

Portable Plasmonic Biosensor for Simultaneously Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in Urine Samples

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Abstract—*Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) remain major causes of sexually transmitted infections (STIs) case worldwide. Rapid and easy to use diagnostic tools will play a crucial role in controlling the spread of the disease. This study presents the introduction of a portable biosensor that has the ability to detect CT and NG in urine samples. The biosensor combines plasmonic nanohole detection with lens-free imaging, allowing for diagnosis at the point of care. It captures images of the diffraction field to detect bacteria. A piezoelectric pump and flow cell enable accurate sample delivery. The whole setup, testing, and image analysis are controlled by graphical user interface. The utilization of control and sensor antibodies in various configurations on the sensor yields the intended level of sensitivity and specificity. Simultaneous NG and CT testing can be conducted independently due to the use of microfluidic channels, which require less battery testing and offer a high-throughput capability. Tests on the control are conducted utilizing plasmonic pixels to minimize background noise. The limits of detection for NG and CT, respectively, using our point-of-care biosensor, are 452 and 121 CFU/mL. This makes it practical, cost-effective, and efficient in bridging the accessibility and affordability gap for STI diagnostics.

Keywords—plasmonic biosensor, sexually transmitted infections, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, point-of-care diagnostics.

I. INTRODUCTION

One of the most prevalent health concerns on a global scale is the transmission of infections through sexual contact. [1]. Chlamydia, gonorrhea, syphilis, and HIV comprise the most prevalent sexually transmitted infections [2]. *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) are bacterial infections among sexually transmitted diseases (STIs), with around one million new cases reported daily. Despite effective treatments, many infections are asymptomatic, leading to severe complications like infertility, pelvic inflammatory disease, ectopic pregnancies, and chronic pelvic pain [3]. Current diagnostic methods, such as nucleic acid amplification tests (NAATs), require specialized laboratories and sample preparations, making them unsuitable for resource-poor settings [4]. There is a need for simultaneous, easy-to-use, and affordable diagnostic tests for NG and CT that can be used

in clinics to improve early detection and treatment access while reducing infection spread. In response to this need, we developed a nanohole array-based plasmonic biosensor that is portable and lens-free, utilizing computational imaging to detect NG and CT in urine samples [5]. This system captures and analyzes diffraction field images, ensuring precise sample delivery via a low-cost pump and flow cell. The setup and image analysis are controlled by software, with control and sensor antibodies ensuring high sensitivity and selectivity. Independent microfluidic channels enable simultaneous NG and CT testing, enhancing throughput and reliability. Plasmonic pixels reduce noise levels, and simple, inexpensive optics support system feasibility. The detection limits for NG and CT are 452 CFU/mL and 121 CFU/mL, respectively, making this biosensor highly efficient for point-of-care diagnostics. This approach, leveraging a camera-based sensing scheme, allows for simultaneous detection at higher throughput, providing better sensitivity and reliability in detecting both bacterial strains in urine.

II. MATERIAL AND METHODOLOGY

A. Plasmonic Biosensor

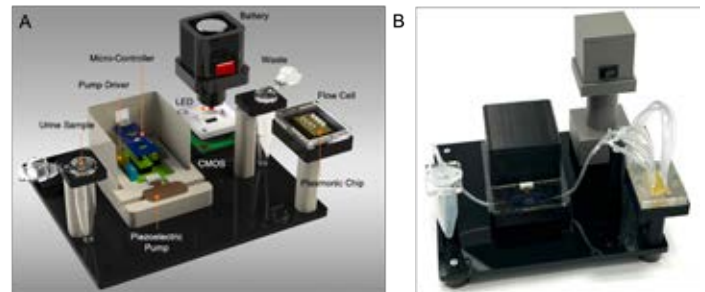


Fig. 1. (A) Schematics and (B) image of the biosensor.

Figures 1A and B present the schematic and photograph of the plasmonic system. We have designed a 3D-printed housing for complete integration of all components of the plasmonic system such that the effect of ambient disturbances and background noise is minimized to the greatest possible extent.

This configuration is comprised of four main subsystems: plasmonic chip (i), sample preparation module (ii), imaging module (iii), and software module (iv). The plasmonic chip features a thin layer of gold film embedded with nanohole lattices, which are modified by attaching antibodies specifically targeting NG and CT bacteria. The imaging module uses an LED light source to illuminate the plasmonic chip, while a CMOS camera captures the resulting diffractive field patterns. Variations in brightness indicate the presence of microorganisms. The sample preparation module includes a flow cell fabricated with Polydimethylsiloxane (PDMS) and a piezoelectric pump for precise sample delivery. This module divides the plasmonic microarray into four different sections: two for detecting CT and NG, and two for control purposes—one with a control antibody and one left unmodified. The sensor sections have a single layer of bioreactive material containing specialized antibodies for NG and CT detection. The control regions are designed to detect changes caused by optical, mechanical, or biological factors. The software module features a GUI that effectively controls and monitors the operations of the optical and microfluidic hardware components. Additionally, it is equipped with an image-processing method that analyses plasmonic diffraction field images in order to detect bacteria present in urine samples. A computer controls the system, establishing a connection between the imaging and sample preparation equipment by USB.

B. Plasmonic Chip

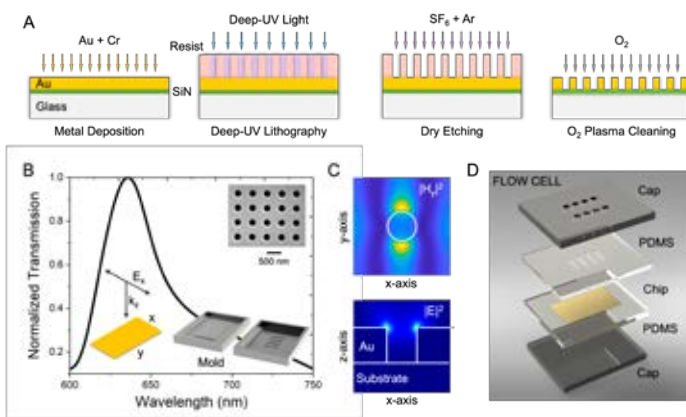


Fig. 2. (A) Fabrication process. (B) The polarization and propagation directions from FDTD simulations are illustrated in the insets of the transmission spectrum of the nanohole arrays. Furthermore, a Scanning electron microscopy (SEM) image of the nanohole arrays and a schematic of the mold are included. (C) The y-component of the magnetic field at the surface of the Au film (top). Cross-sectional profile of the electric field distribution (bottom). (D) The flow cell.

The plasmonic chip is made of a periodically patterned nanohole with a diameter and periodicity of 200 nm and 550 nm, respectively. Figure 2A schematically represents the fabrication procedure. The nanoholes are patterned onto a gold (Au) film with a thickness of 120 nm using the deep-UV lithography method, applied through a high-throughput technique. A 4-inch fused silica wafer, 500 μm thick and

covered with an LPCVD-SiN layer with a thickness of 100 nm, is utilized as the substrate. An adhesion layer of 5 nm chromium (Cr) was placed onto the SiN coating as the first step in the fabrication process, followed by a 120 nm layer of gold. After that, the gold surface was treated with a positive photoresist layer, and deep-UV lithography was carried out. To create the nanoholes, a gas combination of SF₆ and Ar was used to dry-etch the gold film following the lithography process. Residual photoresist was removed with oxygen (O₂) plasma cleaning. Finally, the wafer was segmented into 2 cm $\times$ 1 cm plasmonic chips. Figure 2B inset shows a scanning electron microscope (SEM) image of the nanohole arrays. Symmetrical wave patterns observed along the x-axis indicate the presence of counter-propagating plasmons, whereas the concentrated areas of high intensity at the aperture rims are caused by localized plasmons. The distribution of the electric field (Figure 2C-bottom) shows concentrated increases in the nanohole rims, allowing analytes on the gold surface to access these fields. The nanoholes' circular symmetry justifies the utilization of an unpolarized LED, ensuring uniform field concentration and increased sensitivity in comparison to polarized light.

C. Sample Preparation Module

A piezoelectric pump and a flow cell are the two main components of the sample preparation module. The piezoelectric pump (mp6-liq, Bartels Mikrotechnik GmbH) is controlled by an Arduino microcontroller and an mp6-EVA driver board, as shown in Figure 1B. The driver board controls the flow rates by adjusting the pump's voltage (ranging from 1.0 to 4.5 V) using pulse-width modulation (PWM). Operating at a steady frequency of 100 Hz, the pump maintains a stable relationship between voltage and flow rate. To make sure the analytes were consistently delivered for both the control and sensor tests, a flow cell that had four channels was used. Two control channels minimize noise from optical, mechanical or biological variations. In the dual-layer PDMS construction of the flow cell, the channels are situated on the upper layer, while the plasmonic chip resides in a precisely engineered groove on the lower layer. The molds for these PDMS layers, depicted in the inset of Figure 2B, were fabricated using advanced resin-based 3D printing techniques. To facilitate reusability, we engineered a sophisticated microfluidic system incorporating a holder with dual acrylic caps, thereby obviating the need for PDMS bonding and ensuring a leak-proof assembly. Figure 2D showcases this design, which includes eight holes in the top cap for inlets and outlets, and four screws for stability. The flow cell's four channels deliver urine samples containing NG or CT bacteria to designated regions on the plasmonic chip. For precise bacterial immobilization, we employed flow-cell-assisted functionalization. Molecular printing technology was used to modify plasmonic pixels for control and sensor testing. The sample preparation module facilitated the application of a BSA (Bovine Serum Albumin) coating. The piezoelectric pump gradually delivered the bacteria and BSA over twenty minutes. This procedure involved the binding of protein A/G,

the attachment of antibodies, the coupling of BSA, and the immobilization of bacteria. Afterward, the plasmonic chip was positioned, and the CMOS camera was set up to capture images and analyze the data.

D. Imaging Module

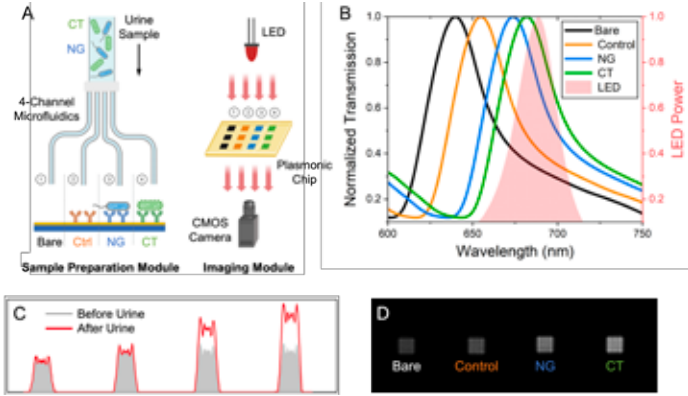


Fig. 3. (A) The sample preparation and imaging modules of the handheld biosensor. Created using BioRender.com. (B) The black curve illustrates the transmission resonance of nanohole arrays functionalized with A/G protein, the orange curve represents the control antibody, the blue curve depicts 10^6 CFU/mL NG, and the green curve corresponds to CT bacteria. The red curve shows the power spectrum of the LED source used in the imaging module. (C) Changes in the diffraction field intensity before (gray line) and after (red line) the plasmonic pixels were exposed to a urine sample containing NG and CT bacteria. (D) A CMOS camera image displaying square-shaped plasmonic pixels: orange represents the control antibody, blue represents NG bacteria, green represents CT bacteria, and bare represents protein A/G.

The portable biosensor utilizes a CMOS camera (Thorlabs, DCC1545M) and LED illumination to capture diffraction field images from the plasmonic chip (Fig. 3A). CMOS camera tracks intensity changes before and after analyte binding to detect spectral fluctuations in nanohole transmission resonance. The detection limit means to the minimum concentration of bacteria that can be detected by observing the changes in diffraction field intensity. Figure 3A depicts the imaging module. The lower PLA component houses the CMOS camera that is powered by USB, while the upper PLA component houses the LED light source that is powered by a battery. An inter-PLA tube provides uniform LED lighting. The upper component comprises a battery, switch, and resistor, which serve the purpose of regulating LED brightness and preventing saturation. The lower section of the device securely holds the plasmonic chip in a customized groove. The alignment of the sensor and control sections of the chip with the active area of the CMOS camera, also referred to as “plasmonic pixels,” is precisely aligned. The light source must have a narrow bandwidth near the nanohole transmission response, and the CMOS camera requires high quantum efficiency in this range. Following the functionalization of protein A/G, a shift of 4 nm in transmission resonance was observed. This shift positioned the LED source at approximately 688.65 nm, which is 33 nm away from the nanohole resonance (Fig. 3B). This alignment enables spectral overlap during the steps of functionalization. The narrow bandwidth of the LED (~ 28 nm) combined

with the consistency of quantum efficiency of CMOS ensures accurate detection of diffraction field intensity changes, even at low analyte concentrations. Successful bacterial binding and the multiplexed detection capabilities of our biosensor for NG and CT bacteria are demonstrated by Figures 3C and 3D, which illustrate the various intensity values and spectral shifts.

E. Software Module

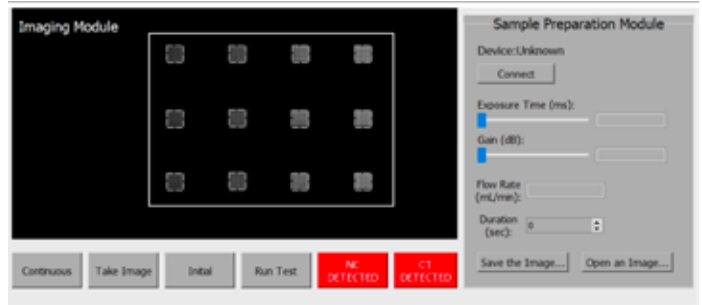


Fig. 4. GUI of the biosensor kit.

A graphical user interface (GUI) based on Python™ was created to assess the plasmonic chip’s performance and transmit sensing data through image processing (Fig. 4). The graphical user interface (GUI) enables users to adjust camera and piezo pump settings, allowing precise customization of test conditions. The plasmonic chip is initially coated with protein A/G and IgG, and then positioned in the flow cell to allow the flow of protein BSA and urine. The chip is placed in front of the CMOS camera after functionalization is complete. The graphical user interface (GUI) shows the diffraction image and computes the average diffraction intensity. The GUI utilizes calibration data to predict the ranges of detection, while also normalizing these outcomes with respect to the control antibody. The system calculates the ratio of the intensity of the diffraction field captured by the sensor to that of the control. It then alerts for the presence of bacteria if the calculated value falls under pre-set thresholds. By concentrating on the diffraction intensity within the specified area of interest, this approach minimizes external disruptions and guarantees data accuracy. Consequently, it is suitable for field use without the necessity of highly trained personnel.

III. RESULTS

A. Detection of NG and CT Bacteria with Plasmonic Imaging

Using anti-NG and anti-CT antibodies (Abcam) targeting the outer membrane proteins of NG and CT, respectively, we developed a method for the identification of bacteria in urine samples. To achieve precise bacterial capture, we utilized a protein A/G fusion to form a functional layer on the detection surface, optimizing the antibody configuration. While culturing CT required host cells, NG was grown on Thayer-Martin agar. For device optimization, the inactivated bacteria were used, which still maintained their membrane integrity for effective capture. By functionalizing plasmonic pixels with protein A/G and IgG and coating other areas

with BSA, we ensured targeted binding of only the bacteria. Four channels characterized our microfluidic system: baseline, control antibody, CT-specific, and NG-specific, which gave us the independence to test for specificity and independent detections. CMOS images of the results depicted prominent spectral shifts at the sensor regions with bacteria, hence successful detection. The NG and CT channels diffraction field intensity increased significantly due to bacterial attachment, unlike baseline and control channels that registered minimal changes. A larger spectral shift in CT, which means more pronounced increase in intensity, proved high sensitivity of the biosensor. These results demonstrated that our biosensor can identify NG and CT in urine samples with high sensitivity and specificity. The platform is ideal for point-of-care diagnostics, as it distinctly differentiates intensity values and spectral shifts to confirm bacterial presence.

B. System LoD for NG and CT Bacteria

The calibration standard curves were constructed through the use of a serial dilution method and tenfold dilutions ranging from 10 to 10^6 CFU/mL for the spiked samples of NG (Fig. 5A) and CT (Fig. 5B). A high concentration, 50 μ g/mL, of protein A/G, as well as antibodies, was used so that the bacteria would be effectively captured on their surface even when such low concentrations are present. The sensitivity and specificity of our designed biosensor were evaluated by targeting the detection of CT and NG bacteria in synthetic urine, mimicking real urine conditions. Each microfluidic channel, functionalized with the same capture agent, contained three plasmonic pixels. The figure presents data from three replicates for each concentration, measured by the three plasmonic pixels. Error bars indicate twice the standard deviation. The obtained intensity values from the diffraction field showed a rising tendency with higher bacteria concentrations, which matched very well with the specific binding reaction between bacteria and their relevant antibodies. Linear regression modeling yielded R^2 values of 0.99474 for NG and 0.99987 for CT, establishment of LoD at 452 CFU/mL in the case of NG, and 121 CFU/mL for CT. Figure 5C shows the diffraction field intensity values for urine samples with 10^5 CFU/mL of only CT bacteria, only NG bacteria, and both bacteria (left, middle, and right plots, respectively). In each experiment, three plasmonic pixels within the same channel were measured. The bars represent the average values, accompanied by error bars indicating twice the standard deviation. The sensor successfully differentiated bacterial presence without any response from the control antibody or the antibodies against other bacteria. This imaging-based readout demonstrated no nonspecific binding, even at high concentrations, confirming the bio-sensor's strength and reliability in detecting NG and CT in urine samples.

IV. CONCLUSION

This portable biosensor was developed to detect CT and NG in urine samples. It label-freely integrates plasmonic nanohole

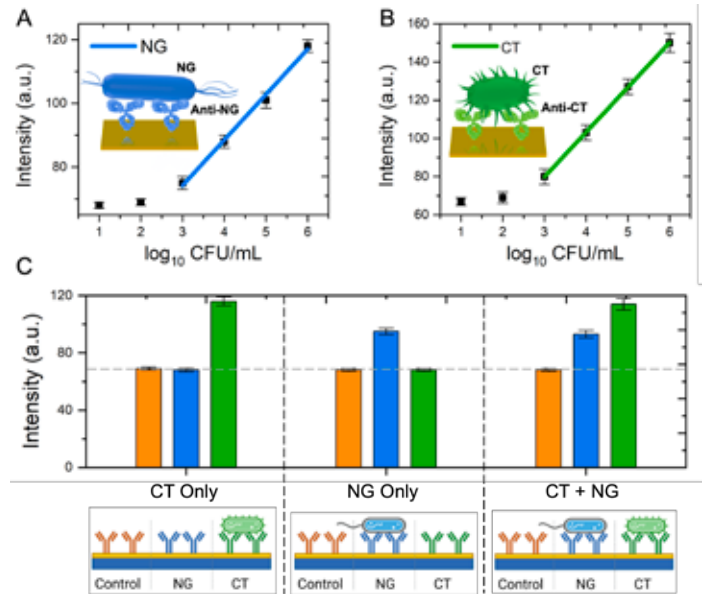


Fig. 5. (A) Calibration graphs for NG bacteria and (B) Calibration graphs for CT bacteria. (C) The intensity values of the diffraction field for urine samples containing only CT bacteria are represented by the green bar on the left. The blue bar in the middle denotes urine samples with only NG bacteria, while the blue and green bars on the right indicate urine samples containing both bacteria. The intensity values are measured at a concentration of 10^5 CFU/mL. The control antibody is represented by the orange bars. Each bar represents the mean value obtained from three separate trials, with error bars indicating twice the standard deviation. Produced using BioRender.com.

sensing with lens-free computational imaging, thereby enabling accurate diagnosis at the point of care. An inexpensive pump and flow cell deliver samples to the plasmonic chip with high precision, while integrated software manages hardware control, testing, and bacterial detection. The system facilitates simultaneous detection of CT and NG through two microfluidic channels. Its high throughput, simple optics, and low-cost parts make the biosensor practical and robust. This handheld device detects NG with a limit of 452 CFU/mL and CT with a limit of 121 CFU/mL.

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