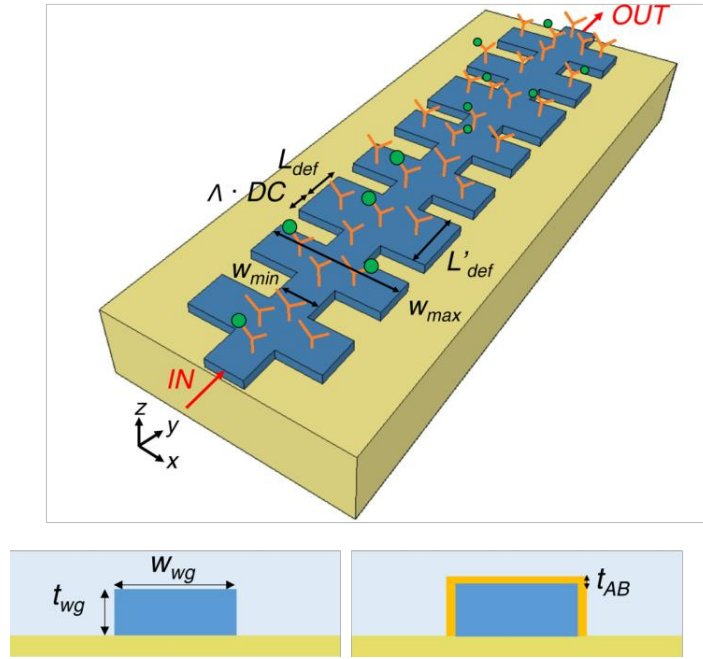


Fig. 3.13 Sidewall grating waveguide with deposited antibodies (labelled as orang Y) and target analytes (green dots) (*top*); cross-section of the rib waveguides without antibody layer (*bottom left*) and with deposited antibody layer (*bottom right*) [171].

Based on the calculated effective indices (n_{eff}) for both grating sections, the optimal design parameters were defined as $\Lambda = 465$ nm and $DC = 50\%$, ensuring resonance operation close to 1550 nm. Overall, this design strategy combines low-loss light propagation with enhanced evanescent-field interaction at the functionalized surface, thereby establishing a robust photonic platform for label-free IgG protein detection within LoC systems.

The transformation of the resonance from a Lorentzian to a box-like profile is enabled by the introduction of engineered defects within the sidewall grating (Fig. 3.15). Each defect, with length L_{def} and width equal to w_{max} , is placed in cascade after a sequence of semi-periods of length $DC \cdot \Lambda$.

The inclusion of multiple defects generates a set of coupled FP cavities. Light propagating along the grating can successively couple from one cavity to the next, producing a transmission spectrum characterized by multiple ripples whose number corresponds to the number of inserted defects. This behaviour is linked to the interactions between the evanescent cavity modes.

To achieve the desired box-like spectral envelope, the defect length L_{def} and the extended defect length L'_{def} were designed to introduce a phase shift of $\pi/2$ at the Bragg wavelength λ_B [173].

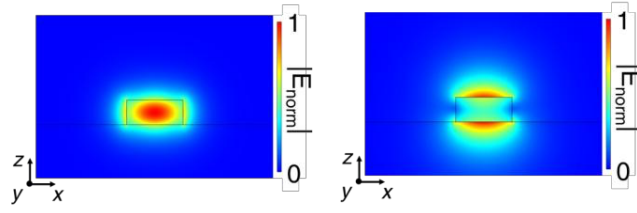


Fig. 3. 14 Electric field distribution of TE ($n_{\text{eff}} = 2.42883$) (left) and TM ($n_{\text{eff}} = 1.70525$) (right) modes at 1550 nm, achieved by FEM simulations [171].

The conditions are expressed as

$$L_{\text{def}} = \frac{4m + 1}{4} \cdot \frac{\lambda_B}{n_{\text{eff-def}}}, \quad m = 1, 2, \dots$$

$$L_{\text{def}}' = DC \cdot \Lambda + L_{\text{def}}$$

Where $\lambda_B \approx 1550$ nm is the Bragg wavelength, m denotes the grating order, and $n_{\text{eff-def}}$ is the effective refractive index of the defect region (1.66381 at 1550 nm). The transmission spectra of the proposed devices were analysed using a custom numerical model combining the Effective Index Method (EIM) [174] and a two-dimensional Transmission Matrix Method (TMM) [175]. The EIM simplified the modelling of the waveguide geometry by reducing the 3D structure into an equivalent 2D cross-section, allowing the extraction of the effective refractive index of the guided modes by solving the wave equation in the transverse plane. The n_{eff} can assume complex values, thereby accounting for both phase propagation and possible modal attenuation arising from losses or evanescent contributions. The Transmission Matrix Method, on the other hand, is particularly suited for structures composed of repeating segments. In this framework, each uniform section of the grating and the defect region is represented by a transfer matrix, and the overall device response is obtained by cascading these matrices. This approach significantly reduces computational cost while retaining the essential physics of wave propagation and coupling within the periodic structure. Within this modelling strategy, each grating semi-period and defect was characterized through its S-parameters. In line with common practice [176, 177], a conservative propagation loss of 10 dB/cm was assumed, accounting for reflections, evanescent coupling, surface roughness, and fabrication tolerances. Defects were inserted within the sidewall grating according to a distribution based on Newton's binomial series [173]. This design yields a set of resonances centred at the Bragg wavelength, producing an overall flat-top spectral envelope resembling a box-like shape. The main grating parameters, order (m), number of defects (N_{def}), and total number of semi-periods (N_t), were optimized through iterative simulations to simultaneously maximize roll-off, extinction ratio (ER), and Q-factor, while preserving the box-like resonance. To quantitatively assess the quality of this spectral shape, the Coincidence Ratio (CR) was defined as the ratio between the area under the actual

transmission curve within the full width at half maximum (FWHM) and that of an ideal box-shaped with the same amplitude. Values above 70% were considered as good indicators of box-like behaviour [178]. Table 3.6 summarized all the grating performance, in terms of Q-factor, ER, CR, and roll-off. As an initial design condition, $N_t = 128$ and $N_{def} = 2$ were adopted. The analysis of the transmission spectra shows that increasing m improves both Q-factor and roll-off but reduces ER and leads to random resonance shifts (Fig. 3.15). These shifts are attributed to rounding of the defect length, which causes red or blue shift of the resonance. A value of $m = 100$ (corresponding to $L_{def}' = 91 \text{ } \mu\text{m}$) was selected as the best compromise, yielding a Q-factor of approximately 1.2×10^4 , compact footprint, and preservation of the box-like profile. Variation of N_t (Fig. 3.15) confirmed that longer gratings improve Q-factor and roll-off in agreement with Fabry-Perot theory [179], but at the expense of reduced ER and higher losses. Consequently, $N_t = 128$ was identified as the most suitable value, balancing Q-factor enhancement with the preservation of the box-like spectral shape. The impact of the number of defects is illustrated in Fig. 3.15. Increasing N_{def} enhances the flatness of the resonance envelope but also reduces the Q-factor while increasing ER. Even numbers of defects produce symmetric structures (e.g. $N_{def} = 2$ with $N_t = 128$), resulting in paired FP cavities that generate symmetrical resonances and a quasi-flat-top profile. Conversely, odd numbers of defects yield asymmetric configurations, leading to non-identical resonances and lower CR values. Based on this parametric study, the configuration with $N_t = 128$, $N_{def} = 2$ and $m = 100$, corresponding to a total grating length of $212 \text{ } \mu\text{m}$, was selected as the optimal design. This geometry guarantees a Q-factor $\approx 1.2 \times 10^4$, ER = 15.35 dB, roll-off = -10.50 dB/oct. , and CR = 74.06%, confirming the achievement of a stable and highly resolvable box-like resonance (Fig. 3.16).

Table 3. 6 Performance comparison by varying m , N_t , and N_{def} [171].

m	N_t	N_{def}	Q-factor	ER (dB)	CR (%)	Roll-off (dB/oct.)
5	128	2	541	27.79	74.71	-11.16
10	128	2	1,218	34.96	74.80	-11.02
50	128	2	2,981	29.32	74.62	-10.97
100	128	2	11,994	15.35	74.06	-10.50
150	128	2	8,334	31.01	74.18	-10.73
100	32	2	707	8.71	73.92	-5.67
100	64	2	1,144	21.15	74.38	-10.11
100	128	2	11,994	15.35	74.06	-10.50
100	256	2	128,531	5.49	63.48	-6.97
100	128	1	77,386	31.76	57.23	-4.70
100	128	2	11,994	15.35	74.06	-10.50
100	128	3	3,398	25.07	63.95	-7.91
100	128	4	2,203	24.28	74.96	-11.06
100	128	5	1,659	27.55	73.02	-11.71

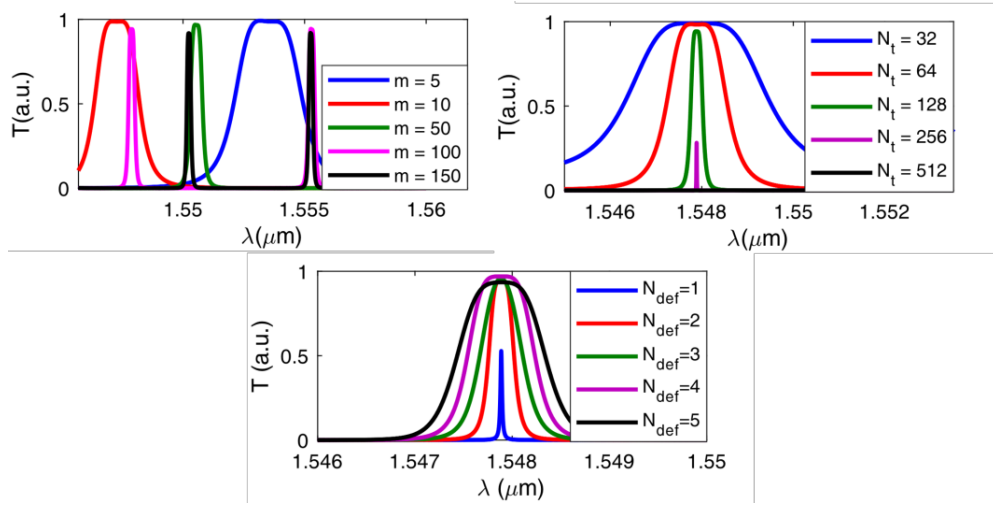


Fig. 3. 15 Transmission spectra of the waveguide by varying m ($N_t=128$, $N_{def}=2$) (top left), (top right) N_t ($m=100$, $N_{def}=2$), and (bottom) N_{def} ($m=100$, $N_t=128$) [171].

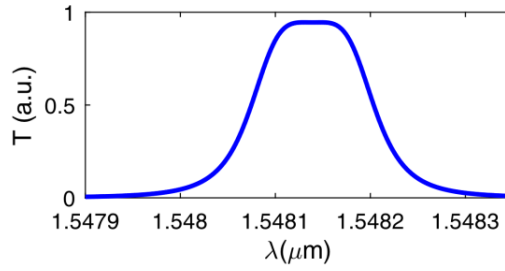


Fig. 3. 16 Transmission spectra of the design grating with $N_t=128$, $N_{def}=22$, $m=100$ ($L_{def}=91\mu m$) and a total length of $212\mu m$ [171].

3.2.2.6.2 Results and Biosensor performance

To enable the detection of protein in biological and aqueous solutions, the sensor surface was functionalized by depositing an anti-IgG layer across the entire grating area. Following the approach described in the literature [165], the antibody coating was modelled as a SiO_2 layer with thickness $t_{AB}=10$ nm and refractive index 1.45, thereby reproducing the typical characteristics of IgG/anti-IgG binding. In this configuration, variations in IgG concentration were assumed to modify the refractive index of the layer without altering its thickness, since the antibody film intrinsically accounts for the anti IgG- IgG binding distance [165, 179]. As illustrated in Fig. 3.17, the strong confinement of the electromagnetic field within the 10 nm-thick functionalized layer ensures high sensing efficiency. Notably, the evanescent field reaches values as high as $\approx 98\%$ in the region where the binding takes place (thickness range 110-120 nm). Although the proposed device was conceived for single-wavelength interrogation, i.e., monitoring amplitude variations at a fixed wavelength, its performance was also evaluated in terms of resonance wavelength shift ($\delta\lambda_{RES}$), which is one of the most widely adopted metrics in biosensing.

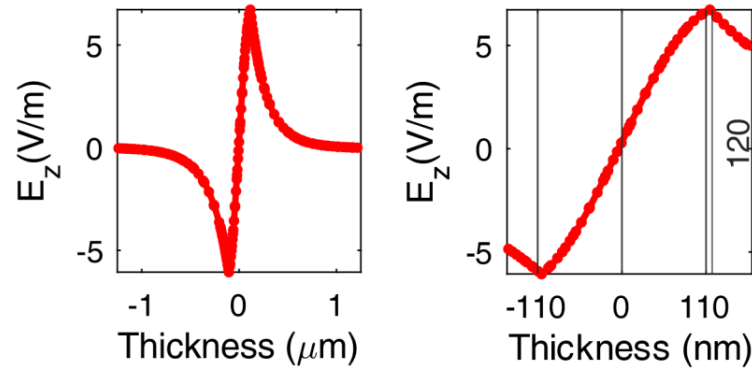


Fig. 3. 17 TM mode electric field z-component versus waveguide thickness (*left*); magnificient of the core region (*right*). The zero position is defined at the center of the waveguide core [171].

The functionalization process induces a redshift of $\delta\lambda_{\text{RES}} = 2.83$ nm, as reported in Fig. 3.18. Based on this shift, the surface sensitivity S was estimated as the ratio between the wavelength shift $\Delta\lambda$ caused by the 10 nm SiO_2 deposition and the effective index change Δn from the water background (bare case, Fig. 3.13 *bottom left*) to the functionalized configuration (Fig. 3.13 *bottom right*). A surface sensitivity of 21.47 nm/RIU was obtained, a value comparable with that of plasmonic architectures ($\approx 30\text{nm/RIU}$ for a 10 nm layer [180]), though accompanied by greater amplitude responses than conventional plasmonic sensors.

The bulk sensitivity S_λ , commonly employed for performance evaluation in the literature, was calculated as 490.49 nm/RIU. However, both surface and bulk sensitivities only quantify the evanescent field interaction with the analyte and do not account for the resonance profile. To integrate both aspects, the FOM was introduced, defined as $\text{FOM} = S_\lambda / \text{FWHM}$, where FWHM represents the resonance linewidth. This parameter provides a more comprehensive assessment of the biosensor's performance.

For conventional SPR-based sensors, reported FOM values typically range between 75 and 150 RIU^{-1} [181, 182]. Further improvements in resonance linewidth (FWHM < 1 nm) observed in photonic crystal (PhC) cavities have enabled even larger FOM values, spanning 525 - 1559 RIU^{-1} , substantially outperforming previously reported platforms. This outstanding performance arises from the combination of very high bulk sensitivity and an exceptionally narrow resonance linewidth (FWHM ≈ 126 pm). To further characterize the sensing capabilities, the effect of varying protein concentration on the resonance wavelength was analysed to estimate both resolution and dynamic range. The antibody-protein layer thickness was fixed at 10 nm, consistent with the comparable dimensions of free and bound antibodies. According to the experimental evidence in [181], protein concentrations of 1 pg/mL and 1 ng/mL correspond to refractive index variations of 1×10^{-4} RIU $^{-1}$ and 1×10^{-3} RIU $^{-1}$, respectively.

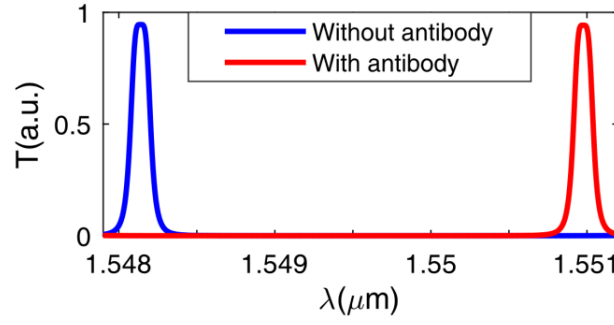


Fig. 3. 18 Spectrum of the designed device before (blue curve) and after (red curve) deposition of antibody layer [171].

As shown in Fig. 3.19, the resonance wavelength shift $\delta\lambda_{\text{RES}}$ increases with protein concentration, starting from the reference resonance of the functionalized but unbound surface. The dependence follows an exponential trend, reflecting the effective refractive index variation in the grating sub-sections. Within the sub-nanogram concentration range (up to 500 pg/mL), the resonance shift reaches 14.46 pm, confirming the high resolution of the sensor in detecting extremely low analyte concentrations.

To validate the modelling assumptions, additional FEM simulations were performed to examine the impact of different functionalization layer distributions. Specifically, the effective refractive index variation under TM polarization was evaluated for two cases: (i) a protein layer uniformly coating the entire waveguide (Fig. 3.20 *top left*), and (ii) a protein layer deposited solely on the top surface (Fig. 3.20 *top right*). The comparison in Fig. 3.20 *bottom* demonstrates similar responses for the two configurations, confirming the robustness of the initial assumption. Nonetheless, covering the entire waveguide surface is preferable, as it enhances interaction with the evanescent field and maximizes the sensing area.

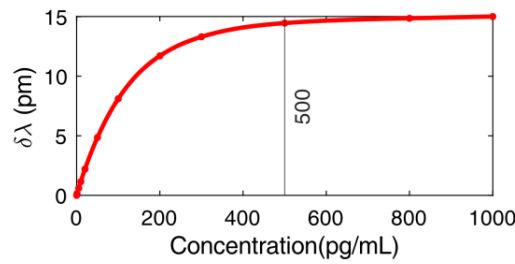
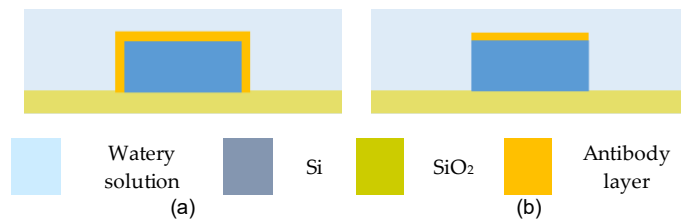


Fig. 3. 19 Resonance wavelength shift ($\delta\lambda_{\text{RES}}$) as a function of antibody concentration [171].



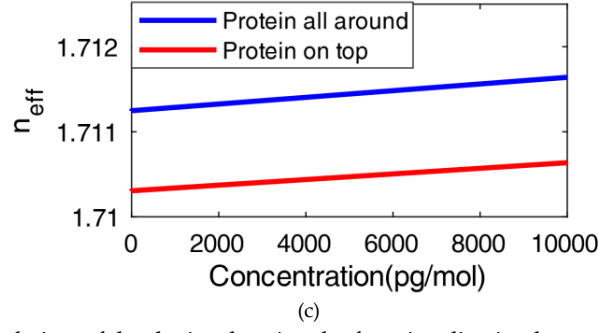


Fig. 3. 20 Cross- sectional view of the device showing the functionalization layer distribution: surrounding the entire waveguide (*top left*) and limited to the top surface (*top right*). Variation of the TM₀ mode effective refractive index as a function of antibody concentration for the two configurations (*bottom*) [171].

In practical scenarios, biosensors are typically interrogated using a single-wavelength laser, where the analyte concentration is inferred from variations in transmitted or reflected power [183]. Nevertheless, when the resonance shift is large, the ability to resolve subtle changes in transmission may be compromised, often resulting in significant crosstalk, a limitation commonly observed in biosensors with a Lorentzian resonance profile. The proposed device overcomes this drawback thanks to the combination of a pronounced resonance shift ($\delta\lambda_{\text{RES}}$) and a steep roll-off.

To validate this advantage, a performance analysis was carried out against a biosensor with a Lorentzian-shape resonance, designed to achieve comparable Q-factors and bulk sensitivity (S_{λ}). Specifically, a sidewall grating, fabricated with the same technological approach, was implemented using parameter $N_t = 64$, $N_{\text{def}} = 1$, and $m = 100$. This design resulted in a resonance with $Q \approx 8.9 \times 10^3$ and extinction ratio $ER \approx 16$ dB. As shown in Fig. 3.21, the Lorentzian resonance exhibits a roll-off nearly twice as steep as that of the proposed box-like profile (-10.50 dB/oct. vs -5.85 dB/oct), directly reflecting its restricted ability in discriminating analyte concentration changes.

By fixing the operating wavelength at the centre of the left resonance edge ($\lambda = 1550.92$ nm), the proposed box-like biosensor exhibits a sharp transmission variation from ≈ -21 dB to -4.2 dB for protein concentrations up to 500 pg/mL, which is significantly larger than the corresponding change for the Lorentzian resonance (-1.8 dB to -2.2 dB). This enhanced response translates into a markedly higher capability to discriminate small protein concentrations (Fig. 3.21 *bottom*). Furthermore, the broader transmission variation improves robustness against noise, as wider noise margins enhance sensing accuracy compared to Lorentzian-based devices.

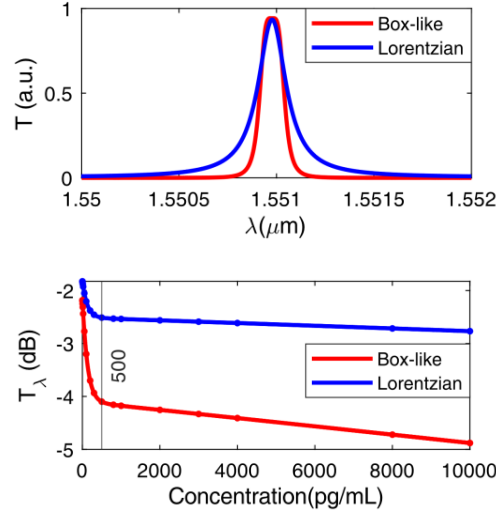


Fig. 3. 21 Transmission spectra of the sidewall grating, comparing the box-like resonance (red curve) with the Lorentzian resonance (blue curve) (*top*); Transmission at $\lambda = 1550.92$ nm as a function of protein concentration for the box-like (blue curve) and Lorentzian (red curve) profiles (*bottom*) [171].

From a technological standpoint, the fabrication of the proposed sensor can be realized using advanced lithographic techniques such as EBL. Following fabrication, the development of a tailored biofunctionalization protocol is essential to ensure the specific interaction with the target biomolecules. In fact, the functionalization of the sensor surface and the stable immobilization of bioreceptors within the active area are critical steps for achieving high-performance label-free biosensing. Biofunctionalization refers to the oriented immobilization of specific antibodies on the sensor surface, enabling efficient binding of target proteins and enhancing detection specificity. This process relies on the chemical modification of the substrate to create a uniform and stable layer covering the sensing area. Terminal functional groups are introduced within this layer to establish covalent cross-linking and high-affinity molecular interactions with bioreceptors [184].

Through this approach, the biorecognition elements are firmly anchored to the surface in a highly controlled and reproducible manner, which is essential for ensuring sensor reliability and repeatability. A critical challenge in surface biofunctionalization is the occurrence of non-specific absorption, which originates from undesired electrostatic and Van der Waals interactions. To minimize this phenomenon, a hydration layer, typically consisting of water molecules, is engineered at the surface, or alternatively, the surface charge of the chemical scaffold is adjusted to reduce ionic interactions. Both strategies aim to suppress non-specific binding, thereby preserving the selectivity of the biosensor [185].

The choice of an appropriate immobilization strategy is therefore a pivotal step in sensor design. Factors such as antibody orientation, pH conditions, target analyte concentration, matrix effects, and overall operating environment can significantly affect the final performance of the biosensor. A carefully optimized functionalization protocol is thus fundamental to maximize both sensitivity and

specificity.

For the silicon-based platform under study, surface modification is typically achieved through silinization [186]. This process consists of three main steps: (i) surface activation to generate reactive hydroxyl groups (-OH) on the substrate, (ii) silane coupling, which can be carried out either in liquid phase or in vapor phase, and (iii) a final polymerization step to cross-link the silane molecules and stabilize the monolayer [187]. Prior to silinization, the surface is cleaned with oxidizing agents to remove organic contaminants and increase the hydroxyl group density ($\approx 10^{15} \text{ cm}^{-2}$), thereby ensuring the effective immobilization of silanes [185]. This cleaning step not only improves surface reactivity but also enhances reproducibility in the subsequent functionalization process. Organofunctional silanes are then used to form stable chemical linkages between the silicon surface and organic moieties. Among them, amino-functional silanes such as 3-aminopropyl(diethoxy)methyl silane (APDEMS) and (3-aminopropyl) triethoxysilane (APTES) are widely employed, as they produce uniform and mechanically stable films across the sensing area [188-190]. Once the silane scaffold is established, bifunctional cross-linkers such as glutaraldehyde $(\text{CH}_2)_3(\text{CHO})_2$ are introduced to provide reactive aldehyde groups for the anchoring of bioreceptors [191, 192]. The functionalization procedure is completed by immobilizing antibodies or antigens on the activated surface, followed by controlled incubation and washing with PBS. This preserves the biological activity of the immobilized biomolecules across the entire sensing area. This guarantees efficient target capture and reliable, specific binding events, which are essential for achieving high-performance biosensing.

The proposed SOI-based biosensor, featuring an engineered sidewall dielectric grating with a box-like resonance, demonstrates exceptional optical performance for label-free detection in liquid biopsy application. The device combines a high Q-factor ($\approx 1.2 \times 10^4$) and an extinction ratio of 15.35 dB with a bulk sensitivity of 490.49 nm/RIU and a roll-off of -10.50 dB/oct., corresponding to a FOM of 3892-78 RIU⁻¹. This performance substantially surpassed that of Lorentzian-shaped resonances with comparable Q-factors, enabling enhanced discrimination of biomolecular interactions even at sub-nanogram concentration. The pronounced amplitude variation ($> 4 \text{ Db}$ for 500 pg/mL of IgG) ensures robust and crosstalk-free signal interrogation under single-wavelength operation. Owing to its compact footprint, high sensitivity, and spectral stability, the device represents photonic platform for the quantitative detection of cancer-related biomarkers in liquid biopsy, supporting the development of integrated lab-on-chip systems for early and minimally invasive diagnostics.

3.2.2.7 Optical Slot-Assisted Metasurface for IgG protein detection

The second photonic biosensor configuration developed in this work builds on the recent advances in all-dielectric metasurfaces, which have emerged as a promising platform for highly sensitive and compact detection of clinically relevant

biomarkers.

Dielectric metasurfaces have emerged as versatile photonic platforms capable of supporting sharp optical resonances, which have been extensively exploited both for wavefront manipulation in the far-field and for sensing and imaging in the near field. Their design typically balances the interplay between extended and localized resonances, which involves a trade-off between the Q-factor and spatial field confinement. High Q-factors are desirable for refractometric sensing, as they enable precise tracking of resonance shifts, while strong localization is advantageous for high-resolution imaging [197]. In simple gratings, GMR illustrates the inherent trade-off between spectral sharpness and near-field confinement: modes with greater penetration depth exhibit higher quality factors yet reduced spatial localization. One possible route to mitigate this compromise is to engineer resonances directly at the unit-cell scale, where structures such as nanodisks, elliptical dipoles, and symmetry-broken geometries have demonstrated promising capabilities [198, 199].

Historically, the concept of refractometric sensing based on resonance shifts was first established with surface plasmon resonance (SPR) in planar gold films during the 1980s [200]. SPR rapidly became a widely adopted analytical method for studying biomolecular interactions [201]. Despite its success, this technique suffers from intrinsic limitations: the detection limit typically lies in the nanomolar to sub-nanomolar range, and the optical configuration requires bulky setups with deep oblique incidence, which significantly restricts portability and system integration. These shortcomings stimulated the exploration of alternative photonic architectures capable of achieving higher sensitivity while relying on simpler and more compact optical readout configurations. In this context, dielectric nanostructures emerged during the 2010s as efficient optical resonators with strongly resonant spectra [202]. Fully dielectric metasurfaces, such as arrays of silicon nanodisks, were subsequently applied to protein detection. For instance, Yavas et al. [203] demonstrated refractometric sensing of PSA, achieving a detection limit of approximately 100 picomolar, a performance comparable to that of commercial ELISA kits. A notable advantage of all-dielectric metasurfaces lies in their compatibility with normal-incidence optical interrogation, which simplifies the setup and facilitates system miniaturization. A more recent advancement involves the exploitation of bound states in the continuum (BICs) in dielectric metasurfaces [204]. By introducing small structural asymmetries, BICs give rise to high-Q resonant modes with narrow spectral linewidths, thereby improving the figure of merit for refractometric detection. Experimental demonstrations have shown that BIC-based metasurfaces can approach 50-70% of the physical sensitivity limit, expressed as $\Delta\lambda = a \cdot \Delta n$, where $\Delta\lambda$ denotes the resonance shift, a the lattice constant, and Δn the variation of the surrounding refractive index [205]. Despite this improvement, the detection limit generally remains in the picomolar range, and pushing the sensitivity toward the attomolar regime appears unrealistic for BIC-based designs.

In parallel, alternative approaches based on molecular absorption spectroscopy have been investigated. Molecules exhibit characteristic absorption lines in the infrared, which can be exploited by engineering metasurfaces with narrowband absorption features. This method is particularly suitable for relatively small molecules, as large biomolecules with molecular weights above 50,000 Da display complex absorption spectra that are difficult to resolve. Tittl et al. [206] demonstrated this concept using arrays of dielectric metasurfaces supporting BIC resonances, each tailored to a specific absorption line. The reported results suggest detection performance in the nanomolar regime, which confines practical use to analytes occurring at relatively high concentrations.

While these approaches have significantly advanced the field, they also highlight the need for alternative mechanisms capable of combining strong field confinement with high-Q resonances. In this regard, the excitation of non-radiative electromagnetic modes, known as anapole states, has recently attracted considerable attention. Anapole modes originate from the destructive interference between electric and toroidal dipole moments, which suppresses far-field radiation while preserving a high energy density in the near field [207]. This non-radiating behaviour greatly enhances light-matter interactions and is particularly advantageous for label-free biosensing, where sensitivity relies on detecting subtle refractive index variations induced by biomolecular binding. Originally introduced by Zel'dovich in the context of nuclear physics, the toroidal dipole moment has only recently found applications in optical and nanophotonics with the advent of metamaterials and nanostructured dielectrics. Dynamic toroidal excitations have been experimentally demonstrated in both plasmonic and dielectric systems across a broad spectral range, from microwaves to the visible. A distinctive advantage of anapole modes lies in their robustness against variations in the angle of incidence, allowing efficient excitation under normal illumination conditions. This angular tolerance makes them particularly attractive for integration into compact photonic platforms. The physical description of anapole mode excitation is provided in [Appendix B](#).

Against this background, the present work focuses on an optical slot- assisted dielectric metasurface, specifically designed to achieve strong field confinement and high Q-factors in the near- infrared regime, aiming at sensitive and compact IgG protein detection. Dielectric metasurfaces offer distinct advantages for biosensing, including sharp spectral features, high Q-factors, and strong spatial field confinement within a subwavelength footprint. These properties stem from engineered modal coupling effects, such as the optical slot effect, which enhance the overlap between the evanescent field and the functionalized surface. Such features not only improve refractive index sensitivity but also provide a scalable and CMOS-compatible route toward integrated diagnostic devices.

3.2.2.7.1 Proposed device and simulation methodology

The proposed device consists of a two-dimensional array of amorphous silicon (a-Si:H) blocks arranged with period Λ and duty cycle DC, patterned on a glass substrate and in direct contact with water as the ambient medium [193]. This configuration defines an $N \times N$ metasurface, designed to operate in the spectral range between 600 and 700 nm, where water absorption is negligible (Fig. 3.22). The refractive indices considered in the design are $n_{\text{a-Si:H}} = 2.4$, $n_{\text{H}_2\text{O}} = 1.33$, and $n_{\text{glass}} = 1.45$. Each cell of the array consists of two silicon dimers separated by a nanoscale gap (g) along the x-axis. The gap region supports the optical slot effect, which enhances the in-plane confinement of the electric field and contributes to an increased Q-factor, thus improving refractive index sensitivity [194, 195]. The unit cell is characterized by a lateral dimension of $DC \cdot \Lambda$ along the x- and y-axes, and by a thickness t set to 10 nm for optimization purposes. Electromagnetic excitation is applied along the z-axis with a TE-polarized wave oriented along the x-axis. The preliminary design phase, including the parametric investigation of Λ and DC, is reported in detail in Appendix C. There, the spectral response of the metasurface is systematically analysed, leading to the identification of the optimal design parameters that ensure high Q-factors, minimized crosstalk, and robust spectral positioning within the targeted operating window. These results provide the foundation for refining the device geometry and guide the subsequent optimization steps. Building upon these preliminary findings, the role of the inter-dimer gap was subsequently investigated, as the strength of the slot effect is inherently determined by the separation between the silicon blocks. Fig. 3.23 (*top*) illustrates how the transmission spectrum evolves as g is varied, while Fig. 3.23 (*bottom*) quantifies its impact on the resonance features.

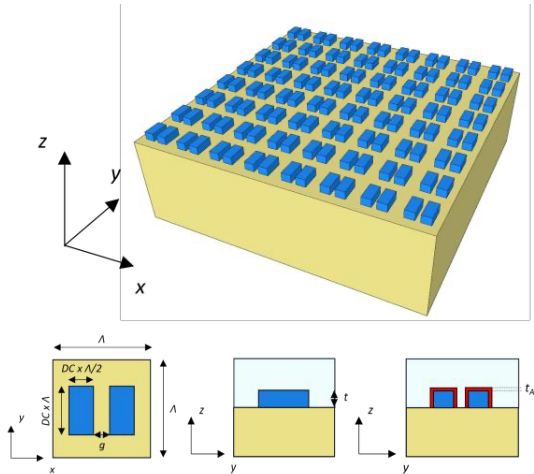


Fig. 3. 22 Proposed optical slot-assisted a-Si:H metasurface on a glass layer (*top*); top-view of a single cell (*bottom left*); cross-section pre and post functionalization with antibody layer, respectively (*bottom right*). Colour legend: blue represents a-Si:H, beige indicates SiO₂, and red corresponds to the antibody layers.

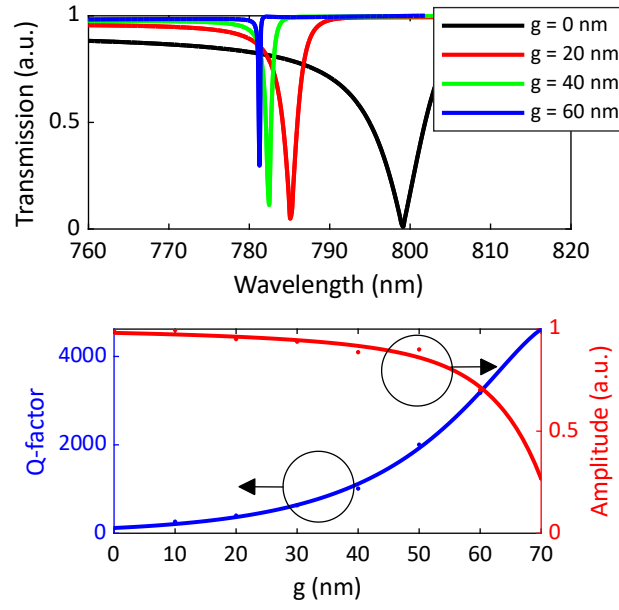


Fig. 3. 23 Transmission spectrum of the device for gap changes (*top*); Evolution of the Q-factor and ER as a function of the gap size (*bottom*) [193].

The simulations reveal that increasing the gap leads to a pronounced rise in the Q-factor, which follows an approximately exponential trend. This improvement, however, is achieved at the expense of a gradual reduction in the resonance amplitude. To establish a design point that ensures both high sensitivity and practical robustness, a trade-off analysis was carried out by jointly considering Q-factor, the resonance amplitude (required to remain above 0.5 to guarantee accurate detection), and fabrication constraints. Based on this evaluation, the configuration with $\Lambda = 500$ nm, DC = 70%, and $g = 60$ nm was selected as the most suitable compromise.

Under these conditions, the device exhibits a Q-factor of 3.19×10^3 together with a resonance amplitude exceeding 0.7, corresponding to an enhancement of more than one order of magnitude in Q when compared to the case with $g = 0$ nm.

3.2.2.7.2 Results and biosensing performance

The biosensing capability of the metasurface was investigated by evaluating its response to the detection of IgG, chosen as the target analyte. The surface sensitivity plays a key role in this context, as it reflects the ability of the resonator to detect refractive index variations caused by IgG molecules absorbed or bound to the functionalized surface. Upon deposition of the antibody layer, the device exhibited a red shift of approximately 3 nm in the resonance wavelength (Fig. 3.24 *top*). From this variation, a surface sensitivity of about 25 nm/RIU was extracted. This value exceeds those typically reported for comparable dielectric metasurface configurations and approaches the performance of conventional plasmonic sensors, while maintaining the advantage of larger resonance amplitudes.

The response of the sensor was further analysed as a function of the analyte concentration. A concentration range of IgG between 1 pg/mL and 1 ng/mL corresponds to a refractive index increment of the antibody layer from 10^{-4} to 10^{-3} a.u. [165]. This change arises from the increase in molecular density of the antibody film with concentration, without altering its physical thickness. Fig. 3.24 *bottom* reports the resonance shift ($\Delta\lambda$) observed at the functionalized resonance wavelength (≈ 784.28 nm) for different IgG concentrations. From these data, an IgG sensing sensitivity of ≈ 1.8 nm/(ng $\cdot$ mL $^{-1}$) was determined, confirming the potential of the proposed metasurface for label-free protein detection at clinically relevant levels.

The metasurface was specifically engineered to sustain an anapole mode in the vicinity of $\lambda \approx 652$ nm. As discussed previously, anapoles represent non-radiating resonances that combine high Q-factor with strong near-field confinement, both of which are essential for enhancing light-matter interaction and, ultimately, sensing performance. The excitation of an anapole arises when the effective scattering cross-sections of the electric dipole C_{sca}^p and the toroidal dipole C_{sca}^t , have equal amplitudes, resulting in destructive interference of their far-field radiation while preserving intense localized fields.

In the proposed geometry, the nanoscale gap plays a pivotal role in tuning both the spectral position of the resonance and the degree of modal leakage outside the unit cell. By carefully adjusting this parameter, the balance between resonance amplitude, Q-factor and near-field energy confinement can be optimized. As shown in Fig. 3.25, the intersection of C_{sca}^p and C_{sca}^t occurs at $\lambda \approx 651.6$ nm, confirming the onset of the anapole regime and giving rise to a pronounced resonant response.

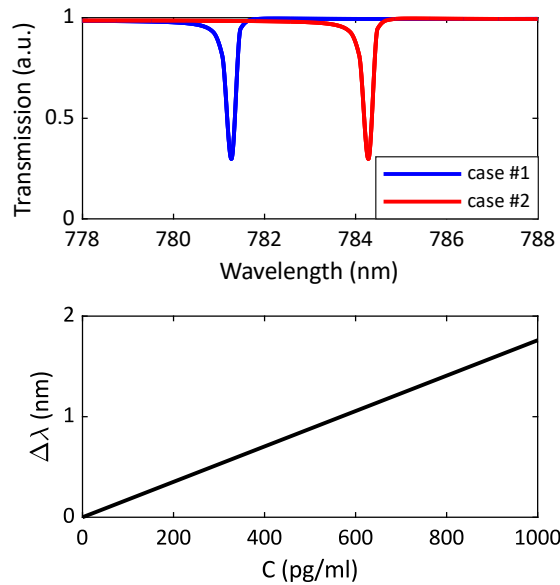


Fig. 3. 24 Transmission spectrum of the metasurface before (blue case) and after (red case) antibody functionalization (*top*); Resonance wavelength shift ($\Delta\lambda$) as a function of IgG concentration (C) (*bottom*) [193].

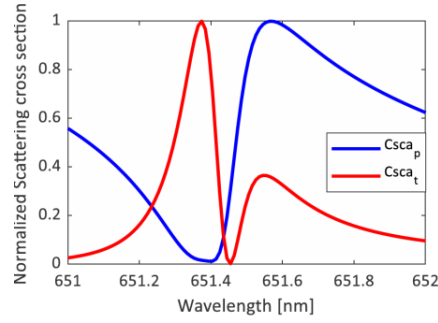


Fig. 3. 25 Scattering cross-sections of the electric dipole C_{sca}^p (blue curve) and toroidal dipole (red curve) in the unit cell [193].

At this wavelength, the electric field exhibits a solenoidal distribution within the plane of the structure, while the corresponding toroidal magnetic field circulates out of plane. This distinctive field topology is responsible for the substantial energy enhancement observed at resonance, with the electromagnetic energy predominantly localized in the gap region. Such confinement mechanisms directly support improved biosensing performance by maximizing the overlap between the functionalized surface and the resonant near field.

The slot-assisted dielectric metasurface presented in this work demonstrated the ability to combine high Q-factor with strong near-field confinement by jointly optimizing the periodic spacing Λ , DC, and inter-dimer gap. This design enabled crosstalk-free operation within the target spectral window and a robust interaction with the functionalized surface. Biosensing experiments with IgG confirmed a resonance red shift of about 3 nm, corresponding to a surface sensitivity of approximately 25 nm/RIU, together with an analytical response of $\sim 1.8 \text{ nm} \cdot (\text{ng} \cdot \text{mL}^{-1})^{-1}$. These results validate the potential of the proposed device as a compact and efficient platform for label-free protein detection.

Looking forward, implementing this concept within a LoC platform offers multiple opportunities for advancing toward practical biosensing devices. A first step concerns the interrogation method: by aligning the resonance of the metasurface with the sharp slope of a LED emission spectrum, small wavelength shifts can be directly converted into measurable variations in transmitted intensity. Such an approach could drastically simplify the optical setup compared to wavelength-scanning lasers or angular interrogation, thus paving the way for low-cost and portable devices. The SNR may be further enhanced by introducing polarization management. System-level integration also requires the development of dedicated microfluidic modules to deliver the analyte to the sensing area with high reproducibility and minimal dead volume. Computational fluid dynamics could guide the design of these channels to prevent bubble trapping and ensure homogeneous flow over the active region. On the hardware side, future implementations should explore board-level LEDs stabilized by constant-current

drivers or simple thermal control units, coupled with low-noise photodiodes and optimized readout electronics, to guarantee stable operation in realistic environments. Equally important is the transition to scalable fabrication and functionalization protocols. Standard PECVD deposition of amorphous silicon on glass, followed by deep-UV lithography and reactive ion etching, offers a CMOS compatible route for large-area manufacturing. Surface chemistry based on silinization and covalent antibody immobilization will need to be optimized for reproducibility, stability and reusability, with careful blocking protocols to minimize non-specific absorption.

Finally, the integrated system could be extended to multiplexed detection, either by spatially patterning the metasurface or by tailoring distinct spectral resonances to different biomarkers. Such advancements would ultimately establish the proposed architecture as a viable candidate for portable, low-power diagnostic devices capable of label-free protein detection in PoC settings.

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Chapter 4

Photonic strategies for optical trapping and destruction of viral bio-objects

The ability to isolate and control viral bio-objects at the nanoscale is increasingly recognized as a cornerstone for next-generation diagnostic and therapeutic technologies. Their nanometric dimensions and strong susceptibility to Brownian motion, however, render stable manipulation particularly challenging under physiological conditions. Addressing this challenge is essential not only for early and accurate detection of viral infections but also for enabling novel applications where viruses act as functional nanocomponents in nanotechnologies.

Building on this premise, the chapter first frames recent progress in photonic routes to single-virus handling, highlighting how advances in light-matter interaction at the nanoscale address the limits of conventional, isolation protocols. It then develops the physical foundations of optical trapping, from tightly focused beams to nanostructured fields, and motivates the transition toward architectures that strengthen gradient forces while mitigating photothermal load. Within this context, an inverted hybrid dielectric-plasmonic nanobowtie is introduced as the core contribution. The design incorporates an embedded metallic layer to enhance near-field localization inside a sub-wavelength gap and is investigated via FEM modelling to quantify electromagnetic energy confinement, thermal response, and trapping performance. Trapping parameters are calculated for viral-scale nanoparticles (diameter of 100 nm), revealing stable confinement at markedly reduced optical power densities while preserving thermal compatibility with the aqueous environment. Beyond stable trapping, the same architecture is further explored by tuning the input optical power to induce localized thermal inactivation of viruses confined at the gap, thereby extending its role from manipulation to selective thermal deactivation. This multifunctional capability broadens the scope of the platform, making it highly attractive for integrated diagnostic and therapeutic LoC systems. Taken together, these results indicate that hybrid dielectric-plasmonic platforms can offer superior performance over purely dielectric or metallic counterparts, offering robust, scalable, and biocompatible virus manipulation.

4.1 Optical trapping of viral bio-objects: background and state of the art

Accurate detection and trapping of single viral particles are of paramount importance for timely diagnosis and early prevention of infectious diseases, particularly in the context of global pandemics, where the ability to isolate and manipulate biological nanocomponents with high spatial and temporal precision is becoming increasingly critical for improve diagnostic accuracy and sensitivity [1].

Although multiple approaches have been developed with sensitivities reaching the single-virus level, including optical methods and immunoassays, robust and scalable solutions for the simultaneous trapping and detection of intact viruses in physiological environments remain elusive. Current protocols for viral isolation and characterization, which have remained largely unchanged for over half a century, are still highly manual, labour-intensive, and dependent on costly high level biosafety facilities [1]. They typically involve sequential steps such as purification from cell debris and bacteria, concentration into reduced sample volumes, culture in host cells, detection of cytopathic effects, and quantification by dilutional titration. While effective, these methods are time-consuming and unsuitable for rapid or PoC diagnostics.

The fundamental challenge lies in the intrinsic nature of viruses [2]. With typical dimensions between 40 and 300 nm, viral particles exhibit low polarizability and are strongly affected by Brownian motion, making stable manipulation particularly difficult. To date, no robust and commercially scalable platform has been established for the controlled manipulation, sorting, and characterization of individual intact viruses. This limitation is especially critical at the early stages of infection, where viral concentrations are extremely low and rapid detection is essential to prevent uncontrolled transmission. In response, researchers have developed a range of single-virus detection strategies, including whispering mode spectral shifts [3], electrical sensing of Influenza A virions [4], cantilever based detection of vaccinia virus [5], differential optical heterodyne detection of HIV [6], Young interferometer sensors for HSV-1 [7], immunolabeling-based fluorescence imaging for HCMV [8], and optical or immunoassay based methods for SARS-CoV-2 [9]. While these examples highlight significant progress in sensitivity, they generally lack the ability to achieve stable trapping in a flowing medium, a key prerequisite for dynamic virus analysis.

Beyond their role as infectious agents, viruses have also attracted growing interest as nanoscale building blocks in materials science and nanotechnology. Since the advent of nanotechnology in the 1980s and its rapid expansion in the 2000s, viruses have been increasingly employed as functional nano-objects [1]. Their well-defined size, shape, and self-assembling properties have been exploited to enhance the design of advanced materials and devices. For instance, viral capsids are highly stable, self-organizing protein shells capable of forming supramolecular

architectures. Although they do not possess unique intrinsic optical properties, these capsids can organize into photonic crystal-like arrangements or serve as scaffolds for photonic and plasmonic materials, thereby imparting novel optical functionalities [10].

These advances in the understanding of nanoscale light-matter interactions have laid the groundwork for the development of photonic strategies specifically tailored to the trapping and manipulation of viral bio-objects, thereby opening the way to next-generation diagnostic and therapeutic platforms.

4.2 Fundamentals and recent advances in optical trapping technologies

Among the photonic strategies, optical trapping has emerged as one of the most powerful approaches, providing a non-contact and label-free means to manipulate biological entities from the microscale down to single viruses and biomolecules [11, 12]. Its integration within LoC systems enables real-time manipulation of single particles, improving the specificity and efficiency of downstream sensing processes.

The fundamental principles of optical trapping were established in the early 1970s, when A. Ashkin and colleagues demonstrated that opposing laser beams could generate stable optical forces capable of confining microscopic particles, such as viruses [13], bacteria [13], and individual living cells [14], thus laying the groundwork for modern optical tweezers. Subsequent advances in piezoelectric actuators, laser modulation, and high-sensitivity photodetectors enabled nanometre-level positioning accuracy and force control in the piconewton regime. Optical trapping typically relies on tightly focusing a single laser beam through a high numerical aperture (NA) objective, generating a strong intensity gradient at the focal point. Particles within this region experience optical gradient forces that draw them into the beam center, achieving stable three-dimensional confinement.

Unlike conventional manipulation techniques that require physical contact with glass microneedles, posing risks of contamination and mechanical damage, optical tweezers operate in a fully contactless manner, minimizing invasiveness and improving biocompatibility [15]. In 1986, the first demonstration of stable three-dimensional trapping of micrometre-sized glass beads in aqueous media using a single focused beam formally established the optical tweezer technique [16]. Shortly thereafter, the trapping of live microorganisms such as *E. Coli* and tobacco mosaic virus marked the entry of optical trapping into biological research [13]. To minimize photothermal effects, especially in living samples, researchers adopted near-infrared (NIR) laser sources (700-1400 nm), which are less absorbed by biological media. For instance, lasers at 1064 nm have enabled extended trapping of live *E. Coli*, yeast, and various bacteria, supporting studies of their motility and growth under physiological conditions [17]. Since then, optical trapping has been employed across a wide spectrum of biological investigations. These include probing the

mechanical properties of red blood cells and neuronal membranes manipulating protozoa and plant organelles and studying the dynamics of single biomolecules. Dual-beam systems have enabled precise force measurements and dynamic mechanical probing. As illustrated in Fig. 4.1, optical tweezers now represent an indispensable tool in cell biomechanics and bio-nanomanipulation, offering non-contact, high resolution access to cellular and subcellular structures.

Driven by the increasing demand for precise control in complex biological environments, optical tweezers have evolved into versatile instruments capable of exerting piconewton-range forces with nanometric spatial resolution [18]. Their ability to probe and modulate the dynamics of single particles has profoundly impacted fields such as single-cell analysis, molecular biomechanics, and targeted manipulation in biological systems. These features are especially relevant for minimally invasive diagnostics, where gentle and accurate handling of delicate bio-objects is required. Consequently, optical trapping technologies are being progressively integrated into microfluidic platforms designed for early cancer detection and real-time biomolecular analysis. Among the nanophotonic architectures explored to overcome the limitations of conventional optical trapping, nanobowtie structures have recently gained significant traction due to their unique ability to combine high field enhancement with minimal invasiveness. Their geometry allows for extreme localization of the electromagnetic field at the nanoscale, especially when the incident light is polarized along the axis of the bowtie gap, resulting in substantial optical gradient forces.

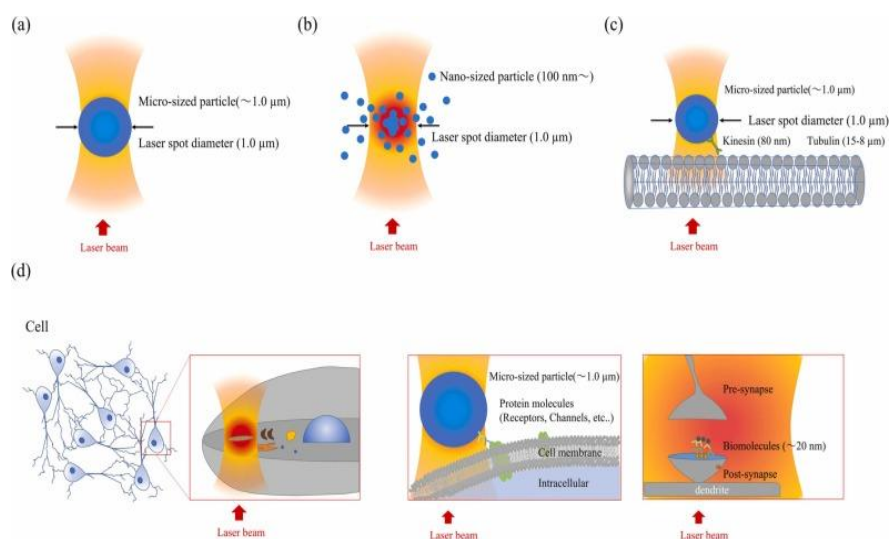


Fig. 4. 1 Conceptual illustration of optical trapping applications: trapping of a single micrometer-sized particle at the laser focus (*top left*); nanoparticle aggregation at the focal point due to optical forces (*top center*); optical control of intracellular transport via coupling of trapped particles to structure such as microtubules (*top right*); examples of intracellular optical manipulation: targeted displacement of vesicular compartments (*bottom left*), interaction of antibody-coated beads with membrane proteins (*bottom center*), and optical assembly of surface biomolecules at synaptic interfaces (*bottom right*) [15].

Numerical simulations have demonstrated that the trapping forces generated by nanobowtie antennas can surpass those achieved by conventional high-NA optical traps by up to an order of magnitude, making them highly attractive for high-precision bioanalytical applications [11, 19]. Nanobowtie configurations offer an advantageous trade-off between performance and integration, as they not only support strong field confinement but also exhibit good compatibility with standard nanofabrication processes and can be easily integrated into more complex photonic platforms.

Traditionally, plasmonic nanostructures have been the most exploited in optical trapping due to their ability to confine optical energy into subwavelength volumes, enabling the manipulation of individual biomolecules, DNA/RNA for subsequent analysis [20], or 3D manipulation with tweezers placed on an optical fiber tip [21] (Fig. 4.2). However, the major drawback of these plasmonic platforms lies in the generation of significant localized heating due to ohmic losses near the metallic components, which can pose a threat to the viability of the biological specimens under investigation. On the other hand, fully dielectric nanostructures, such as ring resonators and dielectric nanoantennas, have emerged as viable alternatives that eliminate photothermal effects, although they typically exhibit lower electromagnetic field confinement compared to their plasmonic counterparts [19, 22] (Fig. 4.2).

To reconcile these trade-offs, hybrid metal-dielectric architectures have emerged as a promising compromise, combining strong field enhancement with reduced thermal dissipation [11]. These hybrid systems outperform both pure metallic and high-refractive index dielectric platforms in terms of light-matter interaction efficiency, making them particularly suitable for optical trapping, surface-enhanced spectroscopy, and nanoscale biosensing. In the context of biological manipulation, this balance is essential to enable efficient, reliable, and non-destructive trapping under physiological conditions. For example, D. Conteduca et al. [23] designed a hybrid platform consisting of a 1D PhC dielectric cavity vertically coupled to a metallic bowtie, enabling the trapping of a single 200 nm Au bead with a P_{in} = 190 μ m. This configuration achieved a high Q-factor and low mode volumes, effectively overcoming the diffraction limit while avoiding direct contact with the trapped particle. While these designs demonstrated excellent trapping performance, they were inherently sensitive to fabrication imperfections and alignment tolerances between the PhC and the plasmonic structure, factors that can drastically affect both light coupling efficiency and overall trapping stability. Moreover, the fabrication of such structures is significantly complex and costly. To address these limitations, a novel inverted hybrid nanobowtie architecture is proposed in the following chapter. This design aims to reduce fabrication and alignment complexity while preserving the high electromagnetic confinement and low thermal impact required for reliable biological nanoparticle trapping.

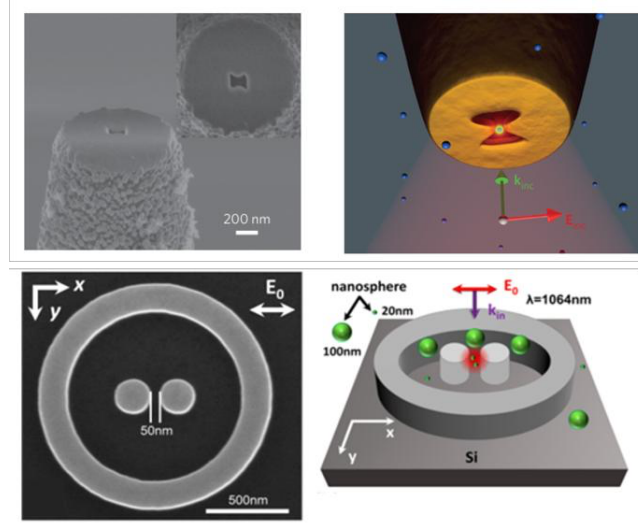


Fig. 4. 2 SEM image of an 85 nm gap bowtie nanoantenna patterned at the extremity of a tapered optical fiber (*top left*) [21]; Schematic of the experimental setup: the nanoantenna is illuminated from the water side using 1.062 nm laser beam (*top right*) [21]; SEM image of a silicon nanoantenna fabricated on a silicon substrate (*bottom left*) [24]; schematic illustration the optical trapping of the dielectric nanospheres using all silicon nanoantenna arrays (*bottom right*) [24].

4.3 Dielectric-Plasmonic Hybrid Nanobowtie dimer: Proposed design

The photonic trapping proposed in this study is a Silicon on Insulator (SOI) nanocavity comprised of two tip-to-tip silicon wedges (TTWSs), which form a bowtie-shaped geometry optimized for electromagnetic field confinement. A metallic layer, either gold or silver, is embedded beneath the silicon elements to enhance the energy confinement and light-matter interaction within the nanoscale gap that acts at the trapping region (Fig. 4.3). Each silicon wedge is characterized by a thickness (t) of 220 nm, a height (h) of 260 nm, and a tip angle (α) of 110° . These structures are separated by a gap measuring 120 nm in width, which was selected to ensure efficient confinement while preventing physical adhesion of the trapped particles to the silicon tips. The two TTWSs are placed above a SiO_2 layer, whose thickness, on the order of the millimeters, is modelled to be several orders of magnitude greater than that of the active cavity region. Specifically, it is approximately four orders of magnitude thicker than the trapping site, allowing the substrate to be treated as infinitely extended by employing Perfectly Matched Layers (PMLs) in COMSOL Multiphysics®. Further details on the PML configuration are provided in [Appendix A](#).

The spatial origin of the simulation domain is set at the base centre of the cylindrical trapping site, located at the dielectric-metal interface. The two TTWSs are symmetrically arranged with respect to the site centre, while the optical excitation is modelled as a normally incident plane wave, propagating along the negative z -axis and linearly polarized along the y -axis, to excite the slot mode within the gap. This polarization orientation enhances transverse electric (quasi-TE) field confinement between the silicon wedges, enabling localized energy accumulation within the trapping region.

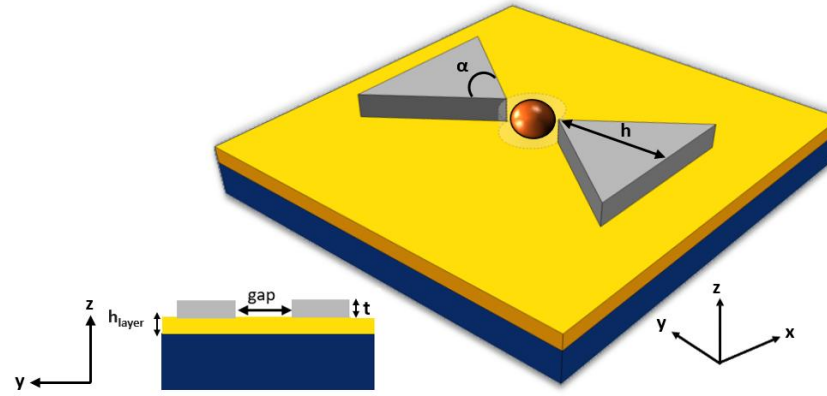


Fig. 4. 3 Schematic of the investigated nanobowtie in SOI platform with a metal layer [25].

The trapping target is modelled as a 100 nm dielectric sphere, corresponding to the characteristic size range of biological particles of interest, such as viruses (e.g., SARS-CoV-2, Influenza A, HIV) [11, 26], and small extracellular vesicles (exosomes) [27]. These bio-objects are increasingly recognized as valuable biomarkers for a variety of diagnostic and prognostic applications. The proposed device is surrounded by an aqueous medium, which is required to model the reaction and transport of multiple biospecies. The operation wavelengths, λ_0 , was set to 1550 nm. Although shorter wavelengths help reduce the scattering forces acting on the particle, this advantage is offset by a corresponding decrease in the gradient force. Conversely, longer wavelengths strengthen the gradient force, thereby enhancing both the precision and stability of particle confinement [28]. For this reason, operating at higher wavelengths enables more effective and controlled optical trapping. Moreover, the use of lasers at telecommunication wavelengths provides high flexibility in polarization control and power handling. Additionally, the moderate absorption coefficient of water at 1550 nm supports the system's biocompatibility, confirming its suitability for trapping biological targets, as demonstrated in studies such as [19, 29 - 31]. The following Table 4.1 shows the refractive indices of the materials at the operating wavelength ($\lambda = 1550$ nm).

Table 4. 1 List of refractive index values of materials used in the device @1550 nm [12].

Materials	Refractive index	Ref.
Silicon (<i>Si</i>)	3.48	[32]
Silicon Oxide (<i>SiO₂</i>)	1.444	[33]
Water (<i>H₂O</i>)	$1.318 + 9.86 \times 10^{-5}i$	[33]
Gold (<i>Au</i>)	$0.52406 + 10.742i$	[34]
Silver (<i>Ag</i>)	$0.40960 + 10.048i$	[35]
Trapped particle	1.50	[36-38]

For spherical particles with a diameter of 100 nm, a refractive index of 1.50 was assumed, consistent with experimental characterization of viruses, such as Influenza A [36, 37] and HIV-1 [36].

4.3.1 Simulation Methodology and numerical Framework

To assess the optical trapping performance of the proposed inverted hybrid dielectric-plasmonic nanobowtie structure, an extensive series of numerical simulations was carried out using the FEM implemented in COMSOL Multiphysics®. In this work, the FEM-based simulation framework was employed to investigate the electromagnetic field distribution and optical trapping forces associated with the proposed nanobowtie cavity. The high flexibility of the platform in coupling different physical domains, such as optical and mechanics, makes it particularly suitable for Multiphysics modelling of nanophotonic systems.

In this research, the simulation framework was developed to capture both the electromagnetic and thermal responses of the device, thereby enabling a Multiphysics analysis essential for evaluating optical field enhancement, heat dissipation, and trapping forces under realistic operating conditions. The optical problem was addressed using the *Wave Optics Module* by solving the time-harmonic Maxwell's equations in the frequency domain.

Perfectly Matched Layers (PMLs) were used to consider the extension to infinity of the substrate SiO₂ (further details are provided in [Appendix A](#)). The excitation source was defined as a linearly polarized plane wave, normally incident along the negative z-direction and polarized along the y-axis, selected to maximize field confinement through the slot effect between the triangular elements. The electromagnetic energy distribution was evaluated by calculating the electric field intensity and the electromagnetic energy density under steady-state conditions. The geometry was discretized using a high-resolution tetrahedral mesh, refined down to 10 nm in regions of high field intensity, such as the slot and tip areas. This approach ensured accurate resolution of local field gradients and near-field effects, which are critical for trapping. The Helmholtz equation was subsequently discretized, resulting in a sparse algebraic system that was numerically solved under steady-state conditions.

To estimate the optical force acting on a trapped nanoparticle (100 nm in diameter, $n = 1.50$), the Maxwell Stress Tensor (MST) formalism was employed [19, 39]. The optical force is generated by momentum transfer due to the interaction between the incident electromagnetic field and the dielectric particles. Specifically, the optical force was calculated by computing the surface integral over a sphere with a diameter 10 nm larger than the particle itself. This integral involved the vector product of the MST and the outward normal vector to the surface. The optical force can be expressed as:

$$F = \oint_s (\mathbf{T}_M \times \mathbf{n}) dS \quad (4.1)$$

where $\mathbf{T}_M$ is the Maxwell stress tensor, S is the particle surface, and $\mathbf{n}$ is the outgoing vector normal to the surface. The force is integrated in one dimension to obtain the trapping potential energy under the simplifying assumption, which is a conservative force. Overall, the MST is often used in a trapping context to evaluate the resultant force on particles, allowing a detailed understanding of the dynamics of optical trapping.

To assess field confinement, the Energy Confinement (EC) factor was calculated, defined as the ratio of the integrated electromagnetic energy density within the trapping site to the total energy within the computational domain. The EC within the gap region was selected as the primary figure of merit, defined as the ratio of electromagnetic energy density integrated over the trapping site to that integrated over the surrounding computational domain, as expressed in Equation (4.2). This metric quantifies the effectiveness of field localization and, indirectly, the trapping capability of the structure.

$$EC = \frac{\iiint_{trapping\ site} W(r) d^3r}{\iiint_{volume} W(r) d^3r} \quad (4.2)$$

Where $W(r)$ is the electromagnetic energy density [24]:

$$W(r) = \frac{1}{2} [\epsilon(r)|E(r)|^2 + \mu(r)|H(r)|^2] \text{ with } r = ax_x + ya_y + za_z \quad (4.3)$$

To maximize electromagnetic confinement at the trapping site, boundary conditions were applied to enforce the continuity of the normal component of energy at high-index-contrast boundaries and to promote strong field localization near the silicon tip regions. In addition to electromagnetic analysis, thermal simulations were carried out by coupling the *Heat Transfer in Solids* and *Electromagnetic Waves physics* interfaces. This approach allowed the estimation of local temperature increases resulting from optical absorption, particularly within the metal layer and the aqueous environment. To demonstrate the suitability of the proposed device as an optical trapping platform for nanoscale biological objects, such as viruses, it was essential to verify that the electromagnetic confinement does not induce critical thermal variations that could compromise the structural integrity and morphological features of the trapped particles. Numerical simulations confirmed that, despite the strong field localization, the temperature rise remains within biologically safe limits, owing to the moderate absorption coefficient of water at the selected wavelength, the efficient dissipation through the SOI substrate, and, most importantly, the careful design of the input optical power, which was tuned to operate below specific thermal thresholds. Furthermore, as will be discussed in the following sections, additional simulations were performed by varying the input power to intentionally increase the temperature at the trapping site. This analysis highlights a dual functionality of the device, enabling not only stable optical trapping but also the potential for thermal inactivation of viruses, a concept that will be further contextualized in the next paragraphs.

4.3.2 Design to enhance the Energy within the trapping site

To improve the trapping performance of the nanocavity, specific structural optimizations were introduced to enhance electromagnetic energy localization within the nanoscale gap. These design strategies included careful tuning of the wedge geometry, gap dimension, and thickness of the metal layer, aiming to strengthen the near-field confinement while limiting radiation losses. The embedded metallic layer is designed to strengthen the near-field confinement while limiting radiation losses. The embedded metallic layer, either silver or gold, was incorporated to leverage plasmonic enhancement effects. Its thickness was varied to identify the optimal trade-off between field amplification and material absorption. Additionally, the sharp wedge tips were engineered to exploit the lightning-rod effect, further concentrating the field in the trapping region. These combined features support high local energy density, which is essential for stable and efficient optical trapping of nanometric biological targets.

This section presents the results of a numerical optimization study aimed at quantifying the energy confinement improvement in energy confinement under different configurations.

The spatial distribution of the electric field intensity within the central plane of the nanocavity was computed under an input optical power density of $10 \text{ mW}/\mu\text{m}^2$, assuming a silver layer thickness (t_{Ag}) of 50 nm. As illustrated in Fig. 4.4, the electric field exhibits a predominant orientation along z-direction, characteristic of a quasi-TE polarization mode within the nanobowtie cavity. The energy remains highly confined in the nanometric gap, with negligible field diffusion into the surrounding silicon wedges. To explore the impact of the metal layer, simulations were carried out using both silver and gold, varying the layer thickness and monitoring the corresponding enhancement in energy confinement (ΔEC) within the trapping region. The energy confinement without a metal layer was $\text{EC}=3.75 \text{ } \mu\text{V}/\text{m}$. As shown in Fig. 4.5, ΔEC increases with metal thickness until reaching saturation at approximately 70 nm for gold and 50 nm for silver. Maximum improvements in EC were observed for $t_{\text{Au}} = 70 \text{ nm}$ and $t_{\text{Ag}} = 50 \text{ nm}$, with respective enhancements of 91.93% and 92.20% relative to the dielectric-only configuration. These findings confirm that the presence of a metal layer substantially strengthens electromagnetic field confinement in the nanocavity, enhancing the coupling between light and matter, an essential feature for effective trapping of biological nanoparticles.

However, further increasing the thickness beyond the identified optima results in diminishing returns, likely due to absorptive losses and internal interference phenomena. Thermal management is a critical aspect in the design of optical trapping systems, especially when handling delicate biological specimens. Excessive heating can affect the structural integrity and biological function of the trapped particles, leading to unreliable results. In this context, the temperature sensitivity of viral envelopes, particularly for enveloped viruses such as SARS-CoV-2, has been well documented, highlighting the need for thermal stability during trapping operations.

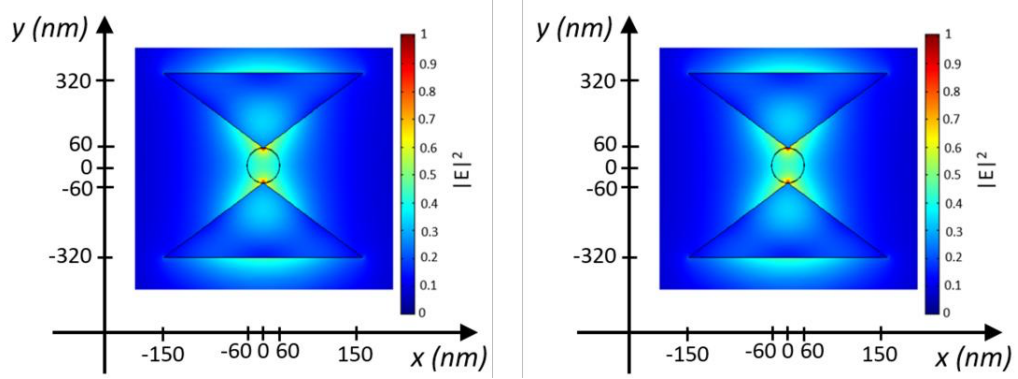


Fig. 4. 4 Electric field intensity profile in the symmetry plane of the nanocavity at $z = 200 \text{ nm} + t_{\text{Ag}} = 50 \text{ nm}$ and $P_{\text{in}} = 10 \text{ mW}/\mu\text{m}^2$ (left); Enlarged view (right) of panel right [12].

The thermal response of the nanocavity was simulated using a coupled optical thermal model in COMSOL Multiphysics, incorporating heat transfer and electromagnetic wave propagation. The absorbed optical power within the particle volume was used as a source term to solve the heat transfer and electromagnetic wave propagation. The absorbed optical power within the particle volume was used as a source term to solve the heat diffusion equation (Fourier's law in differential form), enabling the evaluation of steady-state temperature rise in the trapping site. Fig. 4.6 shows the simulated temperature distribution for the silver-layer configuration ($t_{\text{Ag}} = 50 \text{ nm}$), under an input power of $10 \text{ mW}/\mu\text{m}^2$. The peak temperature rise occurs in the central gap region, directly exposed to the localized field. For $t_{\text{Au}} = 70 \text{ nm}$, temperature increases by $\Delta T = 6.52 \text{ K}$ at $1 \text{ mW}/\mu\text{m}^2$, reaching $\Delta T = 64.17 \text{ K}$ at $10 \text{ mW}/\mu\text{m}^2$. The silver layer results in similar values, with $\Delta T = 6.39 \text{ K}$ at $1 \text{ mW}/\mu\text{m}^2$ and $\Delta T = 63.00 \text{ K}$ at $10 \text{ mW}/\mu\text{m}^2$.

These temperature values remain below critical thresholds known to affect the viability and structural integrity of nanoscale biological objects. As such, the device enables optical trapping while maintaining the thermal safety of the particle. This is particularly relevant considering studies showing that virus inactivation occurs at temperatures exceeding 65°C for exposures longer than 3 minutes. The thermal behaviour of the nanocavity thus plays a pivotal role in ensuring biocompatibility during trapping procedures.

To assess thermal scalability, the temperature variation in the trapping region was computed across a range of optical power densities from 1 to $10 \text{ mW}/\mu\text{m}^2$ (Fig. 4.7). Both gold and silver configurations exhibit a linear relationship between input power and temperature rise, facilitating predictable thermal tuning. The values achieved remain well below the inactivation threshold for typical biological samples, confirming the feasibility of using the proposed nanobowtie system in diagnostic or therapeutic applications without compromising particle integrity.

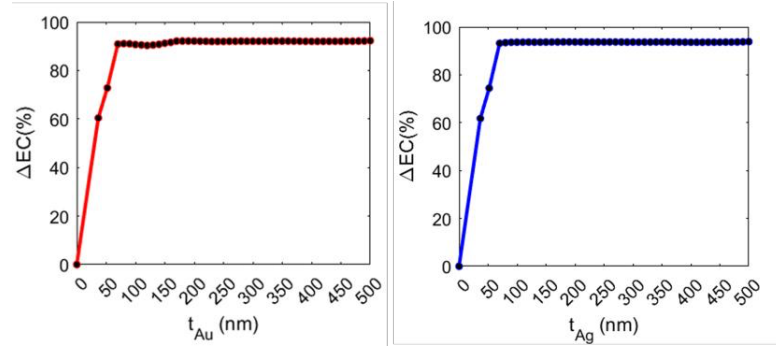


Fig. 4. 5 EC enhancement (%) versus metal thickness for gold (*left*); for silver (*right*) [12].

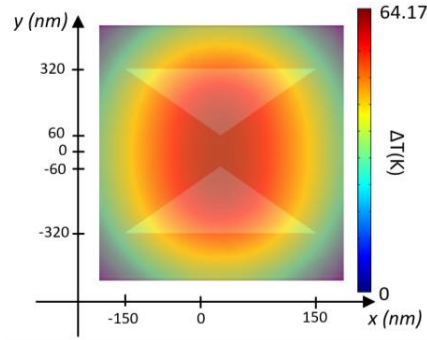


Fig. 4. 6 Steady-state temperature rise ΔT (K) = $T - T_0$ ($T_0 = 293.15$ K) for the silver-layer configuration at $z = 200$ nm + t_{Ag} under $P_{in} = 10$ mW/ μm^2 [12].

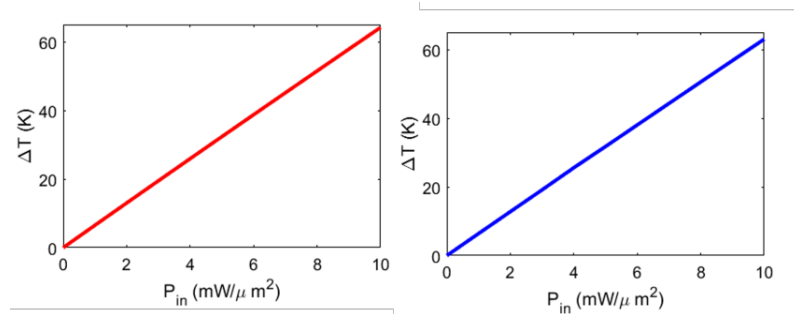


Fig. 4. 7 Temperature increase ΔT as a function of optical power for gold-coated nanocavity (*left*); for silver-coated nanocavity (*right*) [12].

4.3.3 Trapping Performance

To quantitatively assess the optical trapping capabilities of the proposed hybrid dielectric-plasmonic nanobowtie configurations, the optical force exerted on a test particle was computed as a function of its position along z-axis. This approach enabled the evaluation of the trapping behaviour and the influence of the embedded metal layer at the working wavelength of 1550 nm. Fig. 4.8 illustrates a schematic side view (z-y plane) of the device, including a nanoparticle ($d = 100$ nm) located at the trapping site.

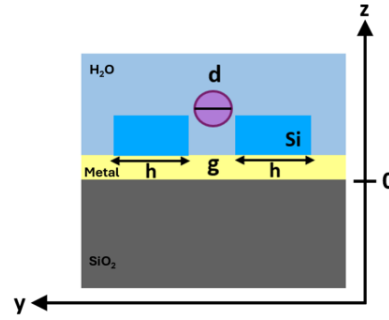


Fig. 4. 8 Schematic and lateral view of the nanocavity (z-y direction) with a trapped particle [12].

The analysis was conducted in the Rayleigh regime [28], which is appropriate for particles smaller than the illumination wavelength and is widely adopted in the optical manipulation of nanoscale biological targets such as viruses [40]. As shown in Fig. 4.9 *top*, the electric field distribution within the trapping region displays a clear intensity maximum at the apexes of the silicon tips, decaying symmetrically along the z-axis. This decay follows a Gaussian-like profile with a standard deviation $\sigma = 6.2789$ nm, consistent with the evanescent nature of the confined optical field.

To assess the trapping performance of the proposed nanocavity, the electric field intensity was analysed at five different positions along the z-axis. The strongest confinement occurred near $z = 100$ nm, where the particle is ideally trapped. Beyond this point, the electric field gradually decreases, reducing the optical force acting on the particle. Figures 46 *bottom right* and Fig. 4.9 *bottom left* show how the vertical component of the optical force (F_z) changes with the particle's displacement along the z-axis when an input power density of $10 \text{ mW}/\mu\text{m}^2$ is applied.

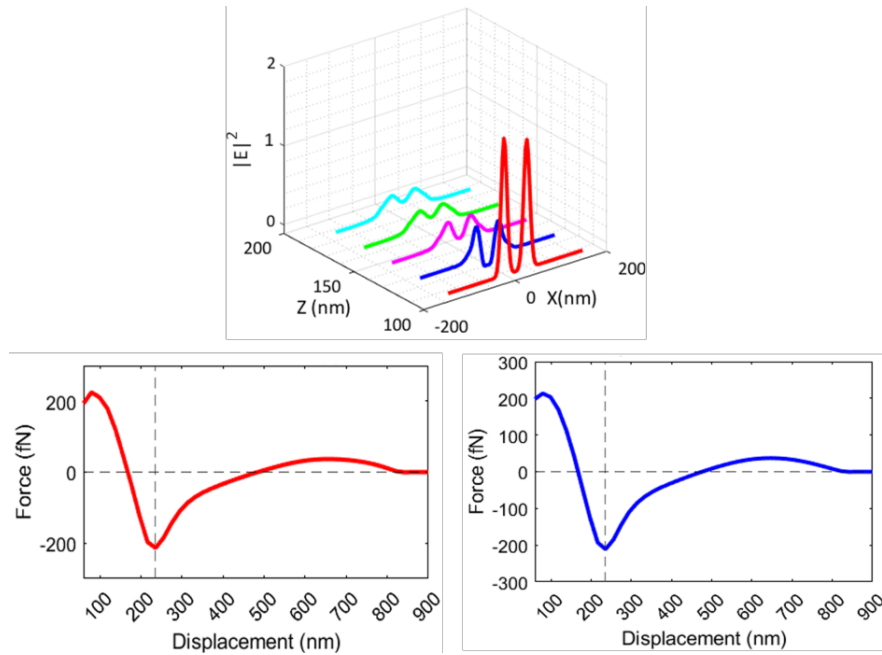


Fig. 4. 9 Representation of the intensity of electric field along the trapping site of the nanocavity (*top*); Optical force trend vs displacement of nanoparticle in configuration with gold layer (*bottom left*) and silver layer (*bottom right*) [12].

In both nanocavity versions (with gold and silver layers), the optical force follows the same trend: it is positive for $z < 170$ nm, pushing the particle upward, and becomes negative beyond that, pulling it back toward the equilibrium. The maximum force reached 2.96 fN for the gold-based cavity and 2.14 fN for the silver-based one, both peaking at $z = 80$ nm. This position ($x = 0$, $y = 0$, $z = 80$ nm) represents a stable equilibrium where attractive and repulsive forces are balanced.

The electric field gradient is strongest at this point, supporting efficient trapping without requiring excessive input power. Outside this region, the force becomes too weak to counteract thermal motion, and the particle is more likely to escape.

To better characterize the trap, two key performance indicators were evaluated: trapping stability and trapping stiffness. Stability refers to the ratio between the optical potential energy (U) and the thermal energy ($k_B T$) and is used to estimate how long a particle can remain trapped against thermal disturbances. The stability trapping (S) is expressed in Equation (4.4):

$$S = \frac{U}{k_B T} \quad (4.4)$$

Here, U [J] is the energy needed to bring the particle from far away into the trap and is expressed in Equation (4.5):

$$U(r) = \int_{-\infty}^{r_0} F(r) \cdot dr \quad (4.5)$$

with $F(r)$ denoting the optical force exerted on the particle moving along the cylinder representing the trapping site. K_b is the Boltzmann constant [J/K], and T_c is the temperature in Kelvin [K], also considering the temperature rise ΔT due to the trapping effect [22]. In a well-designed optical trap, a nanoparticle subjected to minor external perturbations, such as mechanical or thermal fluctuations, tends to remain localized around its equilibrium position.

However, the trap's ability to maintain stable confinement is not absolute and can be significantly influenced by multiple factors, including the geometry of the trapping region, the physical characteristics of the target particle (notably its size and shape), the strength and spatial distribution of the optical forces and, most critically, the local temperature rise at the trapping site. Fig. 4.10 presents the results of a parametric analysis evaluating the influence of thermal fluctuations on trap stability.

A clear linear correlation emerges between the temperature increase and the enhancement of trapping stability for both the gold- and silver-coated nanobowtie configurations, highlighting the temperature dependence of the trapping mechanism.

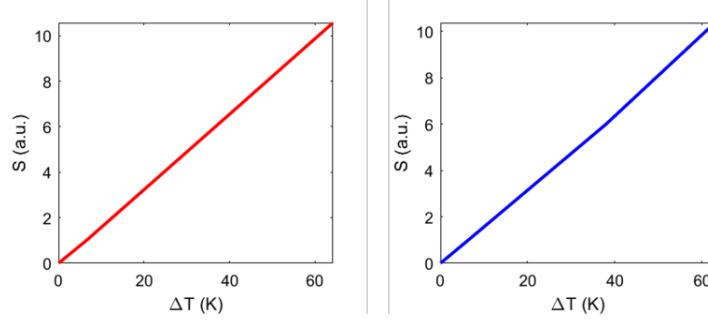


Fig. 4.10 Trapping stability as a function of ΔT for the nanobowtie with a gold layer (*left*); corresponding results for the nanobowtie with a silver layer (*right*) [12].

To further investigate the dynamic stability of the trap, the retention time of a nanoparticle within the potential well can be described through Kramer's theory [41]. In this framework, the trapping time t_{trap} increases exponentially with the ratio between the optical potential depth U and the thermal energy $k_B T$, as expressed in equation (4.6)

$$t_{\text{trap}} \propto \exp\left(\frac{U}{k_B T}\right) \quad (4.6)$$

A value of $U > k_B T$ is generally required to initiate stable trapping. Nevertheless, when this ratio, commonly referred to as the stability factor S , falls below unity, Brownian motion dominates, and the particle rapidly escapes the trap. Conversely, values of $S > 1$ indicate that optical forces outweigh thermal agitation, though this condition alone may not ensure long-time trapping, as retention times in this regime are often limited to just a few seconds, as observed in previous implementations of dielectric nanotweezers [19, 24, 41].

To counteract the destabilizing effect of thermal agitation, it is widely accepted that the potential depth should exceed $10k_B T$ to enable stable confinement under typical Brownian conditions [40, 42, 43]. Such a criterion ensures that the optical trap is resilient against rapid thermally induced displacements, and thus suitable for sustained manipulation of nanoparticles. As a result, maximizing the trap depth relative to thermal energy becomes essential for maintaining nanoparticle retention over longer durations. Accordingly, a stability factor $S = 10$ implies that the optical potential is ten times stronger than the competing thermal forces. This threshold is commonly used in the literature as a reference value for effective and persistent trapping [10, 44-46]. In reference [47], a threshold condition of $S \geq 1$ was introduced and successfully employed to trap silica beads with a diameter of 26 nm using a laser power of 1.5 W. Since the input optical power is a crucial parameter for overcoming Brownian motion and enhancing the stability of optical trapping, we conducted a series of simulations by varying the input power P_{in} up to 10 mW, following a similar approach to that in [47]. The objective was to achieve a trapping stability value equal to or slightly exceeding 10, while simultaneously ensuring that the associated temperature rise does not lead to viral denaturation. An input power of 10 mW is sufficient to increase the optical forces at the trapping site, thus improving the stability of confinement. This enhancement is particularly relevant for nanoscale particles, such as viruses or small vesicles, that require stronger

trapping forces for long term immobilization. As particle dimensions decrease, their polarizability diminishes, necessitating higher optical intensities for effective trapping. Therefore, the optical intensity, defined as power density at the focal spot, must be adapted to the particle radius, especially for particles smaller than the wavelength of light. For instance, a 10mW laser focused on a $1 \mu\text{m}^2$ area can effectively trap particles with a radius of at least 56 nm, whereas a 100mW beam is required to stably trap particles as small as 25 nm in radius [16].

Increasing the input power density also elevates the local temperature within the trapping volume, which in turn improves the system's robustness against external disturbances such as mechanical vibrations. This contributes to the mechanical and thermal stability of the nanocavity, improving control over the trapped nanoparticle. This level of precision is particularly critical in applications requiring long-duration trapping or high reproducibility. However, the drawbacks of elevated power density must be considered, including the potential thermal damage to biological materials. For example, latex spheres with a diameter of 109 nm were reported to be destroyed within 25 seconds under a 15mW beam, raising concerns when dealing with fragile biological samples [48]. Accordingly, the design of a trapping nanocavity requires a careful balance between enhancing trapping performance and avoiding adverse thermal effects.

In our simulations, a power density of $10 \text{ mW}/\mu\text{m}^2$ resulted in a local temperature increase of approximately 63 K with a silver layer and 64.17 K with a gold layer, values that strike an optimal compromise between mechanical stability and thermal safety. Notably, [49] achieved stable trapping ($S=10$) of a 75 nm nanoparticle using a 250mW laser. In contrast, in our work, a trapping stability factor of 10.370 and 10.565 was obtained with a silver and gold-based nanobowtie configuration, respectively, under an input power density of only $10 \text{ mW}/\mu\text{m}^2$, more than a tenfold improvement over the performance reported in previous works [19, 24, 42].

A comparative overview of these results is summarized in Table 4.2, which contrasts the achieved stability factors with those reported in previous experimental and numerical works, along with the associate particle sizes and input power densities.

Table 4. 2 Comparison of trapping stability for various configurations, input powers, and nanoparticles sizes [12].

	Configuration	Type of Work	Trapping Stability	Size of the Trapped Particle	Input Power Density	Ref.
1.	Dielectric Nanobowtie	Simulated device	1.2	100 nm	$6 \text{ mW}/\mu\text{m}^2$	[19]
2.	Slot Silicon Waveguides	Experimental device	10	75 nm	$>250 \text{ mW}/\mu\text{m}^2$	[49]
3.	All dielectric Nanobowtie	Simulated device	≈ 3	10 nm	$1 \text{ mW}/\mu\text{m}^2$	[50]
3.	Dielectric-Plasmonic Nanobowtie	Simulated device	10.565	100 nm	$10 \text{ mW}/\mu\text{m}^2$	Proposed Au
4.	Dielectric-Plasmonic Nanobowtie	Simulated device	10.370	100 nm	$10 \text{ mW}/\mu\text{m}^2$	Proposed Ag

Fig. 4.11 illustrates the trapping stability changes with increasing input power. A critical threshold is identified below which the optical forces are no longer sufficient to overcome Brownian motion, and the trapping stability factor falls below unity. For both configurations, this lower limit is approximately $0.7 \text{ mW}/\mu\text{m}^2$.

Importantly, the proposed nanobowtie exhibits substantial performance improvements over earlier approaches. For instance, while the configuration in [19] achieves $S = 1$ using an input of $6 \text{ mW}/\mu\text{m}^2$, the hybrid plasmonic-dielectric architecture presented here reaches the same stability with just $1 \text{ mW}/\mu\text{m}^2$.

This enhancement is primarily due to the localized electric field between the facing silicon tips, which intensifies the interaction between the electromagnetic field and the nanoparticle. The efficiency of the trapping mechanism is also closely linked to the physical dimensions of the trapped particle.

Trapping nanoparticles with diameters below the wavelength of light (i.e., $d < \lambda$) is inherently more challenging, as the optical force scales with the cube of the particle radius. The total optical force comprises a scattering component, directed along the beam propagation, and a gradient component, directed toward the region of highest field intensity.

As the particle size increases, the scattering force dominates, potentially overpowering the gradient force and destabilizing the trap. Furthermore, the thickness of the dielectric silicon arms significantly influences the field distribution and, consequently, the trapping efficiency.

To evaluate these effects, a parametric study was conducted by varying both the particle diameter d and the silicon layer thickness t , as illustrated in Fig. 4.12. The results demonstrate that smaller particles are associated with higher stability values, and thinner dielectric layers moderately enhance the trapping strength by concentrating the electromagnetic field more effectively. Nonetheless, the particle diameter plays a more decisive role than dielectric thickness in determining trap stability.

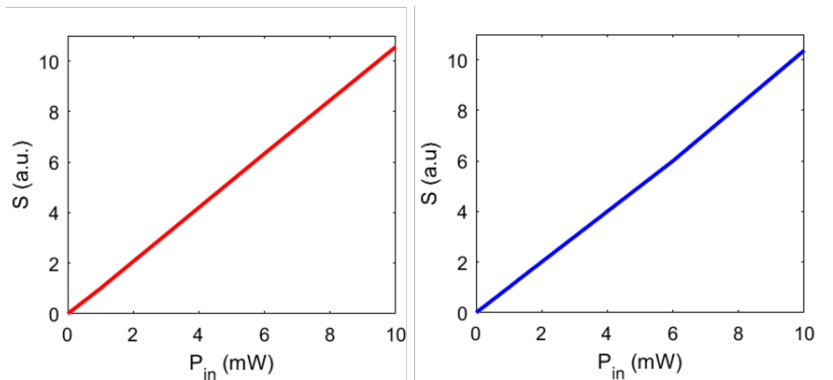


Fig. 4.11 Trapping Stability vs input power density: gold-layer configuration (left); (b) silver-layer configuration (right) [12].

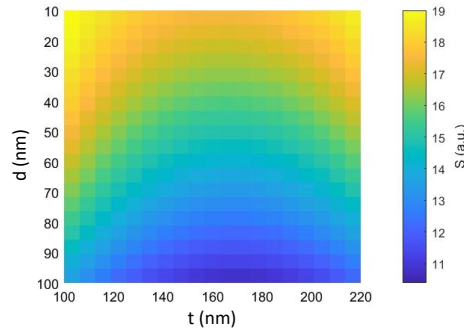


Fig. 4. 12 3D surface plot of trapping stability as a function of particle diameter and dielectric layer thickness with $P_{in} = 10 \text{ mW}/\mu\text{m}^2$ (silver-coated configuration) [12].

For the proposed geometry, a diameter of 100 nm and a dielectric thickness of 220 nm were selected as a trade-off between fabrication feasibility and compatibility with biologically relevant particles, consistent with values used in prior studies [19, 23, 24]. To further characterize the trap, the stiffness k_i was calculated as the derivative of the restoring optical force with respect to the particle displacement along each spatial direction ($i = x, y, z$). This metric reflects the strength of confinement near the equilibrium position and is commonly used to assess trap robustness. Previous optical tweezers designed for polystyrene beads with diameters of 20 – 100 nm reported stiffness values of $\sim 0.01 \text{ fN}/(\text{nm} \cdot \text{mW})$ [24] and up to $0.3 \text{ fN}/\text{nm}$ for 100 nm beads at 10 mW input power [10, 45, 51, 52]. In this work, significantly higher values were obtained: $k_z = 2.238 \text{ fN}/\text{nm}$ for the silver-coated device and $k_z = 2.293 \text{ fN}/\text{nm}$ for the gold-coated version, over a displacement range from 60 to 500 nm. These results clearly demonstrate the superior confinement performance of the proposed device, particularly for nanoparticles around 100 nm in diameter, a size range characteristic of several viruses. The combination of high stability and high stiffness indicates that this platform is well-suited for bio-nanomanipulation tasks, enabling long-duration, high-precision trapping of biological particles under realistic operating conditions.

4.3.4 Trapping and destruction of virus through dielectric-plasmonic hybrid nanobowtie dimer

The same hybrid dielectric-plasmonic nanobowtie can also be exploited for focalized thermal inactivation of viruses confined at the gap by precisely tuning the input optical power. Increasing the power density enhances near-field absorption, leading to a controlled temperature rise within the trapping region. This section introduces the dual functionality of the device, nanoparticle manipulation and selective thermal deactivation, and describes the simulation employed to correlate input power with local temperature, while identifying operating windows that maintain confinement stability and ensure thermal safety for the surrounding medium.

4.3.4.1 Thermal inactivation of virus

Thermal inactivation represents a fundamental mechanism to reduce or eliminate viral infectivity, and it has gained increasing attention as an alternative or complementary strategy to pharmacological and immunological approaches. While vaccines and antiviral drugs are being developed for viral diseases such as SARS-CoV-2, viral mutations remain a critical challenge, underscoring the importance of efficient inactivation and disinfection methods. Environmental conditions, including temperature, humidity, and airflow, play a central role in determining viral persistence and transmission dynamics.

Respiratory infections often follow seasonal patterns, reflecting the periodic variability of humidity and temperature [53]. It has been shown that influenza viruses are more stable in cold and dry conditions, or in highly humid environments, where their inactivation rate is reduced [54]. Similarly, SARS-CoV-2 is unable to survive outside a host organism without suitable environmental conditions. In general, most viruses persist longer at very low ($\leq 30\%$) or very high ($\geq 84\%$) relative humidity, whereas intermediate values (40-60 %) lead to significant inactivation [55]. Moreover, viral infectivity decreases exponentially as temperature rises from 14 °C to 34 °C [56], and elevated temperatures have consistently been associated with high inactivation rates. The mechanisms are primarily related to protein denaturation and disruption of weak molecular interactions that sustain the viral capsid and envelope, resulting in the loss of structural integrity and reduced infectivity.

Overheating also induces degradation of the viral capsid, with denaturation of proteins leading to the collapse of the spherical morphology. For example, in the case of SARS-CoV-2, the phospholipid membrane is particularly sensitive to temperature and may rupture under thermal stress, exposing the nucleic acids (RNA or DNA) contained inside the envelope. This facilitates genomic analysis, mutation tracking, and the development of infection control strategies. The outer spike glycoproteins, which confer the typical crown-like appearance, are also highly temperature sensitive, and their destabilization further compromises viral infectivity (Fig. 4.13).

Conventional methods of thermal inactivation, such as steam sterilization at 121-135 °C, are highly effective against a wide range of pathogens. However, such extreme conditions are not always compatible with sensitive materials and may not be accessible in all settings. More recent studies have shown that SARS-CoV-2 can be effectively inactivated at significantly lower temperatures (around 65°C), highlighting the potential for milder thermal treatments [57, 58].

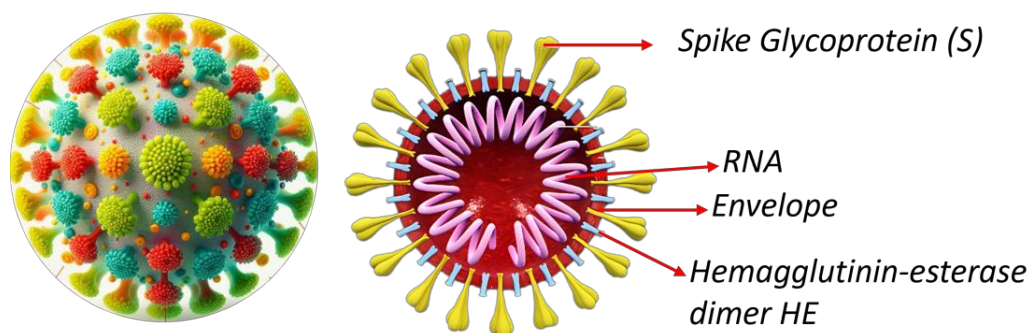


Fig. 4. 13 Schematic representation of a complete and sectioned SARS-CoV-2 virion illustrating the structural elements most affected by thermal stress.

Among emerging approaches, infrared (IR) radiation has been investigated as a promising physical technique for viral inactivation. Compared with ultraviolet (UV) irradiation or chemical disinfectants such as alcohol IR offers health and environmental benefits and is already widely used in therapeutic applications. IR heating transfers thermal energy via electromagnetic waves, with sources emitting across a spectrum ranging from near-IR (0.7 μm) to far-IR (1.0 nm). FIR sources are particularly effective surface heaters, capable of raising local temperatures without significantly affecting the ambient environment. A recent study characterized multiple IR sources and demonstrated that FIR radiation could achieve a 90% inactivation rate of bacteriophage Phi8 (a model for enveloped viruses) in a closed chamber at 50% relative humidity after three hours for exposure, while 45.7 % inactivation was observed under open-air conditions with airflow [59]. The FIR source used in these experiments emitted radiation at a wavelength of approximately 7.9 μm and generated surface temperature around 120 $^{\circ}\text{C}$.

In this context, the hybrid dielectric-plasmonic nanobowtie investigated in this work was conceived to operate not only as an efficient optical trapping device but also as a localized thermal inactivation tool. By carefully tuning the input optical power, the structure enables precise temperature modulation at the trapping site, thereby preserving the integrity of the biological nanoparticle under standard operating conditions while providing the possibility of inducing viral deactivation when required. This dual functionality is particularly advantageous for LoC system diagnostic applications, where compactness, integration with microfluidics, and multifunctionality represent key requirements. The following section present 3D FEM simulations that validate this approach, highlighting the corresponding thermal response under different excitation conditions.

4.3.4.2 Results

3D FEM simulations confirmed that embedding a metallic layer beneath the silicon wedges significantly enhances electromagnetic confinement in the trapping site. A 70 nm gold layer yielded an increase of approximately 90.93% in the local field energy density compared with the purely dielectric configuration.

A parametric variation of the input optical power density (P_{in}) was then performed with the dual objective of preserving trapping stability while simultaneously inducing a temperature rise sufficient to inactivate viral particles. The trapping stability S , introduced in the previous section, was employed as the primary performance indicator. Values of $S > 10$ are generally required to counteract thermal fluctuations and to ensure sustainable confinement of nanoscale bio-objects [22]. Simulation results demonstrated that $P_{in} = 10 \text{ mW}/\mu\text{m}^2$ satisfies both conditions, yielding $S > 10$ and a temperature increase $\Delta T > 65 \text{ K}$, corresponding to the inactivation threshold for many enveloped viruses. The relationship between ΔT and P_{in} followed an approximately linear trend (Fig. 4.14), with ΔT remaining below $\sim 140 \text{ K}$ for $P_{in} < 20 \text{ mW}/\mu\text{m}^2$. Within this operating range, the device enables stable trapping while simultaneously supporting photothermal viral deactivation.

Importantly, the required power densities are more than one order of magnitude lower than those typically reported for conventional photothermal inactivation methods [60], underscoring the efficiency of the proposed hybrid nanobowtie. Such energy savings are particularly advantageous for LoC implementations, where compactness, minimized heat generation, and compatibility with microfluidic integration are essential for clinical applicability.

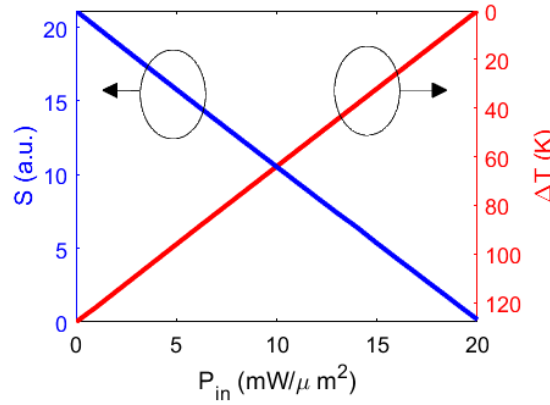


Fig. 4. 14 Simulated temperature increase (ΔT , red curve) in the trapping site and trapping stability factor (S , blue curve) as a function of input optical power density for the nanocavity incorporating a gold layer [25].

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Chapter 5

Conclusions

The dissertation advances nanophotonics to address two key constraints in healthcare technology: the development of sensitive, readily integrable photonic biosensors for liquid biopsy in oncology and the controlled trapping and manipulation of viral scale bio-objects for infectious-disease workflows. It advances both strands, from device design to system integration, demonstrating that photonic technologies can underpin decentralised, real-time, patient-centred diagnostics.

On the oncology front, two complementary sensing strategies were conceived and developed to enable LoC integration and wafer-level manufacturability. The first platform, a 1D photonic crystal waveguide with engineered defects, was designed to yield a flat, box-like resonance that enhances spectral interrogation while reducing susceptibility to intensity noise and drift, two practical limitations that often compromise refractometric readout in real samples. A systematic design study identified an optimal configuration delivering $Q \approx 1.2 \times 10^4$, $ER = 15.35$ dB, roll-off = - 10.50 dB/oct, $CR = 74.06$ %, thereby achieving a stable, highly resolvable flat-top profile. Surface and bulk sensitivities were estimated as $S = 21.47$ nm/RIU and $S_\lambda = 490.49$ nm/RIU, respectively; the FWHM ≈ 126 pm supports a figure of merit well beyond typical SPR values, indicating strong potential for label-free biosensing.

The second platform, the slot-assisted metasurface, exploits the optical-slot effect and an anapole-mode condition to enhance near-field confinement while preserving a compact, wafer-scalable footprint. A trade-off design achieves $Q = 3.19 \times 10^3$ with a resonance amplitude > 0.7 . Upon antibody surface functionalization, the device exhibits a ≈ 3 nm red-shift ($S \approx 25$ nm/RIU) and an IgG sensing slope of ≈ 1.8 nm/(ng·mL⁻¹) over 1pg/mL– 1 ng/mL⁻¹, approaching plasmonic-sensor performance while retaining high signal amplitude. The anapole regime is confirmed near $\lambda \approx 651.6$ nm, providing the field localization required for refractometric transduction at clinically relevant concentrations.

Both platforms were designed for label-free detection, with IgG employed as a model tumour-associated biomarker at diagnostically relevant concentrations. All

design choices – from the box-like resonance shaping of the PhC for stable fixed wavelength amplitude tracking, to the anapole assisted slot confinement in the metasurface for enhanced near-field sensitivity under normal incidence operation – were conceived to optimise the device performance as a biosensing platform. While IgG served as demonstrator, the derived design principles are not biomarker specific. They can be directly extended to other tumour associated proteins, cells, and nucleic acids of clinical interest, enabling multiplexed LoC systems capable of simultaneously screening biomarkers signatures. From this perspective, the thesis contributes not only two high performance photonic devices but also a transferable design framework that can be leveraged in future work toward on-chip functionalisation strategies, microfluidic integration, and validation with clinically derived samples, thereby consolidating the role of nanophotonic in advancing early cancer diagnostics through liquid biopsy.

Despite the advances achieved, the work to date is rooted in Multiphysics modelling under idealised boundary conditions for materials, interfaces and biological fluids flow. The next phase will involve experimental realisation and system-level integration within a LoC stack so that device-level figures of merit translate into end-to-end diagnostic performance. For the PhC sensor, a CMOS compatible SOI process flow (deep-UV or e-beam lithography, sidewall grating/defect definition, and low-loss passivation) will be exercised through wafer-level fabrication. For the slot-assisted metasurface, Plasma Enhanced Chemical Vapor Deposition (PECVD) of a-Si:H on glass, followed by dry etch patterning, will enable targeted normal-incident operation and a compact footprint. Both dies will be packaged with alignment features for chip-to fluidic bonding and optical I/O (fiber stubs or windowed free space coupling), anticipating wafer-scale throughput and repeatable assembly.

Readout will be streamlined to match the resonance physics of each platform : (i) the PhC's engineered flat-top line shape will be optimised for single wavelength amplitude tracking at a fixed bias point on the flat-top plateau, with an on-chip signal/reference photodiode pair for radiometric normalisation; (ii) the metasurface will support normal incidence interrogation using either a miniature spectrometer or a single-wavelength lock operated near the maximum-slope point. In both cases, intensity stabilisation and background subtraction will be implemented in mixed-signal ASICs collocated with the sensor to suppress source drift and fiber-coupling fluctuations.

At system level, the LoC will implement the traditional modules: sample introduction, transport/mixing, reaction/incubation, separation/enrichment, and optical detection, with electronics for actuation and signal processing. This system architecture is already outlined in the thesis and forms the foundation for the proposed sensors as transducers. Practical measures will include plasma on-chip or blood-to-plasma conversion, low-volume mixers for reagent exchange, antifouling channel coatings, and bubble management to protect immobilised capture layers.

An air bypass geometry will be adopted to purge interstitial air during solution exchanges, thereby preventing scattering artefacts and antibody denaturation, while preserving sub- μL working volumes and keeping the total assay time below thirty minutes when, for example, ELISA-style incubation is required. For label-free operation in complex biofluids, the sensing surface will require robust immobilisation (e.g. silane/amine coupling or polymer) and non-specific absorption control (PEG or zwitterionic layers), plus on-chip regeneration protocols (low pH/high-salt cycles) qualified for repeated use. Channel designs will isolate the sensing chamber to confine functionalisation and avoid reagent losses, with antibody deposition restricted to the chamber. To move beyond IgG, the same chip will host multiple sensing sites, each functionalised with a different capture probe (e.g. HER2, CEA, PSA), while a common microfluidic path will deliver the sample to all sites and a dedicated reference site will stabilise the readout against drift.

Overall, two LoC prototypes are outlined below:

- (i) PhC – LoC: single wavelength amplitude tracking on the engineered flat-top resonance, with on chip signal/reference photodiodes and simple reagent exchange in microfluidic chambers.
- (ii) Metasurface – LoC: normal-incidence interrogation with compact LED/photodiode readout and a solution exchange layout that prevents air intrusion during rapid switching.

Both configurations are designed to operate with sub- μL working volumes and short total assay times.

In parallel with the development of biosensors for liquid biopsy, the thesis also addresses the challenge of manipulating viral scale bio-objects, introducing an inverted hybrid dielectric-plasmonic nanobowtie cavity tailored for controlled trapping and thermal inactivation. By combining plasmonic field enhancement from an embedded Au/Ag layer with low-loss dielectric confinement in a bowtie shaped SOI cavity, the device achieves stable trapping of ~ 100 nm bio-objects under physiologically compatible conditions. Numerical simulations confirmed an optical force of 2.9 fN with a stability trapping > 20 and stiffness exceeding 2.2 fN/nm for trapping of a 100 nm nanoparticle, representative of viral families such as Coronaviridae, Influenza A, HIV, as well as extracellular vesicles. Beyond enhanced stability compared to purely dielectric or plasmonic counterparts, controlled modulation of the incident power enables a localised temperature rise, providing a dual functionality: electromagnetic confinement for long term trapping and selective thermal deactivation of pathogens without device reconfiguration. This combined capability outlines a compact route to rapid functional characterisation and control of pathogens within microfluidic environments, reinforcing the broader claim of this thesis for integrated photonic platforms that seamlessly couple measurement with manipulation. Subsequent work will refine the nanobowtie at the pattern level, e.g., multi element dimer geometries, to increase optical force and

stiffness at constant input power while preserving readout compatibility. The ΔT – P_{in} operating window will be characterised experimentally and used to parameterise closed-loop power control, maintaining $S > 10$ for stable trapping and reaching $\Delta T \geq 65$ K only when thermal inactivation is required. To this end, on-chip thermometry (micro-RTD or fluorescence probes) and duty-cycle excitation will limit peak temperature and calibrate the dose, response surface linking exposure time, temperature rise, and optical power for virus-like particles. To raise throughput, wafer level arrays of nanobowties will be fabricated for parallel trapping and manipulation of 100 nm targets, extending to extracellular vesicles and outer-membrane vesicles; array-to-array uniformity in field distribution and thermal response will be verified against single-site metrics. The cavity will be embedded in microfluidic systems (fluidic conditioning, low-volume exchange, antifouling and bubble control).

Multimodal analytics will be integrated by coupling the trap with single molecule fluorescence and SERS, enabling a compact capture, spectral characterization, controlled thermal inactivation workflow in the same chamber. Validation will proceed in stages: fabrication and packaging; quantitative mapping of stiffness and stability versus P_{in} and ΔT in physiological buffers; demonstration of long-retention trapping of ~100-nm particles; and determination of inactivation thresholds on viral surrogates. Reporting efficiency figures (e.g., P_{in} per stability unit, ΔT per $\text{mW} \cdot \mu\text{m}^{-2}$) will substantiate the advantage over purely dielectric or plasmonic configurations.

Finally, application-oriented studies in infectious disease and oncology will exploit the ability to detect, trap, and inactivate nanoscale bio-objects on-chip to support diagnostics, therapy monitoring, and drug-response assays at the point of need.

Appendix A

COMSOL Multiphysics environment and FEM-based Simulation Approach

COMSOL Multiphysics® software environment provides both fully coupled Multiphysics capabilities and dedicated single-physics modules, ensuring a rigorous and flexible modelling framework.

The Model Builder includes all the steps in the modelling workflow from defining geometries, material properties, and the physics that describe specific phenomena to performing computations and evaluating the results. Among the available numerical methods, the FEM stands out as one of the most established and versatile numerical techniques for solving partial differential equations (PDEs) that govern complex physical phenomena in engineering and applied physics [A.1]. FEM enables the numerical resolution of boundary value problems by discretizing the computational domain into a finite number of subdomains, referred to as finite elements, over which the governing equations are approximated. This spatial discretization process is implemented through the generation of a computational mesh, which defines the nodes and elements used to evaluate field quantities. By transforming continuous differential equations into a set of algebraic equations, the FEM allows for the approximation of the unknown field variables throughout the domain. The solution process involves minimizing a global error functional derived from the variational formulation of the governing equations. This approach ensures accurate numerical solutions even in geometrically complex and heterogeneous domains, such as those encountered in hybrid dielectric-plasmonic nanostructures.

A.1 Perfectly matched layer (PML)

PML are artificial absorbing regions that surround the physical domain and allow for the simulation of open boundaries by preventing non-physical reflections at the outer interface. This technique is particularly effective for all wave types and angles of incidence, as it absorbs outgoing electromagnetic radiation regardless of direction or waveform. In the frequency domain, the PML achieves this behaviour through a complex coordinate transformation that maintains the wave impedance across the boundary, thereby suppressing reflections at the interface [A.2].

In the context of the design of the device proposed in [Chapter 4](#), the substrate thickness was modelled to be on the order of millimeters, i.e., several orders of magnitude larger than the trapping cavity, to ensure the consistent implementation

of the PMLs and the appropriate decay of the radiated fields.

References

- [A.1] I. Ahmed, "Numerical Methods for Solving Partial Differential Equations in Applied Physics," *Frontiers in Applied Physics and Mathematics*, vol. 1, no. 1, 2024, pp. 79-96
- [A.2] J. P. Bérenger, "*Perfectly matched layer (PML) for computational electromagnetics*," San Francisco: Morgan & Claypool Publishers, vol. 8, 2007

Appendix B

Mathematical description of Anapole Modes in dielectric nanostructures

From a physical standpoint, the excitation of anapole modes can be described through the analysis of the polarization current density $\mathbf{J}(\mathbf{r})$, induced in the nanostructure as a response to the incident field [B.1, B.2]. This current is given by:

$$\mathbf{J}(\mathbf{r}) = j\omega(\epsilon_p - \epsilon_b)\mathbf{E}(\mathbf{r})$$

Where $\mathbf{E}(\mathbf{r})$ is the electric field, ω is the angular frequency, and $\epsilon_p = \epsilon_{r,p}\epsilon_0$ and $\epsilon_b = \epsilon_{r,b}\epsilon_0$ denote the permittivity of the nanocuboid and the background medium, respectively, with ϵ_0 being the vacuum permittivity. The corresponding electric ($\mathbf{p}$), magnetic ($\mathbf{m}$), and toroidal ($\mathbf{t}$) dipole moments can be expressed as:

$$\begin{aligned}\mathbf{p} &= \frac{1}{j\omega} \int_V \mathbf{J}(\mathbf{r}) d\mathbf{r} \\ \mathbf{m} &= \frac{1}{2v_b} \int_V [\mathbf{r} \times \mathbf{J}(\mathbf{r})] d\mathbf{r} \\ \mathbf{t} &= \frac{1}{10 v_b} \int_V [(\mathbf{r} \times \mathbf{J}(\mathbf{r})) \mathbf{r} - 2r^2 \mathbf{J}(\mathbf{r})] d\mathbf{r}\end{aligned}$$

Electric and magnetic toroidal dipole excitations in core-shell spheres.

Where $v_b = c/n_b$ is the speed of light in the surrounding medium, and the integral is calculated over the volume of the nanoparticles. The electric magnetic and toroidal quadrupoles are calculated as:

$$\begin{aligned}Q_{\alpha\beta}^e &= \frac{1}{j2\omega} \int_V [r_\alpha J_\beta + r_\beta J_\alpha - \frac{2}{3} \delta_{\alpha\beta} (\mathbf{r} \times \mathbf{J}(\mathbf{r}))] d\mathbf{r} \\ Q_{\alpha\beta}^m &= \frac{1}{3v_b} \int_V \{[\mathbf{r} \times \mathbf{J}(\mathbf{r})]_\alpha r_\beta + [\mathbf{r} \times \mathbf{J}(\mathbf{r})]_\beta r_\alpha\} d\mathbf{r} \\ Q_{\alpha\beta}^t &= \frac{1}{28v_b} \int_V [4r_\alpha r_\beta (\mathbf{r} \times \mathbf{J}(\mathbf{r})) - 5r^2 (r_\alpha J_\beta + r_\beta J_\alpha) \\ &\quad + 2r^2 \delta_{\alpha\beta} (\mathbf{r} \times \mathbf{J}(\mathbf{r}))] d\mathbf{r}\end{aligned}$$

Where δ is the Dirac delta function. The mean square radii for the magnetic and toroidal dipoles are:

$$\overline{\mathbf{R}_m^2} = \frac{1}{2v_b} \int_V [\mathbf{r} \times \mathbf{J}(\mathbf{r})] r^2 d\mathbf{r}$$

$$\overline{\mathbf{R}_t^2} = \frac{1}{28v_b} \int_V [3r^2 \mathbf{J}(\mathbf{r}) - 2r(\mathbf{r} \times \mathbf{J}(\mathbf{r}))] r^2 d\mathbf{r}$$

The cross-sections corresponding to each multipole moment are given by:

$$C_{sca}^{(ed)} = \frac{k_b^4}{6\pi\epsilon_b^2} \left| \mathbf{p} - jk_b \left(\mathbf{t} + \frac{k_b^2}{10} \overline{\mathbf{R}_t^2} \right) \right|^2$$

$$C_{sca}^{(md)} = \frac{k_b^4}{6\pi\epsilon_b^2} \left| \left(\mathbf{m} - \frac{k_b^2}{10} \overline{\mathbf{R}_m^2} \right) \right|^2$$

$$C_{sca}^{(eq)} = \frac{k_b^6}{20\pi\epsilon_b^2} \sum_{\alpha\beta} |Q_{\alpha\beta}^e - j\frac{k_b}{3} Q_{\alpha\beta}^T|^2$$

$$C_{sca}^{(mq)} = \frac{k_b^6}{80\pi\epsilon_b^2} \sum_{\alpha\beta} |Q_{\alpha\beta}^m|^2$$

An incident plane wave with electric field amplitude of $|E_0| = 1 \text{ V/m}$ is considered, with $k_b = \frac{2\pi n_b}{\lambda}$ representing the wavevector in the host medium. In the analysis of anapole modes, it is useful to introduce the quantities C_{sca}^p and C_{sca}^t

$$C_{sca}^p = \frac{k_b^4}{6\pi\epsilon_b^2} |\mathbf{p}|^2$$

$$C_{sca}^t = \frac{k_b^6}{6\pi\epsilon_b^2} \left| \left(\mathbf{t} + \frac{k_b^2}{10} \overline{\mathbf{R}_t^2} \right) \right|^2$$

These terms represent the effective scattering cross-sections of the Cartesian electric and toroidal dipoles. Depending on their relative phase, the two contributions can interfere either constructively or destructively, with their combined effect on the total electric dipole resonance given in Equation (3.10). Evaluating Equations (3.14) -(3.25) separately is useful for device optimization and for clarifying the origin of anapole excitation, which arises at the intersection of C_{sca}^p and C_{sca}^t . The toroidal moment $\mathbf{t}$ contributes to $C_{sca}^{(ed)}$ through the factor $-\frac{jk_b}{10v_b}$, making it sensitive to the permittivity. This dependence highlights the potential to tune anapole modes via the surrounding environment, a feature of relevance for refractometric sensing.

References

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- [B.2] J. Wang, J. Yang, Y. Mei, "Non-radiating anapole state in dielectric nanostructures and metamaterials," *Journal of Physics D: Applied Physics*, vol. 58, no. 20, 2025, pp. 203001, doi: <https://doi.org/10.1088/1361-6463/adb590>

Appendix C

Parametric Analysis and Optimization of the dielectric metasurface

Preliminary simulations were carried out considering an infinite array ($N = \infty$), with $\Lambda = 300$ nm and $DC = 50\%$ as initial design parameters. Under these conditions, two distinct resonances arise, associated with different spatial distributions of the electric field within the unit cell. The mode exhibiting in-plane confinement is of particular interest, since it yields a higher Q-factor compared to the resonance dominated by out-of-plane fields, thereby paving the way to enhanced sensitivity. The separation between the two resonance wavelengths was maximize by adjusting the duty cycle to avoid spectral overlap and to preserve the signal to noise ratio. To ensure resonant operation within the target spectral window, a parametric analysis of the Λ was carried out. Different values of Λ were investigated, namely 420 nm, 440 nm, 450 nm, 460 nm, and 480 nm. Fig. C.1 illustrates the transmission spectra of the metasurface as a function of the period, while Fig. C.2 reports the evolution of the resonance wavelength corresponding to the maximum ER. As expected, increasing Λ results in a progressive red shift of the resonance, consistent with Bragg's law.

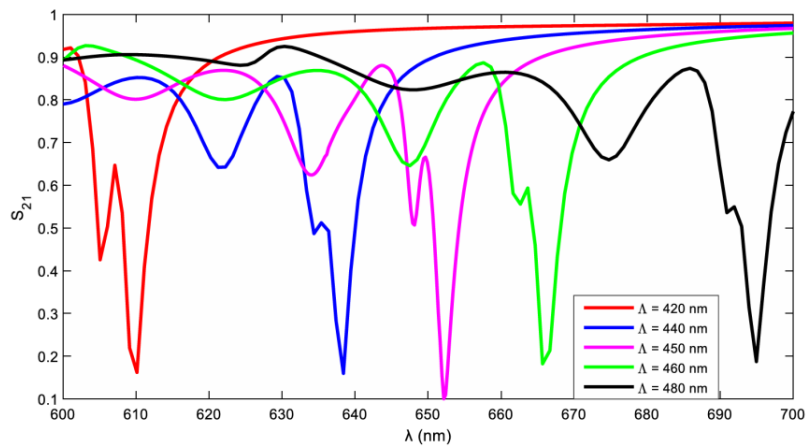


Fig. C. 1 Transmission spectrum as the parameter Λ changes.

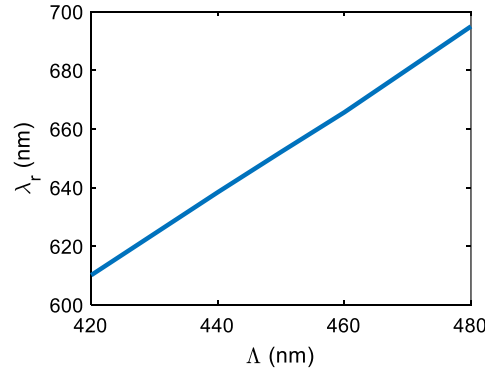


Fig. C. 2 Resonance wavelength shifts as a function of the period (Λ).

Among the tested configurations, a period of $\Lambda = 450$ nm was identified as the optimal design point, yielding a resonance spectrum centred within the desired wavelength range. The corresponding transmission response is shown in Fig. C.3, where three distinct resonance peaks can be observed at $\lambda_1^* = 634.1$ nm, $\lambda_2^* = 648.1$ nm, and $\lambda_3^* = 652.1$ nm.

The spatial distribution of the electric field associated with these resonances is reported in Fig. C.4. At λ_1^* , the field profile reveals a TE-like mode, whereas at λ_2^* the resonance corresponds to a TM-like mode. Finally, λ_3^* is characterized by the excitation of a leaky mode, in which part of the energy radiates into the surrounding medium. These results confirm the multimodal nature of the proposed structure and highlight the relevance of mode selection in optimizing the sensor performance. To further refine the spectral response of the proposed device, particular attention was devoted to the separation between the resonances λ_2^* and λ_3^* . As highlighted in Fig. C.2, the proximity of these two peaks may lead to crosstalk effects, thereby complicating the reliable identification of the resonance of interest. To mitigate this issue, the influence of duty cycle (DC) on the spectral behaviour was systematically investigated.

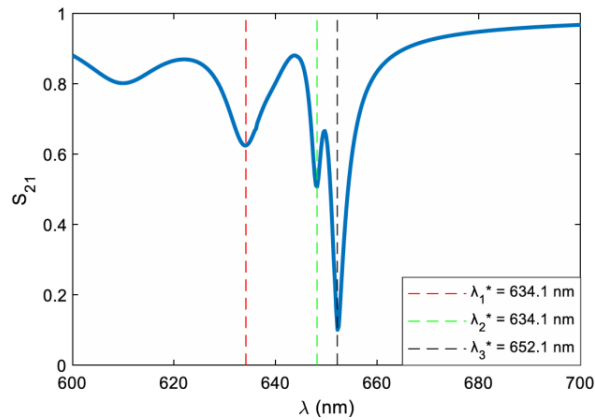


Fig. C. 3 Transmission spectrum of the metasurface for a $\Lambda = 450$ nm.

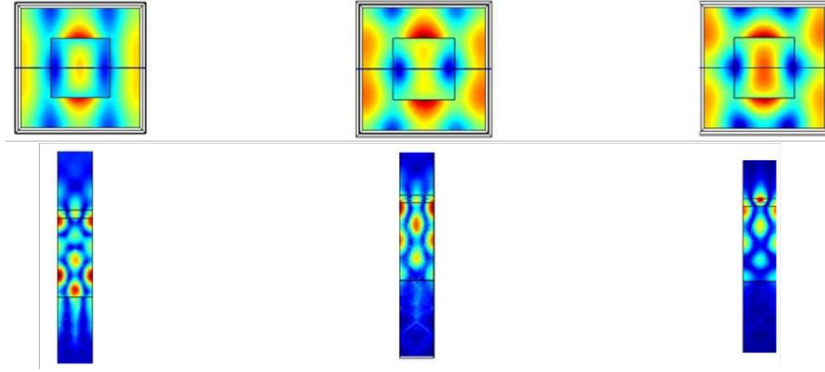


Fig. C. 4 Normalized electric field distribution at $\lambda_1^* = 634.1$ nm (*left*); at $\lambda_2^* = 648.1$ nm (*center*); $\lambda_3^* = 652.1$ nm (*right*), corresponding to TE-like, TM-like, and leaky modes, respectively.

The analysis was carried out for $\Lambda = 450$ nm while varying the DC in the range between 20% and 80%, a choice that also accounts for fabrication feasibility. The transmission spectra corresponding to representative values of DC = 20%, 50% and 80% are reported in Fig. C.5 *left*, whereas Fig. C.5 (*right*) quantifies the dependence of the resonance separation $\Delta = |\lambda_3^* - \lambda_2^*|$ on the duty cycle. The results reveal that increasing DC leads to a progressive enlargement of Δ , thus facilitating clearer discrimination between the two resonances. At DC = 80% the separation reaches $\Delta = 22$ nm, a value sufficient to suppress crosstalk and ensure unambiguous resonance tracking. The transmission spectrum associated with this configuration is shown in Fig. C.6 *left*.

Despite this improvement, the resonances remain close to the spectral boundaries of the targeted operating window, which may reduce sensing robustness under realistic conditions. To address this limitation, the periodicity was slightly reduced to $\Lambda = 430$ nm. The resulting transmission spectrum, displayed in Fig. C.6 (*right*), confirms the effectiveness of this adjustment. At this design point, the resonance λ_2^* exhibits ER of 10 dB and Q-factor $\approx 1.36 \times 10^3$, demonstrating the potential of the structure to deliver both high spectral selectivity and enhanced refractometric sensitivity.

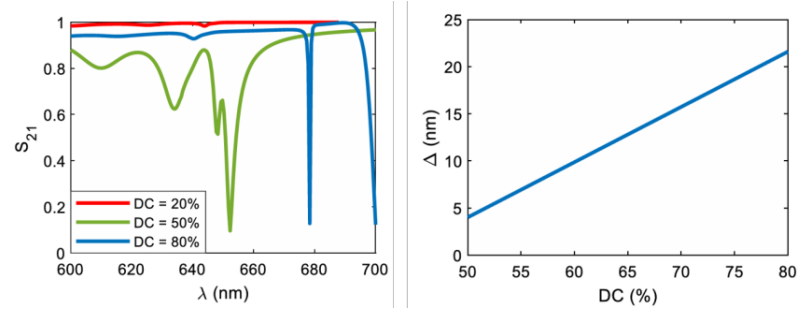


Fig. C. 5 Transmission spectrum of the metasurface for DC values of 20%,50%, and 80% (*left*); Evolution of the resonance separation Δ as a function of the DC (*right*).

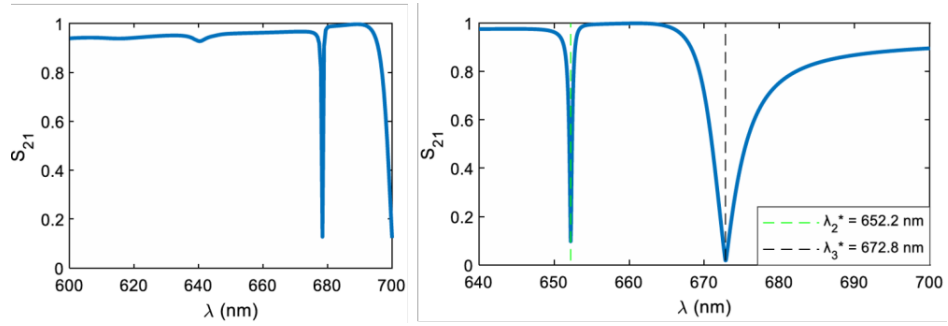


Fig. C. 6 Transmission spectrum of the metasurface for a duty cycle of 80% (*left*); Transmission spectrum of the metasurface for DC = 80% and $\Lambda = 430$ nm (*right*).