Photonic Crystal Enhanced Fluorescence Emission for Ultrasensitive Biosensing

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Abstract: We present advanced biosensing methods with photonics crystal enhanced fluorescence emission from Quantum Dots, Plasmonic Fluorophores, and DNA Nano-grippers for nucleic acid, protein, and pathogen detection.

1. Introduction: The pursuit of higher sensitivity and specificity in biosensors has been driven by the growing need for early and accurate disease detection, monitoring of health conditions, and the advancement of personalized medicine. Conventional molecular and viral diagnostic technologies used in laboratory settings often face limitations in terms of sensitivity, the need for large sample volumes, complex and cost. Photonic crystals, with their unique ability to manipulate light at the nanoscale, have opened new frontiers in enhancing the capabilities of fluorescent-based biosensors. By harnessing the multiplicative enhancement effects during the photon generation and detection process, PCs are able to amplify weak signals from biomolecular interactions, thus pushing the limits of detection to previously unattainable levels without complex assay workflows or sophisticated optics.

2. Methods and Results: We established a PCEF framework that can enhance excitation, highly directional emission extraction, quantum efficiency improvement, and blinking suppression. PCs amplify electromagnetic fields at resonant frequencies of pump laser excitation and provide enhanced fluorescent molecule absorption rates. This, combined with the ability of PCs to direct photon emission spatially, enhances photon collection efficiency, crucial for biosensing applications. Additionally, the Purcell effect boosts the quantum efficiency of emitters, reducing emission lifetimes and increasing fluorescence spontaneous emission rate. Furthermore, the enhanced radiative decay process outcompetes non-radiative decay, mitigates blinking issues, ensuring consistent and reliable signal output, vital for single-molecule detection.

2.1 Photonic Crystal Enhanced Quantum Dot Emission for Detecting Cancer-associated miRNAs:

PCEF-QDs represent the first successful application of PCs in biosensing, achieving single-molecule resolution. This technology leverages the signal amplification capabilities of PCs, resulting in a near 3,000-fold increase in signal intensity for QDs. The PC substrate, combined with a bioassay that employs a bridge pull-down mechanism, where QDs, linked to target molecules, are selectively pulled down onto the PC surface. This selective binding dramatically amplifies the fluorescence of surface-bound QDs, enabling the detection of single quantum dot tags with high specificity. In

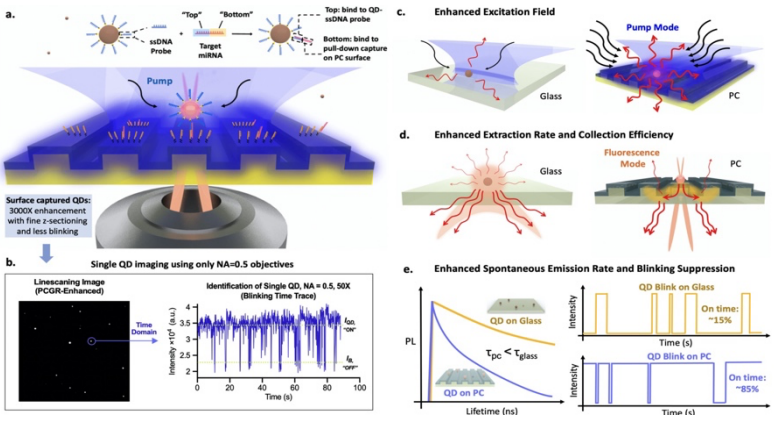

Figure 1. *PC enhanced QD emission enables single QD digital counting for single miRNA detection.*

contrast, unbound QDs in the solution remain unenhanced and undetectable. This selective amplification is critical for detecting low-abundance biomolecular targets, such as microRNA biomarkers, offering significant potential for early cancer diagnostics. The technique's high signal-to-noise ratio and single quantum dot sensitivity facilitate the tracking of rapid particle movements at the single-particle level, underscoring its utility in advanced biosensing applications. This methodology has demonstrated remarkable efficacy in detecting cancer-associated miRNA biomarkers, achieving a detection limit as low as 10 attomoles [1].

2.2 Plasmonic-Photonic Hybrid System for Enhanced Biosensing of Small Proteins:

Building upon the foundational PCEF-QD technology, the Plasmonic-Photonic Hybrid system was developed to optimize and confine enhanced E-fields. This technology synergizes the spatial control of plasmonic nanoantennas with the high-quality factor of PCs, resulting in efficient and targeted amplification of fluorescence signals. Employing heterometallic Ag@Au core-shell plasmonic nanostructures and a dielectric spacer layer, this method results in a 10,000-fold brightness increase compared to a single fluorophore. The system is exemplified in the detection of inflammation and immune response-related biomarkers, such as Interleukin-6, in human plasma. This advancement provides a significant improvement over standard immunoassays, offering a promising avenue for the detection of various biomolecules[2].

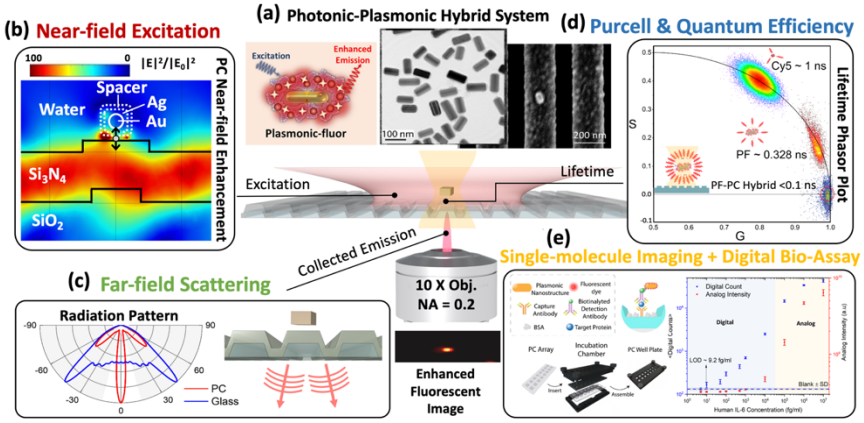

Figure 2. Plasmonic-photonic hybrid enhanced fluorescence for digital resolution immunoassay.

2.3 Photonic Crystal Enhanced Fluorescence with DNA-based Nano-gripper for SARS-CoV-2 Biosensing:

In order to further improve the detection limit by reducing the non-zero read-out from non-specific binding effects, DNA-based Nano-gripper (NG) technology introduces a novel dimension to biosensing functional specificity. These Nano-grippers are designed to generate a signal only upon encountering their target, enhancing the accuracy of biosensing. This feature is particularly beneficial in pathogen detection, as demonstrated by the efficient and specific detection of SARS-CoV-2. Enhanced by PC technology, the Nano-grippers achieve a 10^4-fold signal enhancement compared to traditional fluorophore reporters, enabling ultra-sensitive detection with a limit of detection of 100 viral genome copies/mL in human saliva [3].

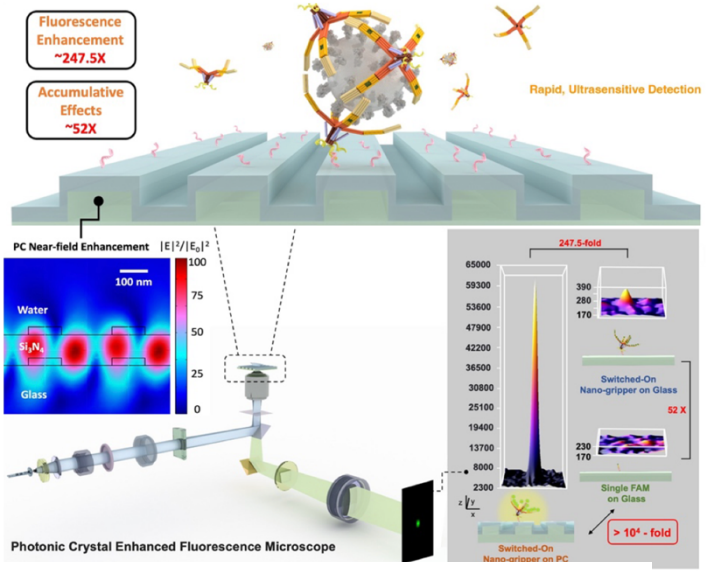

Figure 3. Photonic crystal enhanced detection of SARS-CoV-2 by DNA NG sensor.

3. Conclusion:

These photonic crystal enhanced fluorescent technologies represent a significant leap in biosensing capabilities, offering high sensitivity, specificity, and versatility for detecting a broad range of biomolecular analytes. The integration of these technologies into practical biosensing platforms promises to transform the field of liquid biopsy diagnostics, enabling rapid, accurate, and cost-effective disease detection and monitoring.

References

[1] Y. Xiong *et al.*, "Photonic crystal enhanced fluorescence emission and blinking suppression for single quantum dot digital resolution biosensing," *Nat Commun,* vol. 13, no. 1, p. 4647, Aug 8 2022, doi: 10.1038/s41467-022-32387-w.

[2] P. Barya, Y. Xiong *et al.*, "Photonic-Plasmonic Coupling Enhanced Fluorescence Enabling Digital-Resolution Ultrasensitive Protein Detection," *bioRxiv,* p. 2022.10.29.514360, 2022, doi: 10.1101/2022.10.29.514360.

[3] L. Zhou, Y. Xiong *et al.*, "Designer DNA NanoGripper," *bioRxiv,* p. 2023.04.26.538490, 2023, doi: 10.1101/2023.04.26.538490.