# Photonic Crystal Enhanced Fluorescence with DNA-based Nano-gripper for Ultrasensitive SARS-CoV-2 Biosensing

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***Abstract*—DNA-origami based nano-grippers, integrated with aptamer-based nanoswitches, generate fluorescent signals when detecting SARS-CoV-2. The integration of Photonic Crystal Enhanced Fluorescence Microscope enables a 104-fold enhancement compared to a single fluorophore reporter on glass substrate, providing a promising tool for ultrasensitive detection and rapid diagnostics.**

***Keywords—Photonic Crystal, Enhanced Fluorescence, DNA-origami, Biosensing***

## INTRODUCTION

Photonic crystal enhanced fluorescence technologies have been a focal point of interest in recent decades due to the unique interactions between light and matter that occur at the nanoscale. These technologies have found a strong foothold in the broad arena of biosensing, thanks to their versatility for ultrasensitive, rapid, analyte sensing without the need for extensive sample pre-treatment steps or sophisticated optics. Fluorescence-based bioanalytical techniques, widely used in liquid-biopsy diagnostics applications, typically require many labeled target molecules to combine their emission output to achieve a practically useful signal-to-noise ratio. Approaches capable of amplifying fluorescence signals can provide signal-to-noise sufficient for digitally counting single emitters for ultrasensitive assays that are detected with simple and inexpensive instruments. However, one urgent need in the field of single molecule direct counting for biosensing is to improve the limit of detection (LoD) by avoiding the non-specific binding issue of the fluorescent reporter, which gives non-zero background counts when there are no target molecules present. We tackled this issue by reprograming the function of DNA nano-machine as a designed nano-switch with sensing abilities and only generating the fluorescent signal while capturing a target. Herein, we presented the first-ever design and construction of a DNA-based Nano-gripper (~150nm when fully open) with four fingers and flexible joints inspired by nature bird claws, human hands, and bacteriophages with the ability to capture nanoscale items.

## I. PHOTONIC CRYSTAL ENHANCED FLUORESCENCE (PCEF)

The field of fluorescence microscopy has witnessed a surge of interest in the development of techniques to enhance the absorption of individual fluorescent reporters. The primary goal is to effectively harness their photon emission and mitigate off-focus background to bolster the signal-to-noise ratio (SNR). Total Internal Reflection Fluorescence (TIRF) microscopy, employing high numerical aperture (NA) oil-immersion objectives and Electron-Multiplying Charge-Coupled Device (EM-CCD) cameras, has been a significant player in this arena. However, the high cost and complexity of these systems have driven researchers to explore alternative methods, such as the use of plasmonic nanostructures and dielectric optical microcavities. Despite their potential, these methods have been limited by issues such as significant non-radiative decay, low photon directionality, and the need for precise alignment between cavity and fluorophore. To overcome these challenges, we use construct a newly designed PC nanostructured surface that can serve as a general-purpose macroscopic substrate for widefield microscopy of fluorescence tags with a simple low-cost fabrication process and greatly improved enhancement factor. As illustrated in Fig. 1, we have engineered an instrument designed to enhance the emission intensity of FAM fluorophores through radiative engineering. This results in an average enhancement of around 250× for FAM dyes situated 20-80nm above the Photonic Crystal (PC) surface. This enhancement process involves the meticulous tuning of two quality factors: radiation (Qr) and non-radiation (Qnr). The latter is associated with absorption or inevitable losses caused by fabrication imperfections. To optimize the enhancement factor towards theoretical limits, it is essential to meet the Q-matching requirements (Qr = Qnr) for two PC resonance modes, namely the pump-mode and fluorescence-mode. This significant enhancement is credited to the integration of multiplicative enhancement factors that provide comprehensive enhancement, starting from the fluorescence generation process

(absorption, excited-state lifetime, radiative fluorescence emission) to the collection process (far-field distribution and blinking reduction) after the emission photons are generated. This approach offers a promising tool for rapid diagnostics[1].

## II. Design of the NanoGripper Biosensor

### A. *Design, synthesis and characterization of DNA NanoGripper*

The design of the DNA NanoGripper (NG) was inspired by naturally occurring structures such as bird claws, human hands, and bacteriophages. The DNA NG was designed with four fingers, each composed of three phalanges connected by two flexible joints. The dimensions of the NG were optimized to grasp objects approximately 100 nm in diameter or smaller. The DNA NG was assembled using a long M13mp18 derived "scaffold" DNA with 229 short "staple" strands. The successful formation of the NG structure was confirmed through atomic force microscopy (AFM) imaging, transmission electron microscopy (TEM) imaging, and cryo-EM imaging[2]. The NG's fingers project outward from the central palm, suggesting its potential to grab other 3D objects with matching dimensions.

### B. *Capture of SARS-CoV-2 virus by DNA NanoGripper*

Cryo-EM images confirm the flexibility of the DNA NanoGripper (NG), with its fingers able to bend at various angles. The NG's fingers were decorated with DNA aptamers targeting the spike proteins on SARS-CoV-2. AFM and TEM images show that the NG can bend its fingers to capture spherical-shaped gold nanoparticles (AuNPs) of different sizes. Furthermore, when multiple SARS-CoV-2 spike protein-targeting aptamers were attached to the NG's fingers, cryo-EM images showed that multiple NGs could adhere their fingers to the viral particle outer surface in various binding poses. This effective NG-virus interaction paves the way for using the DNA NG in the ultrasensitive detection.

## III. Intigrating DNA NanoGripper with PCEF Systerm

The development of nanoscale machines has opened up new possibilities in the field of biomedical applications. One such advancement is the programmable DNA nano-machine, designed as a nano-switch that generates a fluorescent signal only upon capturing a target. This design improves the limit of detection (LoD) by circumventing non-specific binding issues of the fluorescent reporter.

To achieve the bio-sensing ability as a virus detector, an aptamer nano-switch was designed and introduced for robust fluorescent signal release, in which the SARS-CoV-2 spike-specific binding aptamer was tagged with a fluorescent reporter, along with a quencher-labeled "lock" DNA that forms a partial duplex with the aptamer. These functional Nano-gripper with aptamer-quenched fluorophore pairs (52 pairs/gripper) serve as a nano-switch and only generate a fluorescent signal when it captures a target SARS-CoV-2 virion. Photonics crystal enhanced fluorescence and designed nano-machine enabled fluorophore accumulation can function in synergy to amplify virus-actived fluorescence signals. By concentrating multiple fluorophores on one nanogripper theoretically provide 52-folds fluorescence signal compare to single FAM reporter while only generate signal by virus activation. Photonic crystals (PC) as dielectric microcavities can provide much stronger local field enhancement, far-field directional emission, large Purcell enhancement, and high quantum efficiency. However, its fluorescent enhancement is non-selective to all fluorescent reporters that close to surface, and usually generate inevitable high background signal that caused by non-specific binding of fluorescent reporters. By employing this Photonic-DNA nanomachine hybrid system, we reporter a nearly 104-flod signal enhancement compare to a FAM reporter on glass substrate. Different than surface-based ELISA assay, the capture reaction between Nano-gripper and SARS-COV-2 virus happens in solution at a fast rate which reaches equilibrium after 10 minutes of mixing. After the target virus had activated the fluorescent signal, the mixture was then incubated on functionalized photonic crystal surface with ssDNA pull-down tethers for signal enhancement and direct counting. As a consequence of nearly-zero off-target signal, we achieve a limit of detection (LoD) of 100 viral genome copies/mL in human saliva solution to achieve ultral-high detection sensitivity.

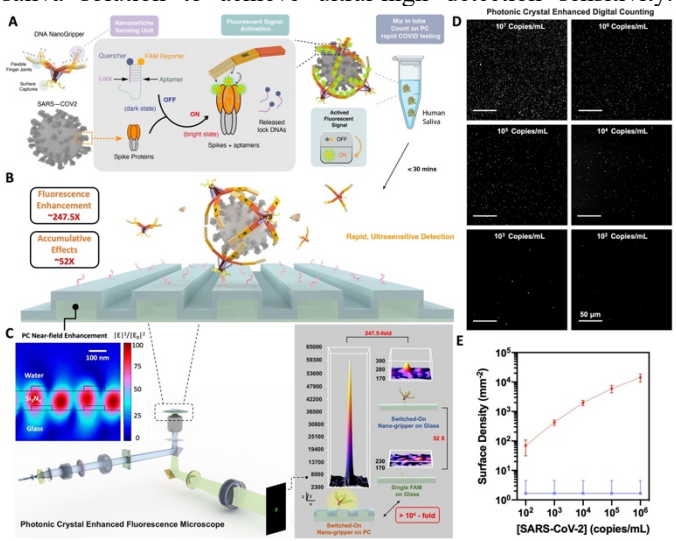


Fig. 1. Photonic crystal enhanced detection of SARS-CoV-2 by DNA NG sensor.

To mimic the clinic samples, we spiked SARS-CoV-2 pseudo viruses into human salvia and then mixed with our aptamer functionalized nanogripper for about 30 min at room temperature. Then, the mixture was dropped to the surface of the ssDNA tethers coated photonic crystal. The nanogripper can be immobilized through the hybridization between the ssDNA tethers and ssDNA links at the bottom of the nanogripper's palm. PCEF microscopy was used to image the light spots of stimulated FAM fluorophores and the images of readout were processed by our developed algorithms. The number of light spots were counted as the detection results which were summarized below. Here, we achieved the detection of SARS-CoV-2 in human saliva as low as 100 copies/mL. This demonstrated our nanogripper can work with photonic crystal-based biosensing platform very well and achieve much high detection sensitivity.

## References


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

[2] 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.