# Design Strategies and Chemical Modifications in Fluorescence Imaging and Molecular Sensing: A Survey

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#### **Abstract**

This survey explores the advancements in design strategies and chemical modifications that have significantly enhanced fluorescence imaging and molecular sensing technologies. Emphasizing the integration of advanced materials such as graphene-based plasmonic devices and semiconducting single-walled carbon nanotubes (SWNTs), the survey highlights improvements in imaging resolution and sensing precision, crucial for applications in environmental monitoring and biomedical diagnostics. Key developments include optimizing SWNT synthesis for increased brightness and exploring diverse biomedical applications. The role of chemical modifications, including solvent effects and doping strategies, is underscored for optimizing platform performance. Despite progress, challenges such as low signal-to-noise ratios, material limitations, and environmental interferences persist. The complexity of integrating new technologies into existing systems and computational challenges in real-time data analysis further complicate the field. Future research should focus on optimizing imaging frameworks, improving detector efficiency, and enhancing data quality assessment. Additionally, exploring novel materials and engineering specific defects in graphene to optimize plasmonic properties and sensor performance presents promising research opportunities. The survey concludes by emphasizing the dynamic nature of the field, offering prospects for continued innovation and impactful applications across scientific and medical disciplines, aiming to address existing challenges and explore novel research directions for significant advancements in diagnostic and therapeutic applications.

# 1 Introduction

#### 1.1 Significance of Fluorescence Imaging and Molecular Sensing

Fluorescence imaging and molecular sensing are vital in biomedical and scientific research, providing critical insights into the molecular dynamics of live cells and complex biological systems [1]. These techniques facilitate high-resolution visualization of cellular and subcellular structures, essential for comprehending intricate biological processes [2]. In the biomedical domain, fluorescence-guided surgery has proven indispensable for tumor resection, highlighting the role of fluorescence imaging in improving surgical precision and outcomes [3].

The NIR-IIb imaging method significantly enhances the visualization of lipid droplets within living cells and tissues by minimizing photon scattering and autofluorescence, allowing deeper tissue penetration and improved spatial resolution. This advancement is crucial for applications necessitating deep tissue imaging, such as centimeter-deep fluorescence microscopy, which uncovers vital in vivo micro-information. However, challenges like phototoxicity and imaging through scattering continue to limit the depth and quality of fluorescence microscopy [4].

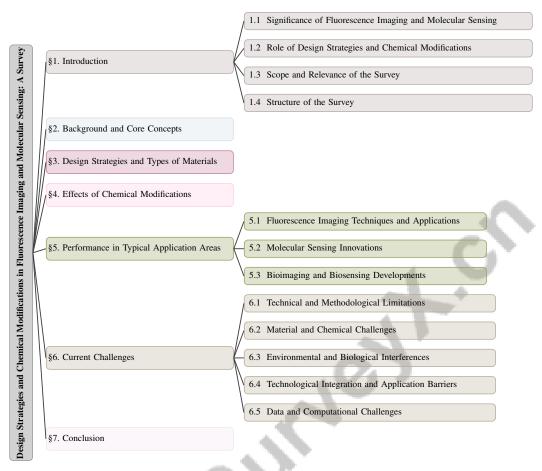


Figure 1: chapter structure

Despite its importance in exploring subcellular structures, fluorescence microscopy often entails time-consuming sample preparation, high costs, and potential image misinterpretation due to photobleaching [4]. The integration of fluorescence imaging with molecular sensing has revolutionized the understanding of material properties and biological processes, driving innovations in medical diagnostics and therapeutic monitoring [2]. These techniques are pivotal in enhancing diagnostic accuracy, reinforcing their essential role in scientific research and medical advancements.

## 1.2 Role of Design Strategies and Chemical Modifications

Advancements in imaging and sensing techniques hinge on design strategies and chemical modifications that optimize sensitivity, specificity, and resolution. Enhancing light-matter coupling is critical for improving imaging and sensing efficiency, achieved through strategic design and modifications [2]. One innovative approach involves leveraging solvent effects to develop efficient two-photon absorption molecules with aggregation-induced emission characteristics, thus enhancing imaging capabilities [5].

The incorporation of machine learning, exemplified by the VirFluoNet model using a conditional generative adversarial network (cGAN), streamlines the imaging process by predicting fluorescence images from phase contrast or other fluorescence images, thereby facilitating more efficient workflows [4]. Such advancements underscore the importance of design strategies in refining imaging techniques.

Chemical modifications significantly enhance the functionality of imaging and sensing materials. For instance, introducing structural defects in materials like graphene can markedly improve their performance in plasmonic applications, which are crucial for molecular sensing by enhancing light interaction and thereby increasing sensitivity and specificity [2].

The integration of advanced imaging and sensing strategies, including x-ray fluorescence sectioning for elemental analysis, fluorescence imaging for intraoperative tissue characterization, and wearable biosensors for continuous molecular monitoring, is paramount for the evolution of these technologies. These innovations are driving significant advancements in scientific research and medical applications, enabling accurate diagnostics and real-time health monitoring [6, 7, 8, 9, 10].

#### 1.3 Scope and Relevance of the Survey

This survey aims to provide a comprehensive analysis of fluorescence imaging and molecular sensing methodologies, emphasizing their significance in advancing scientific research and applications. It explores the synthesis, properties, and applications of highly fluorescent copper nanoclusters (CuNCs), which are essential for sensing and bioimaging due to their unique optical properties [11]. Additionally, the survey examines the temporal resolution of fluorescence imaging, crucial for accurately capturing dynamic biological processes [12].

In the context of quantum sensing, the survey investigates diamond surface properties and NV-center physics, focusing on engineering strategies to enhance quantum sensing capabilities [13]. It also highlights nanopore-based DNA sequencing sensors, covering both established and emerging modalities integral to molecular measurement and characterization [14]. Furthermore, the survey addresses next-generation transmission electron microscopy, emphasizing data-driven approaches vital for material characterization [15].

The design and application of hybrid xanthene dyes are examined, focusing on their photophysical properties and potential use in cancer imaging and photodynamic therapy [16]. Low-light optical imaging techniques, including the performance of advanced cameras like EMCCD, qCMOS, and sCMOS, are analyzed for their relevance in biological applications [17]. The survey also discusses physiological imaging modalities, particularly fluorescence imaging, and the challenges associated with data collection and extraction [6].

Advancements in aptamer chemistry are explored to enhance pharmacological properties, highlighting their significance in current scientific research [18]. The survey covers plasmon-free surface-enhanced Raman scattering (SERS) substrates, detailing their mechanisms, enhancement strategies, and biomedical and environmental sensing applications [19]. The optimization of gene expression sensors in E. coli for synthetic biology and metabolic engineering applications underscores the survey's relevance to biotechnological innovations [20].

The survey encompasses various device configurations for DNA sequencing, specifically focusing on detecting transverse electrical currents, pertinent to current scientific research [21]. FRET-based probes are included, emphasizing their relevance in biological applications and the necessity for specific donor-acceptor pair configurations [22]. The focus on calix[4]arenes and their derivatives illustrates their applications in sensing metal ions, anions, and biomolecules, enriching the survey's comprehensive scope [23].

Advancements in graphene-based terahertz (THz) devices are discussed, highlighting their applications in molecular sensing and wave modulation, which emphasizes the survey's relevance to current scientific research [24]. The survey specifically addresses nanodiamonds, focusing on production methods, surface functionalization, and biomedical imaging applications, bridging knowledge gaps in this area [25]. Additionally, the introduction of solutions achieving real-time pixel readout rates faster than modern EMCCDs is discussed, showcasing technological advancements [26].

This survey aims to synthesize the latest advancements and ongoing challenges in fluorescence imaging and molecular sensing by exploring interconnected topics. It highlights recent breakthroughs such as ultrasensitive microscopy for single-molecule imaging, the development of FRET-based small-molecule sensors, and the optimization of low-light imaging techniques. By integrating insights from these areas, the survey seeks to illuminate future research directions and potential applications in biological systems, particularly in enhancing signal extraction and analysis in noisy environments, improving the design of responsive imaging agents, and refining quantitative measurement techniques in live-cell studies [7, 22, 17, 1].

## 1.4 Structure of the Survey

This survey is meticulously structured to explore design strategies and chemical modifications in fluorescence imaging and molecular sensing. The **Introduction** establishes the significance of these technologies in biomedical and scientific fields, emphasizing their transformative role in enhancing diagnostic and therapeutic applications.

The second section, **Background and Core Concepts**, provides foundational knowledge of imaging techniques and the integration of various methodologies to optimize performance, contextualizing subsequent discussions on material design and synthesis.

In the third section, **Design Strategies and Types of Materials**, the survey delves into specific approaches for designing nanoparticles and advanced materials, emphasizing their synthesis and application in imaging and sensing, drawing on recent advancements to illustrate the diversity of materials employed.

The fourth section, **Effects of Chemical Modifications**, examines how chemical modifications impact the properties and functionality of imaging and sensing materials, highlighting their role in enhancing imaging techniques and molecular sensing capabilities.

The fifth section, **Performance in Typical Application Areas**, evaluates how these materials and techniques perform across various applications, including fluorescence imaging, molecular sensing, and bioimaging, underscoring their practical implications.

The sixth section, **Current Challenges**, identifies and discusses technical, methodological, and environmental challenges that hinder the optimization of these technologies, exploring barriers related to technological integration and data handling.

Finally, the **Conclusion** synthesizes the survey's key findings, emphasizing the importance of ongoing research and innovation in design strategies and chemical modifications. It outlines potential avenues for future research, aiming to foster innovations in fluorescence imaging and molecular sensing technologies, including advanced FRET-based small-molecule sensors, enhanced analytical tools for single-molecule imaging in live cells, and the development of far-field fluorescence microscopy techniques that surpass diffraction limits. Addressing these areas can significantly improve the sensitivity, resolution, and applicability of fluorescence-based methods in biological contexts [27, 7, 22, 28, 1]. The following sections are organized as shown in Figure 1.

## 2 Background and Core Concepts

#### 2.1 Fundamental Imaging Techniques

Fundamental imaging techniques are pivotal in fluorescence imaging and molecular sensing, enabling detailed visualization and analysis of complex biological and chemical systems. Single Molecule Fluorescence Imaging (SMFI) stands out for its ability to detect and analyze individual fluorescent molecules with high spatial resolution, offering critical insights into molecular dynamics and interactions at the molecular level [3]. Traditional fluorescence microscopy, while prevalent, faces challenges such as extensive sample preparation and image distortion, which can compromise accuracy [4]. Advanced techniques like the quantum-confined Stark effect (QCSE) enhance light emission by manipulating internal electric fields, thereby improving imaging capabilities [2]. NIR-IIb fluorescence imaging, using semiconducting single-walled carbon nanotubes (SWNTs) emitting in the 1500-1700 nm range, provides high-resolution in vivo imaging, beneficial for deep tissue applications due to reduced photon scattering and autofluorescence [29]. These advancements are crucial for precise imaging of complex biological structures, including distinguishing cancerous from healthy tissues during surgery [3]. Moreover, integrating fluorescence imaging with Raman spectroscopy enhances molecular specificity and clinical applicability, particularly in tumor margin delineation [5]. These foundational techniques drive the field of fluorescence imaging and molecular sensing forward, expanding their application in complex biological and chemical analyses and paving the way for future innovations.

## 2.2 Integration of Imaging Techniques

Integrating various imaging techniques is crucial for enhancing the performance and applicability of fluorescence imaging and molecular sensing technologies. Combining modalities allows researchers to overcome the limitations of individual techniques, achieving improved sensitivity, specificity, and resolution. The fusion of fluorescence imaging with Raman spectroscopy exemplifies this, providing complementary data that enhances molecular specificity and clinical utility, especially in accurately delineating tumor margins for surgical oncology [5]. Additionally, advanced computational models like VirFluoNet, which employs a conditional generative adversarial network (cGAN) to predict fluorescence images from phase contrast or other fluorescence images, significantly advance imaging technology. This model streamlines the imaging process and improves data acquisition and analysis efficiency [4]. Such integration enhances imaging workflows and enables real-time analysis, essential for studying dynamic biological processes. Furthermore, combining NIR-IIb fluorescence imaging with SWNTs facilitates high-resolution in vivo imaging while minimizing photon scattering and autofluorescence, particularly beneficial for deep tissue imaging and precise visualization of complex biological structures [29]. These multidisciplinary strategies underscore the importance of integrated approaches in advancing fluorescence imaging and molecular sensing capabilities, paving the way for innovative applications in biomedical and scientific domains.

In recent years, the advancements in imaging and sensing technologies have been significantly influenced by the development of innovative design strategies and materials. To elucidate this complex interplay, Figure 2 illustrates the hierarchical categorization of design strategies and types of materials utilized in these technologies. This figure encompasses nanoparticle design strategies and advanced synthesis methods, as well as a variety of advanced imaging and sensing materials. Each category is meticulously detailed, highlighting the techniques, innovative designs, and applications that underpin the current landscape of imaging and sensing technologies. Such a comprehensive framework not only aids in understanding the interrelationships between different strategies and materials but also underscores the importance of innovation in driving the field forward.

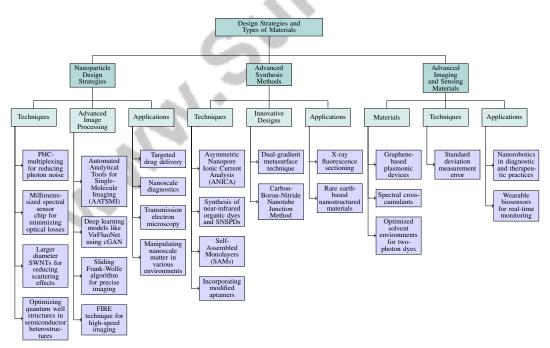


Figure 2: This figure illustrates the hierarchical categorization of design strategies and types of materials used in imaging and sensing technologies. It includes nanoparticle design strategies, advanced synthesis methods, and advanced imaging and sensing materials, highlighting techniques, innovative designs, and applications within each category.

# 3 Design Strategies and Types of Materials

# 3.1 Nanoparticle Design Strategies

Method Name	Enhancement Techniques	Technological Applications	Optimization Methods
PHC[30]	Multiplexing Matrices	Biomedical Applications	Select Multiplexing Matrix
MFSS[3]	Spectral Sensor Chip	Tumour Detection Site	Reduce Optical Losses
QCSE[2]	Polarization Field Harnessing	Biomedical Applications	Machine Learning
AATSMI[1]	Image Processing Techniques	Biomedical Applications	Robust Algorithms
VirFluoNet[4]	Deep Learning Framework	Biomedical Applications	Deep Learning Models
SFW[31]	Continuous Domain	Super-resolution Microscopy	Non-convex Solver
FIRE[26]	Beat Frequency Multiplexing	Biomedical Applications	Deep Learning Models

Table 1: Summary of nanoparticle design strategies focusing on enhancement techniques, technological applications, and optimization methods. The table includes various methods such as PHC, MFSS, and QCSE, highlighting their specific applications in biomedical fields and the optimization strategies employed.

Nanoparticle design strategies are essential for enhancing imaging and sensing technologies by improving sensitivity, specificity, and resolution. Table 1 presents a comprehensive overview of nanoparticle design strategies, detailing the enhancement techniques, technological applications, and optimization methods that contribute to advancements in imaging and sensing technologies. PHC-multiplexing is an innovative approach that enhances signal detection in low-light conditions by reducing photon noise, particularly in brighter pixels [30]. A millimetre-sized spectral sensor chip minimizes optical losses, thereby increasing the efficiency of nanoparticle-based materials [3]. Larger diameter single-walled carbon nanotubes (SWNTs) are designed to reduce scattering effects, enhancing fluorescence imaging clarity and depth [29]. In semiconductor heterostructures, optimizing quantum well structures is crucial for enhancing light emission and imaging performance [2].

Advanced image processing, such as the Automated Analytical Tools for Single-Molecule Imaging (AATSMI), provides enhanced analysis capabilities for single-molecule fluorescence data [1]. Deep learning models like VirFluoNet, using conditional generative adversarial networks (cGAN), transform fluorescence imaging by converting images across molecular labels, broadening imaging applicability [4]. The Sliding Frank-Wolfe algorithm improves recovery accuracy for precise imaging applications by addressing limitations of traditional grid-based nanoparticle design [31]. The FIRE technique enables high-speed imaging with low photon counts, allowing observation of fast dynamic phenomena without blur [26].

These strategies enhance nanoparticle functionality for biomedical applications such as targeted drug delivery and nanoscale diagnostics, as well as scientific fields relying on advanced characterization techniques like transmission electron microscopy. Addressing challenges such as biocompatibility and precise control, these strategies pave the way for breakthroughs in manipulating nanoscale matter across various environments, including air and vacuum [32, 33, 15].

#### 3.2 Advanced Synthesis Methods

Advanced synthesis methods are critical for developing novel imaging and sensing materials, enhancing performance and expanding functional capabilities. The Asymmetric Nanopore Ionic Current Analysis (ANICA) method uses glass nanopores with specific geometries for precise ionic current control, improving sensing applications [34]. The synthesis of near-infrared organic dyes and a 6x6 array of superconducting nanowire single-photon detectors (SNSPDs) significantly advances deep-tissue imaging, enhancing depth and resolution in complex biological environments [35]. Self-Assembled Monolayers (SAMs) optimize interfacial properties, crucial for sensor performance [36].

Incorporating modified aptamers through advanced synthesis methods, including nanoparticle conjugation, enhances targeting and binding capabilities, increasing the sensitivity and specificity of sensing platforms [18]. The dual-gradient metasurface technique integrates adjustable spectral and coupling gradients into a single design, enabling extensive coverage of light-matter interaction parameters [37]. The Carbon-Boron-Nitride Nanotube Junction Method uses CNT-BN-CNT structures for distinct analyte fingerprints based on conductance changes, addressing limitations of pure carbon nanotubes [38].

Advanced synthesis methods like x-ray fluorescence sectioning and rare earth-based nanostructured materials enhance imaging and sensing capabilities by facilitating precise elemental analysis and bioimaging applications, leveraging unique nanoparticle properties such as luminescence and magnetic resonance [39, 10].

## 3.3 Advanced Imaging and Sensing Materials

The development of advanced imaging and sensing materials is crucial for enhancing fluorescence imaging and molecular sensing technologies. Engineered to overcome traditional limitations, these materials offer improved sensitivity, specificity, and resolution. Graphene-based plasmonic devices provide tunable plasmons with longer lifetimes than noble metals, making them ideal for molecular sensing applications [40]. The tunability of graphene plasmons allows precise control over light interactions, enhancing molecular detection capabilities.

Spectral cross-cumulants significantly enhance spectral resolution beyond the limitations of physical detection channels, generating virtual spectral channels vital for super-resolved imaging applications [41]. Optimizing solvent environments for two-photon dyes enhances two-photon absorption efficiency and imaging performance [5].

A novel approach to standard deviation measurement error enhances imaging techniques by providing accurate error quantification, essential for ensuring high-quality outcomes in scientific and medical applications [42].

Collectively, these advanced materials and methodologies underscore innovative strategies enhancing imaging and sensing technologies. By integrating novel methodologies, recent advancements in nanorobotics and wearable electronics are poised to transform biomedical applications. Nanorobotics promises to revolutionize diagnostic and therapeutic practices through precise manipulation of biological systems at the nanoscale, while flexible, wearable biosensors enable real-time monitoring of biomarkers, enhancing personalized medicine by providing detailed health insights [33, 8].

## 4 Effects of Chemical Modifications

#### 4.1 Enhancements in Imaging Techniques

Chemical modifications are instrumental in augmenting imaging techniques by enhancing sensitivity, specificity, and resolution across various applications. The AATSMI system plays a crucial role in minimizing noise and improving the reliability of molecular behavior interpretation [1]. In fluorescence imaging, direct collection of emitted fluorescence from tissues, exemplified by miniature fluorescence sensing systems, reduces optical losses and boosts imaging efficacy [3]. Biocompatibility modifications of semiconducting single-walled carbon nanotubes (SWNTs) are vital for effective in vivo imaging [29], while solvent polarity influences the internal conversion processes and fluorescence quantum efficiency of dyes, impacting imaging performance [5]. Adjustments in indium composition within quantum wells enhance light emission efficiency, thereby improving imaging techniques [2]. Deep learning methods, such as those used in virtual organelle self-coding fluorescence, capture complex relationships between imaging modalities, allowing accurate predictions despite noise or distortion [4]. Advancements in imaging techniques, particularly super-resolution microscopy and fluorescence imaging, have transformed our ability to visualize biological and chemical systems with remarkable precision, improving 3D resolution by 1-2 orders of magnitude. New methods for assessing image quality and information content are critical for validating structural models. The integration of dynamic data analysis in surgical settings, such as early assessment of tissue perfusion using indocyanine green (ICG), enables differentiation between cancerous and benign lesions, demonstrating the potential of these enhanced imaging modalities to provide unprecedented insights into complex biological processes [28, 9].

## 4.2 Impact on Molecular Sensing

Chemical modifications are pivotal in advancing molecular sensing technologies, significantly enhancing sensitivity and selectivity for precise detection and analysis of diverse analytes. Cyanographone exhibits high sensitivity and selectivity for specific gas molecules, making it promising for gas sensing applications [43]. These modifications are crucial for developing sensors with improved

performance in detecting volatile compounds. In nanoporous materials, precise control over pore size is essential for applications such as molecular sensing, DNA sequencing, and water desalination [44]. Tailoring pore dimensions through chemical modifications allows selective passage of target molecules, enhancing specificity and efficiency in molecular sensing. Nanorobots with chemically modified surfaces show significant improvements in molecular sensing capabilities, particularly in medical applications, by enhancing interactions between nanorobots and target molecules, facilitating accurate detection and monitoring of biological markers [33]. Molecular imprinting techniques benefit from chemical modifications that enhance the selectivity and stability of imprinted sites, leading to improved recognition of target molecules [45]. Chemical modifications also impact spectroscopic analysis, where site-specific modifications enable precise investigation of intracellular components, such as tau aggregates [46], which is crucial for understanding complex biological processes and developing targeted therapeutic strategies. Challenges in achieving reproducibility and reliability in molecular sensing studies can affect overall technology performance [47]. Background fluorescence introduces uncertainty that degrades the signal-to-background ratio, impacting molecular sensing accuracy [48]. Innovative design strategies, such as the use of CNT-BN-CNT junctions, enhance molecular sensing capabilities by effectively discriminating between different analytes with high sensitivity [38]. Recent advancements in chemical modifications have significantly improved molecular sensing technologies, particularly through organic field-effect transistors (OFETs) and wearable biosensors. These innovations leverage the exceptional chemical sensitivity of organic semiconductors, enabling detection of a wide range of analytes-from small gas-phase molecules with potential health and security implications to biomacromolecules like proteins and nucleic acids that indicate physiological conditions. The integration of flexible electronics in wearable devices allows continuous, non-invasive monitoring of biomarkers in various bodily fluids, opening new avenues for applications in environmental monitoring, medical diagnostics, and personalized medicine. These advancements underscore the versatility of chemical modifications and their crucial role in advancing the precision and applicability of molecular sensing technologies across multiple fields [49, 50, 18, 51, 8].

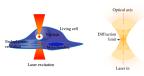
# 5 Performance in Typical Application Areas

## 5.1 Fluorescence Imaging Techniques and Applications

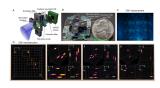
Fluorescence imaging techniques have substantially evolved, enhancing applications across scientific and medical fields. Aggregation-Induced Emission (AIE) nanoparticles demonstrate a high tumor-to-normal tissue signal ratio, proving their efficacy in tumor imaging. Semiconducting single-walled carbon nanotubes (SWNTs), operating in the near-infrared II region, enable high-resolution imaging of deep tissues, crucial for detailed anatomical visualization [29]. The two-photon fluorescence properties of NAPBr in different solvents highlight the versatility of fluorescence techniques [5], while advancements in red-emitting devices underline the potential for precision enhancement in imaging applications [2].

FIRE microscopy achieves high frame rates, surpassing traditional methods and facilitating rapid imaging of dynamic processes. The Sliding Frank-Wolfe algorithm enhances single-molecule fluorescence imaging by improving image reconstruction and accuracy [31]. Self-coding fluorescence methods, integrating advanced computational models, significantly enhance image quality and efficiency, broadening applicability in biological research [4]. The Analytical Tools for Single Molecule Imaging (AATSMI) technique in live-cell fluorescence microscopy effectively tracks single molecules and reconstructs cell shapes, proving invaluable in cellular imaging [1].

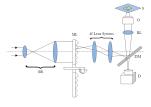
Advancements in microscopy and nanotechnology, driven by innovative materials and cutting-edge technologies, have deepened our understanding of molecular and cellular processes. Transmission electron microscopy (TEM) has provided unprecedented insights into material structures and dynamics, facilitating breakthroughs in quantum computing, drug discovery, and catalysis. The rise of nanorobotics in medicine is transforming diagnostic and therapeutic approaches through precise manipulation of biological systems at the nanoscale. Enhanced fluorescence microscopy techniques make single-molecule imaging in living cells more accessible, allowing detailed analyses of molecular behavior despite data noise challenges. These advancements expand our knowledge of fundamental biological processes and pave the way for innovative applications in medicine and materials science [33, 15, 1]. They underscore the transformative potential of fluorescence imaging in medical diagnostics, environmental monitoring, and therapeutic interventions.







(b) CM2 Imaging System: A Novel Microscopy Device for High-Resolution Imaging [52]



(c) A schematic diagram of a 4f lens system in a scientific or engineering context[12]

Figure 3: Examples of Fluorescence Imaging Techniques and Applications

As shown in Figure 3, fluorescence imaging techniques are crucial in various scientific and engineering applications, offering insights into complex biological and material systems. The figure illustrates three examples: an erroneous citation placeholder, the innovative CM2 Imaging System, and a schematic of a 4f lens system. The CM2 Imaging System marks a significant advance in microscopy technology, featuring a high-resolution setup with a 3D-printed housing, excitation and emission filters, a microlens array, and a CMOS sensor, all designed to enhance imaging capabilities. The 4f lens system diagram highlights the optical components essential for expanding and manipulating light beams, a critical function in scientific and engineering contexts. These examples emphasize the versatility and innovation inherent in fluorescence imaging, underscoring its vital role in advancing research and technology [27, 52, 12].

#### 5.2 Molecular Sensing Innovations

Molecular sensing has undergone significant innovations, driven by advanced materials and techniques that enhance sensitivity, selectivity, and applicability across various domains. The evaluation of  $CoCu_2O_3$  demonstrates its capability to detect low concentrations of pollutants like benzene, showcasing its potential in environmental monitoring [53]. Porous  $Cu_3N$  films, developed via a template-free method, exhibit excellent sensitivity and selectivity for  $NO_2$  detection, marking substantial progress in molecular sensing applications [54].

The fabrication of hBN nanopores with atomic precision is a breakthrough in molecular sensing, offering improved pore size control and stability, crucial for DNA sequencing and water desalination [44]. Recent studies show the ability to tune plasmonic resonances through morphological changes, providing a novel approach to enhance molecular sensing capabilities for complex analytes [55].

Porous nanocarbons have emerged as versatile platforms for molecular sensing, with systematic characterization leading to improved performance across various sensing technologies [56]. The dual-gradient metasurface, capable of capturing both spectral and coupling-strength information, signifies a considerable advancement in the field, offering new possibilities for precise molecular detection and analysis [37].

CNT-BN-CNT junctions effectively detect and differentiate between analytes such as benzene, thiol-capped oligoyne, and pyridyl-capped oligoyne, showcasing their potential in complex molecular sensing applications [38]. These innovations underscore the transformative impact of advanced materials and techniques on molecular sensing, paving the way for novel applications in environmental monitoring and medical diagnostics.

#### 5.3 Bioimaging and Biosensing Developments

Advancements in bioimaging and biosensing technologies have significantly enhanced their potential applications in biomedical and environmental fields. Hybrid xanthene dyes have been pivotal in cancer imaging and photodynamic therapy due to their superior photophysical properties and biocompatibility [16]. These dyes provide enhanced fluorescence efficiency and stability, making them suitable for high-resolution imaging applications.

The integration of low-light optical imaging techniques, utilizing advanced cameras such as EMCCD, qCMOS, and sCMOS, has improved the sensitivity and resolution of bioimaging systems [17]. These

advancements enable detailed visualization of biological processes at low light levels, crucial for applications requiring minimal phototoxicity and photobleaching.

Nanodiamonds have gained attention in biomedical imaging due to their unique optical properties and biocompatibility. Their surface functionalization and production methods have been optimized for enhanced imaging capabilities, bridging knowledge gaps in this area [25]. These developments facilitate more precise imaging of complex biological systems, advancing diagnostic and therapeutic applications.

In biosensing, optimizing gene expression sensors in Escherichia coli has shown significant potential in synthetic biology and metabolic engineering [20]. These sensors enhance the detection and analysis of specific biological markers, improving the functionality and efficiency of biosensing platforms.

Exploration of calix[4] arenes and their derivatives has expanded their application in sensing metal ions, anions, and biomolecules [23]. These compounds exhibit high selectivity and sensitivity, making them valuable for environmental and biomedical sensing applications.

Advancements in graphene-based terahertz (THz) devices have opened new avenues for molecular sensing and wave modulation [24]. These devices provide tunable sensing capabilities essential for detecting and analyzing a wide range of biomolecules.

Recent innovations in bioimaging and biosensing technologies, such as fluorescence imaging for intraoperative tissue characterization and nonlinear optical techniques for rapid, label-free imaging, highlight their potential to revolutionize scientific research and enhance medical applications. For instance, fluorescence imaging with indocyanine green (ICG) allows differentiation between cancerous and benign lesions during surgery, while nonlinear optics facilitate chemically specific imaging of biological samples, offering deeper insights into biological processes. These advancements improve diagnostic accuracy and pave the way for more effective treatment strategies in clinical settings [57, 9]. By enhancing sensitivity, specificity, and resolution, these technologies continue to drive innovative diagnostic and therapeutic solutions.

# 6 Current Challenges

Addressing the challenges affecting fluorescence imaging and molecular sensing technologies involves categorizing these obstacles into distinct yet interrelated areas. Identifying these challenges reveals limitations in existing methodologies and underscores the need for innovation and research. The following subsections explore specific categories of challenges, starting with technical and methodological limitations that hinder optimization and practical application.

#### 6.1 Technical and Methodological Limitations

Fluorescence imaging and molecular sensing technologies are impeded by several technical and methodological limitations. A significant challenge is achieving a high signal-to-noise ratio (SNR) in fluorescence imaging, which affects the extraction of reliable molecular information [1]. The balance between sample preservation, image quality, and data acquisition time further constrains current techniques [4]. Optical losses in remote sensing complicate the detection of low-intensity emissions, impacting tumor diagnosis and monitoring [3]. Additionally, increased water absorption at longer wavelengths affects signal intensity crucial for deep-tissue imaging [29].

The calibration of atomic-scale details in methods like CNT-BN-CNT is essential for performance consistency in molecular sensing [38]. Managing non-radiative recombination due to high internal fields remains challenging, affecting imaging efficiency [2]. The role of solvents in influencing the geometry and photophysical properties of dyes is not fully understood, limiting optimization for specific applications [5].

These challenges necessitate continuous research and innovation. Advances in analytical tools, such as automated high-throughput analysis and improved molecular localization algorithms, are crucial for enhancing these technologies. Developing robust metrics for assessing image quality in super-resolution microscopy is vital for validating structural models and ensuring accurate molecular interpretations [7, 28, 1]. Overcoming these barriers will unlock the full potential of these technologies, enhancing diagnostic and therapeutic applications.

#### 6.2 Material and Chemical Challenges

Material and chemical challenges significantly hinder the advancement of imaging and sensing technologies. Optimizing electrode materials for detecting transverse electrical currents in DNA sequencing is crucial for improving sensitivity and specificity [21]. Precise control over graphene doping levels is essential, as variations can significantly affect its electrical and optical properties [58].

In molecular sensing, achieving high selectivity for target ions in complex matrices remains challenging, as current probes often lack specificity [23]. The scalability of graphene-based terahertz devices and complexities in their fabrication hinder widespread application, emphasizing the need for advancements in material processing techniques [24].

Sensitivity to molecular environments complicates the accuracy of chemical imaging techniques, necessitating improvements for reliable biomedical applications [46]. Structural defects in graphene limit its effectiveness for plasmonic sensing, highlighting the need for engineered defects to optimize performance [40].

Addressing these material and chemical barriers is vital for unlocking the full potential of imaging and sensing technologies. Progress in nanorobotics, fluorescence imaging, and advanced technologies can lead to significant advancements in targeted drug delivery, intraoperative tissue characterization, and precision surgery, ultimately enhancing patient outcomes [59, 33, 9, 10].

## 6.3 Environmental and Biological Interferences

Environmental and biological interferences pose substantial challenges to the accuracy and reliability of fluorescence imaging and molecular sensing technologies. Background fluorescence degrades the signal-to-background ratio, complicating data interpretation and reducing detection precision [48]. These issues are particularly problematic in complex biological environments where autofluorescence obscures signals from targeted probes.

Environmental conditions, such as temperature and pH, affect the stability of imaging and sensing materials. pH changes can alter the photophysical properties of dyes, impacting their efficiency and emission spectra [5]. This necessitates developing robust materials that maintain consistent performance across diverse conditions.

Biological interferences, including proteins and other biomolecules, can affect the binding specificity and sensitivity of sensors. This is evident in tau aggregate formation, where environments influence aggregation, complicating spectroscopic analysis [46]. Advanced design strategies are required to enhance selectivity and stability in sensing platforms.

Interactions between imaging materials and tissues can lead to photobleaching and phototoxicity, impacting imaging quality [4]. Addressing these challenges is crucial for improving the applicability of these technologies in biomedical research and diagnostics.

The complexities introduced by environmental and biological factors underscore the need for ongoing research and innovation. Such advancements are essential for sophisticated applications like real-time plant monitoring, enhancing resource efficiency, and minimizing crop losses. Recent studies in nanobiotechnology demonstrate that integrating smart sensors can translate plant chemical signals into digital data, improving agricultural monitoring systems [7, 60].

#### 6.4 Technological Integration and Application Barriers

Integrating new technologies into existing systems presents several challenges that must be addressed to fully realize their potential in fluorescence imaging and molecular sensing. A significant barrier is incorporating single-molecule techniques into current systems, which face throughput limitations and difficulties operating in native environments, restricting practical applications [61]. Overcoming these limitations requires advancements in technology design and methodologies for seamless integration.

In health monitoring systems, flexible laser sensors encounter challenges related to wireless technology and sensor uniformity, hindering effective deployment in real-world applications [62]. Innovations in sensor design and communication technologies are necessary for robust operation in diverse environments.

Reliance on the accuracy of injected physics knowledge poses limitations, as inaccuracies can lead to suboptimal exploration of new technologies. This highlights the need for precise and validated models to guide integration [63]. Interdisciplinary collaboration is essential to refine models for effective integration.

The long-term stability of fluorescent responses and the efficacy of calixarene probes in real-world samples remain unanswered questions, complicating their application [23]. Ensuring stability and reliability in complex environments is vital for successful imaging and sensing.

Challenges associated with integrating new technologies underscore the interplay of factors like data management, real-time monitoring, and advanced analytical methods, as shown by developments in electron microscopy, nanobiotechnology, and wearable electronics. These advancements necessitate a reimagined approach accommodating vast datasets and multimodal analyses while facilitating seamless communication between devices [64, 15, 65, 8, 60]. Addressing these challenges requires a concerted effort to advance capabilities, improve compatibility, and refine models, paving the way for enhanced applications in research and diagnostics.

#### 6.5 Data and Computational Challenges

Data and computational challenges are critical hurdles in advancing imaging and sensing technologies, affecting real-time monitoring and analysis capabilities. Wearable sensors face challenges in data handling and computational analysis, particularly in real-time applications. Integrating these sensors necessitates robust data processing algorithms to manage vast information volumes, ensuring accurate and timely analysis [8].

The intricate nature of biological and environmental data underscores the need for sophisticated computational models capable of efficiently processing large datasets. These models must address limitations of current imaging techniques, which struggle with spatio-temporal resolution and quantitative accuracy in tracking cellular processes. Leveraging advanced methodologies, such as diffusion-driven percolative clustering and label-free prediction techniques, can enhance understanding of cellular organization and improve data interpretation reliability [28, 65, 66, 9]. The computational burden of processing such data can be substantial, necessitating sophisticated algorithms and high-performance computing resources.

Variability and heterogeneity of data from different platforms pose challenges in data integration and standardization. Ensuring consistency across datasets is crucial for accurate analysis, particularly in wearable biosensors and fluorescence imaging, where standardized protocols can enhance monitoring reliability. Developing these standards is essential for effectively interpreting large-scale health data and improving clinical outcomes through personalized medicine [9, 67, 8].

The demand for real-time data processing complicates the computational landscape, necessitating adaptive algorithms capable of swiftly responding to fluctuating conditions. These algorithms must manage and interpret vast datasets generated by advanced technologies, essential for applications ranging from intraoperative tissue characterization to monitoring health indicators through biosensors [65, 9, 8, 15]. This is particularly challenging in diagnostics and environmental monitoring, where timely decision-making is critical.

Challenges in data extraction and processing highlight the need for continual advancements in data processing techniques and computational methodologies. Such innovation is essential for enhancing the effectiveness and reliability of imaging and sensing technologies, particularly in intraoperative tissue characterization using fluorescence imaging, where precise data analysis can significantly impact surgical outcomes. As physiological imaging technologies evolve, understanding key factors such as pixel size and spatial frequency sampling becomes crucial for reproducible results. In electron microscopy, integrating data-driven approaches is vital for addressing complex materials challenges, enabling transformative discoveries across fields [6, 15, 9]. Addressing these challenges will fully realize the potential of these technologies, enhancing their applicability in research and practical applications.

## 7 Conclusion

This survey underscores the transformative impact of design strategies and chemical modifications on fluorescence imaging and molecular sensing technologies. The integration of cutting-edge materials, such as graphene-based plasmonic devices and semiconducting single-walled carbon nanotubes (SWNTs), has significantly advanced imaging resolution and sensing precision, enhancing applications in environmental monitoring and biomedical diagnostics. Future research should focus on optimizing SWNT synthesis for greater brightness and exploring their diverse biomedical applications.

Chemical modifications, including solvent effects and doping strategies, have proven crucial in enhancing platform performance. Future studies should prioritize the experimental validation of theoretical models and investigate additional solvent systems to further improve imaging and sensing capabilities. However, challenges such as low signal-to-noise ratios, material and chemical constraints, and environmental interferences remain obstacles to progress. The field also faces challenges in integrating new technologies into existing systems and managing the computational complexities of real-time data analysis.

Future research directions should aim at optimizing frameworks for various imaging scenarios, improving detector efficiency, and refining methodologies to enhance data quality. Additionally, advancing the stability and reliability of organic sensors and exploring novel materials are critical for improving performance. There are substantial opportunities in optimizing the plasmonic properties of graphene by engineering specific defects to enhance sensor effectiveness.

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