Large-Scale Functionalized Metasurface-Based SARS-CoV-2 Detection and Quantification

Rajib Ahmed, Carlos F. Guimarães, Jie Wang, Fernando Soto, Asma H. Karim, Zhaowei Zhang, Rui L. Reis, Demir Akin, Ramasamy Paulmurugan,* and Utkan Demirci*

Cite This: https://doi.org/10.1021/acsnano.2c02500

ABSTRACT: Plasmonic metasurfaces consist of metal–dielectric interfaces that are excitable at background and leakage resonant modes. The sharp and plasmonic excitation profile of metal-free electrons on metasurfaces at the nanoscale can be used for practical applications in diverse fields, including optoelectronics, energy harvesting, and biosensing. Currently, Fano resonant metasurface fabrication processes for biosensor applications are costly, need clean room access, and involve limited small-scale surface areas that are not easy for accurate sample placement. Here, we leverage the large-scale active area with uniform surface patterns present on optical disc-based metasurfaces as a cost-effective method to excite asymmetric plasmonic modes, enabling tunable optical Fano resonance interfacing with a microfluidic channel for multiple target detection in the visible wavelength range. We engineered plasmonic metasurfaces for biosensing through efficient layer-by-layer surface functionalization toward real-time measurement of target binding at the molecular scale. Further, we demonstrated the quantitative detection of antibodies, proteins, and the whole viral particles of SARS-CoV-2 with a high sensitivity and specificity, even distinguishing it from similar RNA viruses such as influenza and MERS. This cost-effective plasmonic metasurface platform offers a small-scale light-manipulation system, presenting considerable potential for fast, real-time detection of SARS-CoV-2 and pathogens in resource-limited settings.

KEYWORDS: large-scale metasurface, Fano resonance, microfluidics, surface plasmon resonance, SARS-CoV-2, point-of-care detection

INTRODUCTION

Metasurfaces are metal–dielectric surfaces that interact with light at subwavelength scale1,2 and enable unusual and outstanding photonic behaviors at the micro/nanoscale, such as optical chirality,3 negative reflection,4 ultraband reflection/transmission,5 diffraction-limited focusing,6 polarization routing,7 anomalous diffraction,8 and asymmetric Fano response.9 The plasmonic properties of metasurfaces can be controlled by using metal composites, changing the thickness of coating layers, and engineering structural geometries.10–12 Different geometries are commonly obtained by nanofabrication techniques such as electron beam lithography,13 nanoimprinting,14 ion-beam milling,15 laser-interference lithography,16 chemical synthesis, and self-assembly.17 Plasmonic Fano resonant metasurfaces show asymmetric spectral shift due to isolated metal atoms, which are found to be useful in ultrasensitive biomolecule detection applications.18 Surface plasmons of metasurfaces can exist when the free electrons of nanostructures undergo certain oscillation conditions to couple an external optical field.19 The resonant excitations of these free electrons with response to an external optical field, known as surface plasmon resonances (SPRs), are sensitive to the surrounding medium and act as an optical sensor. Particularly, SPRs in the visible/near-infrared region have been shown to be of more interest in biosensing applications due to significant light confinement based on slow down, which allows light coupling to the free electrons in the surface. SPRs can be generated from localized or propagated plasmons. For example, localized SPRs can be generated from spherical nanoparticles and nanoholes,20 and optical field distribution can be explained based on the Mie scattering principle. Similarly, the propagating SPRs can be generated from waveguide modes through grating structures21 or plasmonic gold-coated flat surfaces using prism coupling.22 Further, terahertz (THz) plasmonic and toroidal metasensors are able to improve classical nanophotonics and their biosensitivity

Received: April 3, 2022
Accepted: September 12, 2022
with high accuracy and reliability in immunosensing applications. Metasurfaces with high-quality factor (Q-factor) resonance were studied in photonic crystal and waveguide arrays. This is due to the well-known physics of optically bound states in the continuum (BICs). Plasmonic BIC metasurfaces found useful applications only in longer wavelengths such as the THz regime, where optical losses by metal are negligible, and they can be considered a perfect conductor. However, BIC plasmonic metastructures in mid-infrared or visible ranges remain challenging due to intrinsic optical losses by noble metals (such as gold or silver). Therefore, a hybrid BIC plasmonic system with minimum radiation losses in the visible range was demonstrated based on the combination of metallic grating and dielectric structures. Light confinement and enhancement properties of plasmonic nanostructures and integration with microfluidics reveal a promising platform for next-generation biosensors. However, the fabrication of cost-effective, large-scale, and uniform Fano resonant nanoplasmonic metasurfaces with predefined optical properties remains a critical engineering challenge. In particular, the requirements of a complex nanofabrication infrastructure with trained personnel, time-consuming processing steps, and bulky optical techniques limit the widespread use of Fano resonant surfaces. Optical disk-based metasurfaces were recently demonstrated as an alternative approach that moves away from traditional silicon substrates to plastic-templated platforms. An optical disk has shown promising reproducibility and uniformity in precise electromagnetic excitation at the nanoscale for rapid and cost-effective biosensing applications, especially for the highly sensitive detection of peptides, proteins, and viruses from a variety of sample sources.

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most recent infectious disease-causing respiratory tract illness to humans in a worldwide pandemic. Different laboratory-based and clinical diagnostic techniques have been used to detect SARS-CoV-2, such as paper-based lateral flow assay (LFA), reverse-transcription polymerase chain reaction (RT-PCR), quantitative RT-PCR, enzyme-linked immunosorbent assay (ELISA), and isothermal PCR. RT-PCR is considered the gold standard technique for the diagnosis of SARS-CoV-2 in clinical samples. Being a highly sensitive molecular assay of viral RNA detection, RT-PCR enables the detection of SARS-CoV-2 with a minimum number of RNA copies. However, RT-PCR diagnosis requires centralized facilities limiting their use in remote or field locations. Similarly, ELISA techniques, although useful, do not provide quantitative diagnostic results while being less sensitive. Therefore, cost-effective, rapid diagnostic tools for SARS-CoV-2 detection with high precision within a limited time frame in a resource-limited setting are urgently required in
point of care (POC). Alternative biosensing tools have been proposed to mitigate current diagnostic problems, such as graphene-oxide-functionalized field-effect transistors (FETs), surface-enhanced Raman spectroscopy (SERS), and localized surface plasmon resonance (LSPR). However, LSPR optical systems are bulky and hard to deploy for the POC and at resource-limited settings. Recently, an optical disc-based portable biosensing platform has been reported for viral detection, utilizing a number of chemical steps for surface etching and clean-room access for coating plasmonic metal layers. Further, previously optical disc-based biosensing approaches have used bit-error measurements through a commercial disc readout and surface-enhanced Raman spectrum measurement using bulky equipment, which limits their transportability for point-of-care detection or use at resource-limited settings.

Here, we employ a cost-effective plastic-templated optical digital versatile disc (DVD) as a large-scale metasurface to detect and quantify SARS-CoV-2. We use commercially available DVD surfaces, which are already coated with plasmonic metal layers, thus avoiding previously reported surface preprocessing. We demonstrate how the large-scale metasurface platform integrated with a microfluidic system can generate optical Fano responses when employed for SARS-CoV-2 detection. These optical responses quantify molecular (antibodies, proteins, and SARS-CoV-2 viral particle) interactions and their binding to targets in real time. The Fano spectral responses present high sensitivity ($S = 1475 \text{ nm/RIU}$) and figure of merit (FOM = 147), comparable with the reported Fano metasurface with a spectrum in the visible range. Further, the high sensitivity of the plasmonic metasensors with exotic electromagnetic response of toroidal dipoles was demonstrated to detect infectious virus surface proteins. Platinum nanoparticle (PtNP)-based nanoprobes have been used to capture and detect virus particles such as hepatitis B virus (HBV) and Zika viruses (ZIKV). Similarly, a thin metallic nanoparticle/porous-based optical platform has also been used to detect specific single molecules and cancer markers. Similar to other platforms, our metasurface-based platform showed specificity and distinguished SARS-CoV-2 when compared with infectious particles from similar respiratory RNA viruses such as Middle East Respiratory Syndrome (MERS) and influenza virus. Overall, we demonstrate (i) a cost-effective large-scale plasmonic metasurface approach for real-time binding of antibodies, proteins, and viruses to their respective targets (Supporting Information, Table S1); (ii) spectral shift, interactions, and binding of protein G with the SARS-CoV-2 antibodies (monoclonal and polyclonal); (iii) detection of SARS-CoV-2 antibodies and proteins based on their spectral binding and validation with gold standard RT-PCR, ELISA, and dot blot techniques; (iv) selectivity or specificity of SARS-CoV-2 antibodies and their interaction and binding against influenza and MERS viruses; (v) detection and quantification of SARS-CoV-2 viral particles (heat-inactivated (HI) and gamma-inactivated (GI)); and (vi) a label-free, optical platform to detect SARS-CoV-2. Therefore, the plastic-template plasmonic metasurface presents a clear potential for biosensing applications, such as SARS-CoV-2, other infectious agents, and other viruses at POC and resource-limited settings due to cost-effectiveness and device portability.

### RESULTS AND DISCUSSION

**Overall Plasmonic Chip-Based Real-Time Detection Concept of SARS-CoV-2.** We used heat- and gamma irradiation-inactivated SARS-CoV-2 samples separately, obtained from BEI Resource Center supported by National Institute of Allergy and Infectious Diseases (NIAID), as shown in Figure 1A (further details are available in the Materials and Methods section). We used a plastic-templated optical disc-based plasmonic metasurface with a built-in nano periodic surface grating (spacing $\sim 750 \text{ nm}$ and width ($W$) $\sim 450 \text{ nm}$) as an optical platform to detect and quantify SARS-CoV-2 particles (Figure 1B). To demonstrate surface plasmon-based biosensing response, we cleaned the already metal-coated plasmonic optical disc-based metasurface and assembled it as a custom microfluidic chip. Further details regarding surface preparation and chip fabrication are provided in the Materials and Methods section. The fabricated plastic-templated microfluidic chip contains metal–dielectric waveguide strips that excite multiple surface plasmon modes. When broadband light is directed to illuminate the microfluidic plasmonic metasurface of the device, a plasmonic response is observed due to the large-scale uniform surface grating structures.

We then employed this plasmonic chip for a single-step detection and quantification of SARS-CoV-2 samples. We used heat- and gamma-inactivated SARS-CoV-2 samples to verify the sensitivity of our cost-effective detection platform. This detection mechanism was based on layer-by-layer or step-by-step surface modification through chemical processes to capture and quantify biomolecules and virus particles (Figure 1B,C). For layer-by-layer surface functionalization, we start with the formation of a gold–thiol interaction-based self-assembled monolayer on our device’s gold surface by overnight incubation in 11-mercaptoundecanonic acid (11-MUA). Then, the carboxyl groups of 11-MUA are covalently functionalized via N-(3-(dimethylamino)propyl)/N-hydroxysuccinimide (EDC/NHS) chemistry. Similarly, EDC/NHS activation helps with the binding of protein G to the device surface to immobilize SARS-CoV-2 antibodies to capture the SARS-CoV-2 viral particles for diagnostic applications. As broadband light is directed toward the modified chip surface, optical resonance can be observed in the reflected light. The resonance response curve moves to higher wavelengths (red shift) as we functionalize the metasurface microfluidic chip with bioanalyte layers (Figure 1C). This optical plasmonic phenomena was then employed for real-time detection of interactions and binding of biomolecules with the surface. As the different analytes flow from input tubing toward output tubing in a microfluidic channel, decorated with a Fano resonant sensor as its bottom channel layer, the resonance peaks shift to higher wavelengths due to the higher refractive index in the immediate vicinity of the metasurface as targets bind to the surface. After washing, resonance peaks are unable to return to their previous level, indicating the effective binding of biomolecules including the virus particles with the surface (Figure 1D). Therefore, layer-by-layer surface sensing with target surface chemistry allows real-time monitoring of the specific binding of mono- and multilayers of biomolecules and virus particles, distinguishing them at the molecular level.

**Surface Characterization and Optical Resonance Validation.** To observe and characterize the optical response, a cost-effective and disposable microfluidic chip was fabricated based on a plastic-templated plasmonic metasurface (Figure 1C). For the detection mechanism, we employ a cost-effective large-scale plasmonic metasurface as an optical platform to detect and quantify SARS-CoV-2. We use commercially available DVD surfaces, which are already coated with plasmonic metal layers, thus avoiding previously reported surface preprocessing. We demonstrate how the large-scale metasurface platform integrated with a microfluidic system can generate optical Fano responses when employed for SARS-CoV-2 detection. These optical responses quantify molecular (antibodies, proteins, and SARS-CoV-2 viral particle) interactions and their binding to targets in real time. The Fano spectral responses present high sensitivity ($S = 1475 \text{ nm/RIU}$) and figure of merit (FOM = 147), comparable with the reported Fano metasurface with a spectrum in the visible range. Further, the high sensitivity of the plasmonic metasensors with exotic electromagnetic response of toroidal dipoles was demonstrated to detect infectious virus surface proteins. Platinum nanoparticle (PtNP)-based nanoprobes have been used to capture and detect virus particles such as hepatitis B virus (HBV) and Zika viruses (ZIKV). Similarly, a thin metallic nanoparticle/porous-based optical platform has also been used to detect specific single molecules and cancer markers. Similar to other platforms, our metasurface-based platform showed specificity and distinguished SARS-CoV-2 when compared with infectious particles from similar respiratory RNA viruses such as Middle East Respiratory Syndrome (MERS) and influenza virus. Overall, we demonstrate (i) a cost-effective large-scale plasmonic metasurface approach for real-time binding of antibodies, proteins, and viruses to their respective targets (Supporting Information, Table S1); (ii) spectral shift, interactions, and binding of protein G with the SARS-CoV-2 antibodies (monoclonal and polyclonal); (iii) detection of SARS-CoV-2 antibodies and proteins based on their spectral binding and validation with gold standard RT-PCR, ELISA, and dot blot techniques; (iv) selectivity or specificity of SARS-CoV-2 antibodies and their interaction and binding against influenza and MERS viruses; (v) detection and quantification of SARS-CoV-2 viral particles (heat-inactivated (HI) and gamma-inactivated (GI)); and (vi) a label-free, optical platform to detect SARS-CoV-2. Therefore, the plastic-template plasmonic metasurface presents a clear potential for biosensing applications, such as SARS-CoV-2, other infectious agents, and other viruses at POC and resource-limited settings due to cost-effectiveness and device portability.
2A). The sample preparation and chip fabrication steps are illustrated in detail in the Supporting Information (Figure S1). The plastic-templated metasurface with broadband light illumination shows scattering in a rainbow pattern due to a large uniform nanostructure and color diffraction easily visible to a naked eye (Supporting Information, Figure S2). This is due to the reason that the plasmonic metasurface shows excitation of bright modes and dark modes due to plasmonic nanoscale surface gratings. The scanning electron microscopic (SEM) image shows a nanoplasmonic grating structure with a uniform surface periodicity of \( \sim 750 \text{ nm} \) (Figure 2B). To observe and characterize the optical response, the fabricated microfluidic chip was illuminated with a broadband light source and the reflected light was collected for analysis. Figure 2C shows the schematic representation of the experimental setups to measure the plasmonic response of the metasurface microfluidic chip. Further, an experimental optical resonance curve followed with numerical modeling shows excitation of optical modes due to light interaction with plasmonic nanoscale surface structures (Figures 2D and S3). The numerical modeling shows excitation of optical modes due to light interaction with plasmonic nanoscale surface structures (Figures 2D and S3). The numerical modeling also shows the electric and magnetic field top and side views with their charge distributions at Fano resonance (Supporting Information, Figure S3). Further details regarding chip fabrication, optical setup, and numerical modeling are described in the Materials and Methods section and Supporting Information in Figure S3. Briefly, the devices are fabricated by laser cutting of poly(methyl methacrylate) (PMMA) and double-side adhesive layers, followed by attachment of these to the sensor area of the DVD surface. The optical resonance field can be observed with normal light illumination, and the experimental results followed medium \((n = 1.0 \text{ and } 1.33)\) refractive index variation (Figure 2D).

We measured the Fano response of the plastic-templated metasurface through a customized optical setup (Supporting Information, eqs 1–3, Figure S4). As we illuminated broadband light onto the microfluidic chip with air medium, we observed a dip plasmonic Fano resonance at 750 nm. The medium-dependent optical Fano resonance shifting was then observed by flowing deionized water (DI) through the microfluidic chip, yielding an asymmetric plasmonic Fano resonance peak at 530 nm (Figure 2D, Supporting Information, eq 2). The shifting of the Fano resonance and shape of the curve change due to the variation of the Fano parameter or \(q\)-value. To verify the optical response as a Fano line-shape, we evaluated resonance spectra through Fano curve fitting (Supporting Information, Figure S5). We calculated Fano fitting parameters \([B, \omega_0, A, \Gamma, q]\) (definitions are provided in the Supporting Information) as \([42.67 \pm 0.64, 750 \pm 0, 1.69 \pm 0.11, 6.91 \pm 0.21, 0.202]\) for air medium \((n = 1.0)\) first. Similarly, for water medium, we calculated Fano fitting parameters \([B, \omega_0, A, \Gamma, q]\) as \([17.34 \pm 0, 552.77 \pm 0.0, 34.63\)
The results confirmed that, as the medium changes, the Fano parameters change their values due to resonance mode coupling or interference with the background mode. Further, to characterize plasmonic behavior of our metasurface, we used the glycerol concentration dilution series and measured the corresponding bulk sensitivity values. As we increased the refractive index (RI) of the dielectric medium, the Fano resonance peak, dip, and middle point of the asymmetric line-shape moved to higher wavelength values as we increased the glycerol concentrations of the medium from 1% to 70% (Figure 2E). This occurs due to the increase in the RI of the surrounding medium and the consequent increase of the resonance mode decay. The waveguide's modes and RI-associated field decay were also studied numerically to explain the resonance wavelength shifting.

Figure 3. Surface sensitivity measurements of the plastic-templated plasmonic metasurface to observe real-time molecular interactions and its binding affinities. (A) Layer-by-layer surface functionalization of the surface plasmonic metasurface (SPM). (B) Real-time binding response of multiple analytes, such as protein G, SARS-CoV-2 antibody, and antigen measured by optical readout. (C) Resonance wavelength shift and binding as a function of protein G concentrations. (D, E) Real-time resonance wavelength as a function of SARS-CoV-2 antigen and antibody dilution.
electric (TE₀₀) and transverse magnetic modes (TM₀₀) are excited based on the waveguide dimensions and surrounding RI medium. The TM₀₀ shows maximum field intensity interaction with the surrounding medium. Therefore, resonance wavelength shifting is observed due to the variation in the RI of the medium. As we increased the glycerol concentration (concentration from 1% to 70%), the RI value increased gradually from 1.33 RIU to 1.43 RIU. Both resonance peak and dip values were thus shifted toward the right (higher wavelength) as we increased the concentration of glycerol percentage values (Figure 2E−G). To evaluate the performance of the plasmonic sensor, we measured the resonance peak/dip wavelength shift (Δλ) and their corresponding full width at half-maximum (fwhm) due to RI variation (Δn). The sensor’s sensitivity (S) and figure of merit (FOM) can be defined as S = Δλ/Δn and FOM = S/fwhm. As we changed the glycerol concentration from 1.0% to 70%, the concentration-dependent change in the RI from 1.336 RIU to 1.428 RIU matched with the Fano response curve shifts toward higher wavelengths. Due to RI variation, the resonance peak moves from 529.94 nm to 571.02 nm, and the peak from 496.09 nm to 528.82 nm. For small RI variation (Δn ≈ 0.007 12), we have observed peak and dip wavelength shifts of 4.03 and 7.69 nm, respectively. However, peak and dip wavelength shifting shows high linearity (R² = 0.99) with RI variation. The linear fitting also shows a maximum sensor sensitivity (S) of 1475 nm/RIU resonance dip wavelength shifting. The fwhm or 3 dB bandwidth of the Fano resonance at 750 nm derives a FOM of 147. Further, to observe the longevity and repeatability of bulk sensing, we have repeated the experiments at multiple times on different days (n = 5). We have found similar optical response of the resonance wavelength shift as a function of varying glycerol concentrations from 2.5% to 20% (v/v) (Supporting Information, Figure S6).

Layer-by-Layer Real-Time Molecular Detection. To build a highly specific detection platform on our photonic plasmonic metasurface, layer-by-layer surface functionalization was performed as illustrated in the conceptual block diagram (Figure 3A). The multiple surface chemistry steps were optimized to capture mono- and multilayers of biomolecules, antibodies, and proteins (Figure 3B, Supporting Information, Figures S6, S6). Finally, optimized chemical steps were used to capture the surface protein of SARS-CoV-2 using anti-spike antibody. Surface functionalization included EDC/NHS, protein G, protein blocking agent, primary SARS-CoV-2 antibody, SARS-CoV-2 spike protein/SARS-CoV-2 viral particles, and secondary anti-SARS-CoV-2 antibody. Detailed chemical steps, incubation periods, and flow rates are defined in Table S2 of the Supporting Information. Figure 3B shows the real-time optical response of our plastic-templated wafer-scale plasmonic metasurface upon capture of distinct biomolecules, evidencing their binding after washing with phosphate buffer saline (PBS) solution. Initially, the plastic-templated metasurface chip with a gold surface was functionalized with carboxyl groups through incubation of 11-MUA.
Afterward, EDC/NHS functionalization was performed by flowing the solution through the chip to provide a stable succinimidyl group on the metasurface that allows the binding of amino groups for further functionalization with proteins (for example, protein G). Protein G-coated surfaces allowed us to immobilize the primary anti-SARS-CoV-2 spike antibody on the devices, which could then bind to the spike protein antigen of the virus and, in turn, with the secondary antibody. Functionalization of our metasurface enables observing molecular interactions, their binding, and consequent capture through the Fano resonance shifting. During EDC/NHS (100 mM:100 mM) solution flow, a Fano resonance wavelength shift of 0.22 ± 0.008 165 nm (mean ± standard error, n = 3) was observed (Figure 3B). Similarly, as we flowed protein G solution, primary anti-SARS-CoV antibody (mAb), and SARS-CoV-2 spike antigen through the microfluidic channel, the cumulative Fano resonance shifts for each step were of 0.36 ± 0.008, 0.66 ± 0.017, and 0.795 ± 0.0125 nm (mean ± standard error, n = 3), respectively. Each chemical step was followed by a thorough PBS wash before flowing the next chemical solution. Atomic-scale molecular interactions and their associated binding were observed at each step by comparing the resonance wavelength shifting (before and after PBS washing). We have also studied variations in protein G concentrations from 25 to 1000 µg/mL and measured the individual Fano resonance shifts during the molecular binding events (Figure 3C). Lower molecular binding and resonance wavelength shifting were observed at the lowest protein G concentration of 25 µg/mL. Maximum Fano resonance shifting was observed with 1000 µg/mL of protein G. However, maximum molecular binding was observed with 500 µg/mL of protein G through the microfluidic channel (comparing the baselines before and after PBS washing) (Figure 3C). Therefore, the optimal 500 µg/mL of protein G was used for functionalizing the plastic-templated metasurface to capture SARS-CoV-2 spike antibody. A study of protein G concentration was also performed over time on the 50 to 500 µg/mL and validated the real-time resonance wavelength shifting due to cumulative molecular binding with no visible saturation (Supporting Information, Figure S8). We then varied the concentration of anti-SARS-CoV-2 spike antibody and SARS-CoV-2 spike antigen to observe their real-time binding and spectral shifting (Figure 3D,E). As we increased the concentration of analytes, the molecular thickness increased gradually, causing the resonance wavelength shift toward higher wavelengths. Similarly, a wavelength shift was also validated using the ELISA-based technique through the spike SARS-CoV-2 antibody and protein concentration variation (Supporting Information, Figure S7).

Optimization of SARS-CoV-2 Binding and Specificity Tests. SARS-CoV-2 presents three main types of surface proteins—spike, envelope, and membrane proteins—as their surface antigens. Among these, spike protein has been considered predominant, for which several candidate vaccines have been developed and administered in humans. Therefore, we functionalized our plastic-templated plasmonic metasurface with anti-spike SARS-CoV-2 antibody to capture SARS-CoV-2 via binding to the viral surface protein. We employed Western blotting to confirm the specificity of antibody–antigen pairs with the used anti-SARS-CoV-2 spike antibody and respective spike protein antigen (Figure 4A). We confirmed our spike SARS-CoV-2 antibody having strong binding with SARS-CoV-2 spike protein (Figure 4A). Minimum binding responses were observed with SARS-CoV-1 and MERS protein. Further, we evaluated the binding affinity between anti-spike SARS-CoV-2 antibody with SARS-CoV-2 viral particles using a fast dot blot analysis (Figure 4B,C) where we observed a virus dose-dependent detection signal (30 × 10^6 to 90 × 10^6 copies per μL).

To improve the specificity of the antibody and to reduce the nonspecific interactions with other related viruses (SARS-CoV-1 and MERS), we introduced a protein blocking agent (Pierce protein-free PBS, Product # 37584, Thermo Scientific) in our system after the functionalization of protein G through the microfluidic channel. Blocking solution passivates protein-free metasurface areas and reduces the nonspecific binding of nontarget molecules. However, the surface treatment with and without blocking agent did not significantly affect the spectral resonance shifting of anti-SARS-CoV-2 antibody (Supporting Information, Figure S9). We have also evaluated spike SARS-CoV-2 monoclonal and polyclonal antibodies of mouse and rabbit sources and tested for their interactions with SARS-CoV-2 antigen. We did not observe any major differences in the binding response by these antibodies, indicating that they have similar recognition ability for surface proteins of SARS-CoV-2 (Supporting Information, Figures S10 and S11). To assess the specificity of binding, we introduced a solution containing the MERS virus in the microfluidic channel (Figure 4D). A small spectral resonance shift was observed with MERS particles compared with SARS-CoV-2 prior to washing, demonstrating specificity. In contrast, negligible binding of MERS anti-SARS-CoV-2 antibody was observed after washing the microfluidic channel with PBS. Yet, a strong molecular binding of SARS-CoV-2 was observed after washing the channel with PBS. Further, we increased the SARS-CoV-2 antibody concentration by 2-fold compared to the initial concentration and showed the resonance wavelength shifting of MERS and SARS-CoV-2 (Supporting Information, Figure S12). A moderate level of nonspecific binding of MERS was observed after the PBS washing but was significantly lower than the specific binding signal observed with SARS-CoV-2 after the washing with PBS. To verify the specific binding of SARS-CoV-2 antibody with SARS-CoV-2 antigen, we have used influenza virus, MERS, and SARS-CoV-2 at the same time (Figure 4E). No spectral shift or molecular binding was observed with influenza virus. However, we observed a small spectral shift and molecular binding with MERS virus compared with SARS-CoV-2. MERS viruses may have spike protein with some homology with the spike protein of SARS-CoV-2. However, maximum resonance shifting with molecular binding was observed with SARS-CoV-2 virus. Therefore, the employed SARS-CoV-2 antibody enabled differentiating influenza, MERS, and SARS-CoV-2 via their associated real-time molecular binding and spectral shifting characteristics. Further, we evaluated our plastic-templated functionalized plasmonic surface with maximum anti-SARS-CoV-2 antibody and varied the virus concentration of MERS and SARS-CoV-2 (Figure 4F). We observed increased cumulative resonance wavelength shifting as a function of spike anti-SARS-CoV-2 dilution from 1:13 to 1:120. Maximum resonance (1.79 times higher) wavelength shifting was observed with SARS-CoV-2 compared with MERS virus (Supporting Information, Figure S12). Therefore, layer-by-layer surface functionalization of a plastic-templated plasmonic metasurface was able to detect their interaction and atomic binding in concordance with virus concentration (Figure 5).
To verify the performance of the plastic-templated plasmonic metasurface, we tested inactivated (heat (HI) and gamma ray (GI)) SARS-CoV-2 particles using our cost-effective optical detection platform. We functionalized the plasmonic metasurface with all optimized concentrations of 11-MUA (10 mM), EDC/NHS (100:100 mM), protein G (500 μg/mL), and anti-SARS-CoV-2 antibody (1:60 dilution of stock). We have shown SARS-CoV-2 viral particle detection through optimized layer-by-layer surface functionalization of our plastic-templated plasmonic metasurface (Supporting Information, Figure S12). Further, incubated (1 h duration) anti-SARS-CoV-2 antibody was used to capture GI and HI SARS-CoV-2 particles (Figure S5A,B).

Quantitative Detection and Capture of SARS-CoV-2 Particles. To verify the performance of the plastic-templated plasmonic metasurface, we tested inactivated (heat (HI) and gamma ray (GI)) SARS-CoV-2 particles using our cost-effective optical detection platform. We functionalized the plasmonic metasurface with all optimized concentrations of 11-MUA (10 mM), EDC/NHS (100:100 mM), protein G (500 μg/mL), and anti-SARS-CoV-2 antibody (1:60 dilution of stock). We have shown SARS-CoV-2 viral particle detection through optimized layer-by-layer surface functionalization of our plastic-templated plasmonic metasurface (Supporting Information, Figure S12). Further, incubated (1 h duration) anti-SARS-CoV-2 antibody was used to capture GI and HI SARS-CoV-2 particles (Figure S5A,B). The stock gamma-inactivated viral particles of $7.2 \times 10^4$ virus copies per microliter in 1:20 dilution were used during GI SARS-CoV-2 experiments. The stock GI SARS-CoV-2 concentration was further diluted to the required concentrations using PBS before flowing them through the microfluidic channel. The real-time response shows a Fano resonance wavelength shift from 533.96 to 532.1 nm due to the molecular binding of SARS-CoV-2 particles on the functionalized surface. Fano resonance wavelength shifting and molecular binding were also observed for HI SARS-CoV-2 particles (Figure S5B). For HI SARS-CoV-2 viral particles, we used a stock concentration of $4.7 \times 10^6$ virus copies per microliter in 1:20 dilution for the experiment. In our experiments, the GI SARS-CoV-2 virus showed higher resonance wavelength shifting and molecular binding com-

---

**Figure 5.** Plastic-templated plasmonic metasurface for SARS-CoV-2 capture, detection, and quantification. (A–C) Real-time detection of GI and HI SARS-CoV-2. (D, E) Resonance wavelength shifting due to logarithmic and serial dilution of the number of viruses. (F) Captured SARS-CoV-2 on our plastic-templated plasmonic metasurface platform. Scale bars = 2 (left) and 0.5 μm (right).
pared to HI SARS-CoV-2 virus particles. This could be likely due to the denaturation of SARS-CoV-2 virus antigen upon exposure to heat during inactivation compared to those inactivated using gamma radiation, as the γ radiation promotes random breakage of genomic biomolecules but not particularly the structural integrity of the viral antigen. 47 Further, we measured the HI and GI SARS-CoV-2 virus copies by real-time RT-PCR to correlate the results (Supporting Information, Figure S13). The CT/Cq (cycle of threshold) value decreased exponentially as we increased the number of virus copies. HI SARS-CoV-2 particles show maximum Cq (approximately two times) compared with GI SARS-CoV-2 particles at the same input virus concentration. To understand this further, we have observed real-time flow and incubation of GI SARS-CoV-2 particles (Figure 5C). Maximum resonance wavelength shift, approximately 0.7067 ± 0.0345 nm, was observed due to the binding of GI virus particles. Real-time Fano resonance wavelength shifting was obtained as a logarithmic function of GI virus concentration (Figure 5D). Continuous virus binding was observed with decreasing dilution (increasing concentration) ranging from 145 to 14.5 × 10^4 copies/mL. A linear spectral response was observed due to the cumulative binding of SARS-CoV-2 particles onto the nanoplasmonic metasurface. Maximum resonance wavelength shifting was observed with maximum dilution of stock concentration, 1.5 × 10^5 copies/mL. Additionally, we also studied a sequential series of higher dilutions from stock concentration (1:10 to 1:160) containing SARS-CoV-2 viral particles ranging from 9 × 10^5 to 1.45 × 10^5 copies/mL. We validated and quantified the virus copies through RT-PCR and their binding confirmation through ELISA as shown in the Supporting Information, Figures S13, S14. In the experiments, the Fano resonance wavelength shifts in a stepwise pattern due to PBS washing steps between the flow of two sequential virus concentrations into the microfluidic channel (Figure 5E). All the SARS-CoV-2 dilutions cause a maintained red shift due to molecular binding after PBS washing. The SARS-CoV-2 viruses are captured randomly at the waveguide surfaces and attached to the spacing between them. The SEM image (Figure 5F) of the captured SARS-CoV-2 virus confirms the highly sensitive capture of the particles on the plasmonic metasurface platform. Further, we also tested the longevity and repeatability of the device using protein G (Figure S15), antibody (Figure S16), and SARS-CoV-2 particles (Figure S17) at multiple times on different days (n = 5 for each experiment), and the results are provided in the Supporting Information.

CONCLUSIONS

We used a commercially available plastic-templated optical disc as a large-scale cost-effective metasurface platform and showed an effective biosensing application to detect and quantify SARS-CoV-2 virus. The demonstrated plasmonic metasurface-based biosensing platform is simple and less time-consuming for nanofabrication or plasmonic material coating and does not require clean room facilities. Our plastic-templated wafer-scale metasurface sensor platform offers an inexpensive alternative SARS-CoV-2 detection tool, can replace silicon substrate-based metasurface fabrication techniques, and does not require any expensive facility for nanostructure fabrication or sample preparation. Additionally, we demonstrate how metal—dielectric layers of the plastic-templated metasurface can be excited at the optical mode to provide a plasmonic Fano response, allowing detection of each analyte layer and quantitative detection of SARS-CoV-2 particles. Some of the limitations of our study are that we have measured only a single point (∼1 mm radius spot size) from a large chip surface (20 × 3 mm²), and surface nonuniformity may cause some shifting of the reference wavelength (during the initial PBS wash). Therefore, the virus can bind nonuniformly everywhere; so to minimize nonspecific virus binding, we used a protein-free blocking agent (further details are in the Methods and Materials section). Furthermore, during the measurements, the PBS signal was observed to stabilize over longer periods of time, and this could potentially be resolved by replacing PBS with other washing solutions such as osmotically balanced buffers. 48

Further, layer-by-layer surface chemistry functionalization and real-time light—matter interaction are employed to measure molecular interactions and binding of the antibodies, proteins, and the antigens, including anti-SARS-CoV-2 antibody, SARS-CoV-2 spike proteins, and the intact viruses. During the surface functionalization of our biosensing platform with anti-SARS-CoV-2 antibody, we have observed nonspecific binding of MERS spike protein due to its similarity to that of SARS-CoV-2. Finally, we have validated our plastic-templated Fano resonance metasurface detection platform with gold-standard RT-PCR, ELISA, and dot blot techniques. The proposed wafer-scale cost-effective metasurface platform was evidently able to detect and quantify heat- and gamma-inactivated SARS-CoV-2 viruses without any significant nonspecific binding with influenza or MERS viruses. Overall, we demonstrate a sensitive, large-scale manufacturable surface biosensing platform using a simple, cost-effective, and disposable plastic-templated metasurface-based microfluidic device. This biosensing platform can be easily adapted for addressing other current and future pathogens and to detect a wide range of biological targets such as viruses, antibodies, and proteins in POC and at resource-limited settings.

MATERIALS AND METHODS

Sample Preparation for Plastic-Templated Metasurface-Based Microfluidic Biosensor Application. We have used a simple procedure to fabricate a cost-effective, large-scale biosensing platform for SARS-CoV-2 detection. We have used commercially available blank DVD disks (16x speed, 4.7 GB, TDK) as a plasmonic platform. The commercial DVD surface consists of different subsequent layers, such as a plastic protection layer (top), metal-grating layer, photoresist layer, and bottom polycarbonate plastic layer. Briefly, we removed the top plastic surface of the DVD and removed the PR layer by cleaning the DVD surface, having the plastic-templated metasurface with plasmonic metal-coated surface gratings ready for microfluidic chip building. Finally, we assembled the layers of PMMA, double-side adhesive, and the plasmonic metasurface to fabricate the microfluidic plasmonic chip ready for liquid analyte (biomolecules) flow (further details are available in the Supporting Information, Figure S1). The fabrication procedure of the microfluidic metasurface-based plasmonic chip consisted of the following steps: (i) top plastic removal by using a sharp blade, (ii) cleaning the DVD surface with ethanol and methanol in equal volume amounts (1:1) at 60 °C for 5 min on an electric hot plate (Corning PC-420D), (iii) plasmonic metasurface surface washing with DI water, drying with air flow, cutting with a laser or sharp blade through the PMMA layer side, and attachment of the double-sided adhesive layer according to the microfluidic chip design, and (iv) final assembly of the microfluidic chip with the attachment of the input and output tubing to form a plasmonic microfluidic chip for biosensing applications (further details are available in the Supporting Information, Figure S1).
Surface Functionalization, Sensitivity, and Specificity of SARS-CoV-2 Detection. To measure the optical biosensing performance of the plastic-templated wafer-scale metasurface, bulk and surface sensitivity measurement techniques were performed. To measure the bulk sensitivity of the metasurfaces, glycerol in DI water was diluted (v/v) from 1% to 70% and flowed through the microfluidic chip, measuring resonance wavelength shifting in real time. To measure the surface sensitivity of the metasurface, layer-by-layer surface functionalization was performed to capture SARS-CoV-2 antibody, protein, and virus particles. Surface functionalization of the metasurface is based on subsequent chemical steps: (i) overnight (12 h) incubation of the metasurface inside the 10 mM 11-MUA (Sigma-Aldrich, MO, USA) solution to generate carboxyl groups. (ii) Finally, the surface was washed with ethanol, dried through airflow, and assembled into the microfluidic chip. The microfluidic chip consisted of three layers: a top PMMA layer (thickness: 3.2 mm, MacMaster Carr, Elmhurst, IL, USA), a middle double-side adhesive (DSA) layer (thickness: 50 μm, iTapetore, Scotch Plains, NJ, USA), and last a metasurface layer. A CO$_2$ laser machine (VL-200, VersaLaser, AZ, US) was used to cut the PMMA and DSA layers.

SARS-CoV-2 antibody (Sino Biological, Cat. # 21199, Thermo Fisher Scientific, MO, USA) was run through the channel. That will reduce unbound protein G in our DVD metasurface to increase target antibody binding. (vi) After washing with PBS, monoclonal spike SARS-CoV-2 antibody (Sino Biological, Cat. # 40150-D003) was bound to the protein G layer. (vii) Then, we captured the spike SARS-CoV-2 antigen (Ray Biotech, Cat. # 230-20406-200) and washed the microfluidic channel. (viii) Finally, we washed the microfluidic channel with PBS, and polyclonal spike SARS-CoV-2 antibody (Sino Biological, Cat. # 40592-T62) was flowed through the channel to confirm the binding of spike SARS-CoV-2 antigen. After observing layer-by-layer surface functionalization to capture SARS-CoV-2 antigen, we have also tested our platform with antigens/intact viruses of influenza (H3N2, BioWorld 22060519) and MERS (Cat. # NR-44260). We followed the same surface functionalization procedure as mentioned above, steps (i) to (vi). After SARS-CoV-2 antibody functionalization, we filled the microfluidic channel with influenza virus and washed the channel with PBS. After washing, we flowed inactivated MERS virus through the channel and washed the channel with PBS. Finally, we flowed the SARS-CoV-2 sample through the channel and washed it with PBS. (Further details are available in the Supporting Information, Table S2).

SARS-CoV-2 Sample Collection, Capture, and Real-Time Quantification. We have obtained heat-inactivated SARS-CoV-2 (Catalog # NR-52286, Isolate USA-WA1/2020) and gamma-inactivated SARS-CoV-2 (Cat. # NR-52287, isolate USA-WA1/2020) virus samples from NIAID BEI Resources (order number: IC2020-1784). SARS-CoV-2 sample collection procedures were based on subsequent steps: SARS-CoV-2 samples were isolated and collected through an oropharyngeal swab from patients. After that, collected oropharyngeal swabs were mixed with sample buffer solvent, and finally the SARS-CoV-2 samples were inactivated with external heat or gamma-ray sources. Similarly, the complete genomic information and other information about heat-inactivated SARS-CoV-2, USA-WA1/2020, and gamma-inactivated SARS-CoV-2, USA-WA1/2020, are also available at BEI Resources. We have diluted the stock concentrations and flowed the solutions through the layer-by-layer surface-functionalized DVD metasurface (same as surface functionalization steps (i) to (vii)). As we flowed lower to higher concentrations of SARS-CoV-2 samples, the optical response (Fano resonance wavelength in nm) shifted to higher values, allowing us to correlate it with the number of virus copies.

Optical Modeling. Optical properties of the plastic-templated metasurface were modeled using a finite element-based computational tool (CST Studio Suite). The 3D model structure was based on nanoporous grating with periods = 750 nm, width = 450 nm, and height = 250 nm. Further, single unit cell and periodic boundary conditions were considered to reduce complexity and computational time. The light was illuminated through an input port, and output was measured through the output port. For simplicity, we considered a plastic-templated grating structure based on polycarbonate ($n = 1.58$) and decorated with gold (Au) material. The grating structure was illuminated through a broadband light source, and reflected light intensity was calculated as a function of incident wavelength. Finally, electric and magnetic field distribution and their associated charge distributions were observed at Fano resonance frequency.

RT-PCR, ELISA, Western Blot, and Dot Blot Based SARS-CoV-2 Quantification. RT-PCR. We used an RT-PCR assay kit designed against the nucleocapsid coding region of SARS-CoV-2 for quantifying the viral RNA from heat- and gamma-inactivated viruses obtained from NIAID-BEI Resource Center for determining the viral titer. We followed the manufacturer’s protocol to perform the assay. Further details are described in Table S3 of the Supporting Information.

ELISA. We used ELISA for measuring the specificity of antibody used in this study for functionalizing the Fano resonance sensor. Sample functionalization and the measurement procedure were described previously. We have functionalized a 96-well plate by incubation at 4 °C overnight with 50 μL of recombinant spike protein. The stock concentration of recombinant spike protein was 5 μg/mL (expressed in E. coli) in 100 mM sodium bicarbonate buffer solution. After the incubation period, we washed the plates three times with PBS solution mixed with 0.05% Tween 20 (PBST). We blocked the plate bottom surfaces with 100 μL of 1% bovine serum albumin in PBST for 2 h of incubation. Further, diluted serum samples were added sequentially and incubated for 1 h at room temperature. After washing three times with PBST, samples were incubated at room temperature with horse radish peroxidase (HRP)-conjugated secondary antibody (1:5000) for 1 h. We washed the sample plate three times with PBST and functionalized it with 100 μL of the substrate TMB (3,3′,5,5′-tetramethylbenzidine)/H$_2$O$_2$ (BD Biosciences, San Jose, CA, USA) in a dark room for 20 min. Finally, the substrate reactions were stopped by adding 100 μL of 2 N H$_2$SO$_4$. We used a Tecan spectrophotometer to measure the optical density (OD) at 570 nm wavelength, and the results are expressed as mean absorbance ± standard deviation (SD). Further details are described in Table S4 of the Supporting Information.

Western Blot Analysis. To test the specificity of antibody used in this study for the Fano resonance sensor purchased from Sino Biologicals, we used the spike protein of SARS-CoV-1 and SARS-CoV-2. In brief, 100 ng of spike protein in 30 μL was mixed with 10 μL of 4X loading buffer (NuPAGE LDS), heated at 95 °C for 5 min, loaded in 4–12% SDS-polyacrylamide electrophoresis gradient gel (Thermo Fisher-Invitrogen, Waltham, MA, USA), and run at 80 V for 2.5 h. We then electroblotted the gels onto a nitrocellulose membrane (0.2 μm pore size, Schleicher & Schuell, Keene, NH, USA). The membrane was blocked with 5% nonfat dry milk in tris-buffered saline containing 0.01% Tween-20 (TBS-T, pH 7.6) for 30 min and then incubated with the anti-rabbit SPK monoclonal antibody overnight at 4 °C on a rocking platform for 24 h. The following day, the membrane was washed with TBS-T, incubated with the 1:10 000 dilution of a peroxidase conjugated goat anti-rabbit IgG secondary antibody (Rockland Immunochemicals, Gilbertsville, PA, USA), and allowed to rock for 2 h at room temperature. The blots were washed three times in PBS-T, developed using Pierce ECL Western blotting substrate (Thermo Fisher Scientific, Waltham, MA, USA), and imaged with the IVIS Lumina III in vivo imaging system. Further details are described in Figure S12 of the Supporting Information.

Dot Blot Assay. Further, we have employed the dot blot technique to verify the binding affinity of our spike SARS-CoV-2 antibody and virus particle in nitrocellulose membranes. This naked-eye colorimetric detection was based on the following subsequent steps: (i) The
paper-based platform was prepared, sized and scaled to square sampling boundaries (≈4 × 4 mm²) of the nitrocellulose membrane (Bio-Rad, Cat. # 1620117) having a pore size of 0.45 μm. (ii) GI SARS-CoV-2 was based on the number of virus copies to see their colorimetric response. (iii) Different virus concentrations (2 μL droplets) were deposited once, twice, and thrice at each square boundary. (iv) The dry nitrocellulose membrane was immersed and incubated for 30 min at room temperature with 5% bovine serum albumin (BSA) (Sigma-Aldrich, Cat. # A2153) in TBS (Tris-buffered saline, Cat. # 94158)-T (0.05% Tween 20) (Sigma-Aldrich, Cat. # P7949) in TBS. (v) The nitrocellulose membrane was blocked and washed with PBS overnight, then incubated at 4°C with spike SARS-CoV-2 primary antibody (mouse anti-spike, 1:500 dilution, Sino Biologicals Cat. # 40150-D003-50) solutions. (vi) Finally, membranes were washed three times with TBS-T and incubated for 1 h at room temperature with secondary antibody (goat anti-mouse, alkaline phosphatase conjugate, Invitrogen Cat. # G-21060, 1:1000 dilution). (vii) Finally, the membrane was washed three times with TBS-T and immersed in a solution containing AP-conjugate kit (Bio-Rad, Cat. # 1706432). The membrane was removed from the solution when the dots became visible to the naked eye (20−30 min maximum) (further details are available in the Supporting Information, Table S5).

Optical Setup and Biosensing Experiment. A customized optical setup was used to measure the optical Fano response of our plastic-templated DVD metasurface. This optical setup consisted of a broadband light source (OSL2 fiber illuminator, Thorlabs, wavelength: 380 to 800 nm), customized spectrometer (Ocean Optics, HR4000+, resolution: ∼0.02 nm fwhm, wavelength range: 380−800 nm), light illumination subsystem (convex lens, optical polarizer, shutter, input optical fiber), and light collection subsystem (convex lens, shutter, output optical fiber). Light illuminated from the source passed through the input convex lens, linear polarizer, optical shutter (50 μm), beam splitter (50/50), and focusing objective lens (4×). Incident light was normally illuminated on the DVD chip, and reflected resonance spectra passed through the objective and beam splitter and measured through the customized spectrometer. Biomolecules or analyte was flowed through the input tubing of the microfluidic chip using a syringe pump system. The Refractive Index (RI) of the analyte/dielectric medium causes Fano resonance shifts from left to right. Customized software was used to measure the peak/dip (in nm) of the Fano resonance as a function of time (in seconds). Therefore, real-time molecular interactions and their binding event were measured using MATLAB-interfaced Omindrive software.

Statistical Analysis. Statistical analysis (median and standard deviation of samples) was performed using Microsoft excel software. Further details on the statistical analysis are discussed in the text and figure captions. Normalization procedure involved taking the median of the PBS washing steps before and after each molecular binding event and taking the respective ratios.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.2c02500.

Plasmonic resonance of metasurface, metasurface-based microfluidic chip fabrication steps, SEM and large-scale surface structures, experiment setup, Fano fit curves, resonance wavelength shifting with analytes, longevity and repeatability of biosensing, and experiment steps and procedures (PDF)

AUTHOR INFORMATION

Corresponding Authors
Ramasamy Paulmurugan — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; orcid.org/0000-0001-7155-4738; Email: paulmur8@stanford.edu

Utkan Demirci — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; orcid.org/0000-0003-2784-1590; Email: utkan@stanford.edu

Authors
Rajib Ahmed — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; orcid.org/0000-0002-9184-2400

Carlos F. Guimarães — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; 3B’s Research Group—Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães 4805-017, Portugal; ICVS/3B’s—PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal

Jie Wang — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; orcid.org/0000-0003-2223-5794

Fernando Soto — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States; orcid.org/0000-0001-8494-9325

Asma H. Karim — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States

Zhaowei Zhang — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States

Rui L. Reis — 3B’s Research Group—Biomaterials, Biodegradables and Biomimetics, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, University of Minho, Guimarães 4805-017, Portugal; ICVS/3B’s—PT Government Associate Laboratory, Braga/Guimarães 4805-017, Portugal

Demir Akin — Canary Center at Stanford for Cancer Early Detection, Stanford School of Medicine, Stanford University, Palo Alto, California 94304, United States

Complete contact information is available at: https://pubs.acs.org/doi/10.1021/acsnano.2c02500

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge support from Stanford RISE COVID-19 Crisis Response Faculty Seed Grant Program and Stanford PHIND Seed Grant Program. C.F.G. acknowledges support from Fundação para a Ciência e Tecnologia (Grant No. PD/BD/135253/2017) as well as Fundação LusoAmericana Para o Desenvolvimento (FLAD). F.S. was supported by the Schmidt Science Fellows in partnership with the Rhodes
Tunable fano-resonant metasurfaces on a disposable plastic-template for multimodal and multiplex biosensing. Adv. Mater. 2020, 32 (19), 1907160.

(19) Brolo, A. G. Plasmonics for future biosensors. Nat. Photonics 2012, 6 (11), 709–713.

(20) Valsecchi, C.; Jones, T.; Wang, C.; Lochbiller, H.; Menezes, J. W.; Brolo, A. G. Low-cost leukemic serum marker screening using large area nanohole arrays on plastic substrates. ACS Sensors 2016, 1 (9), 1103–1109.

(21) Sair, N.; Escobedo, C.; Sabat, R. G. Crossed surface relief gratings as nanoplasmonic biosensors. ACS sensors 2017, 2 (3), 379–385.

(22) Estevez, M. C.; Alvarez, M.; Lechuga, L. M. Integrated optical devices for lab-on-a-chip biosensing applications. Laser Photonics Rev. 2012, 6 (4), 463–487.

(23) Ahmadivand, A.; Gerislioglu, B.; Abuja, R.; Mishra, Y. K. Terahertz plasmonics: The rise of toroidal metadevices towards immunobiosensing. Mater. Today 2020, 32, 108–130.

(24) Ahmadivand, A.; Gerislioglu, B.; Manickam, P.; Kaushik, A.; Bhansali, S.; Nair, M.; Pala, N. Rapid detection of infectious envelope proteins by magnetoplasmonic toroidal metasensors. ACS Sensors 2017, 2 (9), 1359–1368.

(25) Li, G.; Fan, Y.; Lai, Y.; Han, T.; Li, Z.; Zhou, P.; Pan, P.; Wang, W.; Hu, D.; Liu, X. Coronavirus infections and immune responses. Journal of medical virology 2020, 92 (4), 424–432.

(26) Kabir, A.; Ahmad, R.; Iqbal, S. M. A.; Chowdhury, R.; Paulmurugan, R.; Demirci, U.; Asghar, W. Diagnosis for COVID-19: current status and future prospects. Expert Review Mol. Diagnostics 2021, 21, 269.

(27) Corman, V. M.; Landt, O.; Kaiser, M.; Molenkamp, R.; Meijer, A.; Chu, D. K.; Bleicker, T.; Bruinink, S.; Schneider, J.; Schmidt, M. L. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 2020, 25 (3), 2000485.

(28) Seo, G.; Lee, G.; Kim, M. J.; Baek, S.-H.; Choi, M.; Ku, K. B.; Lee, C.-S.; Jun, S.; Park, D.; Kim, H. G.; et al. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 2020, 14 (4), 5135–5142.

(29) Song, J.; Cheng, W.; Nie, M.; He, X.; Nam, W.; Cheng, J.; Zhou, W. Partial Leidenfrost Evaporation-Assisted Ultrasensitive Surface-Enhanced Raman Spectroscopy in a Janus Water Droplet on Hierarchical Plasmonic Micro-/Nanostructures. ACS Nano 2020, 14 (8), 9521–9531.

(30) Matricardi, C.; Hanske, C.; Garcia-Pomar, J. L.; Langer, J.; Mihi, A.; Liz-Marzan, L. M. Gold nanoparticle plasmonic superlattices as surface-enhanced Raman spectroscopy substrates. ACS Nano 2018, 12 (8), 8531–8539.

(31) Langer, J.; Jimenez de Abarrasturi, D.; Aizpurua, J.; Alvarez-Puebla, R. A.; Auguié, B.; Baumberg, J. J.; Bazan, G. C.; Bell, S. E.; Boisen, A.; Brolo, A. G.; et al. Present and future of surface-enhanced Raman scattering. ACS Nano 2020, 14 (1), 28–117.

(32) Moitra, P.; Alafeef, M.; Dighe, K.; Frieman, M.; Pan, D. Selective Naked-Eye Detection of SARS-CoV-2 Mediated by N Gene Targeted Antisense Oligonucleotide Capped Plasmonic Nano- particles. ACS Nano 2020, 14, 7617.

(33) Qiu, G.; Gai, Z.; Tao, Y.; Schmitt, J.; Kullak-Ublick, G. A.; Wang, J. Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. ACS Nano 2020, 14 (5), 5268–5277.

(34) Weng, S.; Li, X.; Niu, M.; Ge, B.; Yu, H.-Z. Blu-ray technology-based quantitative assays for cardiac markers: from disc activation to multiplex detection. Anal. chem. 2016, 88 (13), 6889–6896.

(35) Gopinath, S. C.; Awaizu, K.; Tominaga, J.; Kumar, P. K. Monitoring biomolecular interactions on a digital versatile disk: A BioDVD platform technology. ACS Nano 2008, 2 (9), 1885–1895.
(37) Radu, A.; Ussembayev, Y. Y.; Jahn, M.; Schubert, U.; Weber, K.; Cialla-May, D.; Hoeppener, S.; Heisterkamp, A.; Popp, J. HD DVD substrates for surface enhanced Raman spectroscopy analysis: fabrication, theoretical predictions and practical performance. RSC Adv. 2016, 6 (50), 44163–44169.

(38) Morais, S.; Tortajada-Genaro, L.; Maquieira, Á. Array-on-a-disk? How Blu-ray technology can be applied to molecular diagnostics. Expert review of molecular diagnostics 2014, 14 (7), 773–775.

(39) Sun, Y.; Sun, S.; Wu, M.; Gao, S.; Cao, J. Refractive index sensing using the metal layer in DVD-R discs. RSC Adv. 2018, 8 (48), 27423–27428.

(40) Ngamaroonchote, A.; Chotsuwan, C.; Tantisantisom, K.; Laochaorensub, R. Patterned gold electrode prepared from optical discs display largely enhanced electrochemical sensitivity as exemplified in a sensor for hydrogen peroxide. Microchimica Acta 2017, 184 (1), 211–218.

(41) Kuznetsov, A. I.; Evlyukhin, A. B.; Gonçalves, M. R.; Reinhardt, C.; Koroleva, A.; Arnedillo, M. L.; Kiyan, R.; Marti, O.; Chichkov, B. N. Laser fabrication of large-scale nanoparticle arrays for sensing applications. ACS Nano 2011, 5 (6), 4843–4849.

(42) Chen, H.; Kou, X.; Yang, Z.; Ni, W.; Wang, J. Shape-and size-dependent refractive index sensitivity of gold nanoparticles. Langmuir 2008, 24 (10), 5233–5237.

(43) Miroshnichenko, A. E.; Kivshar, Y. S. Fano resonances in all-dielectric oligomers. Nano Lett. 2012, 12 (12), 6459–6463.

(44) Ahmadivand, A.; Semmlinger, M.; Dong, L.; Gerislioglu, B.; Nordlander, P.; Halas, N. J. Toroidal dipole-enhanced third harmonic generation of deep ultraviolet light using plasmonic meta-atoms. Nano Lett. 2019, 19 (1), 605–611.

(45) Draz, M. S.; Vasan, A.; Muthupandian, A.; Kanakasabapathy, M. K.; Thirumalaraju, P.; Sreeram, A.; Krishnakumar, S.; Yogesh, V.; Lin, W.; Yu, X. G.; et al. Virus detection using nanoparticles and deep neural network-enabled smartphone system. Sci. Adv. 2020, 6 (51), No. eabd5354.

(46) Yang, Y.; Kravchenko, I. I.; Briggs, D. P.; Valentine, J. All-dielectric metasurface analogue of electromagnetically induced transparency. Nat. Commun. 2014, 5 (1), 1–7.

(47) Hume, A. J.; Ames, J.; Rennick, L. J.; Duprex, W. P.; Marzi, A.; Tonkiss, J.; Mühlerberger, E. Inactivation of RNA viruses by gamma irradiation: a study on mitigating factors. Viruses 2016, 8 (7), 204.

(48) Cheng, X.; Liu, Y.-s.; Irimia, D.; Demirci, U.; Yang, L.; Zamir, L.; Rodriguez, W. R.; Toner, M.; Bashir, R. Cell detection and counting through cell lysate impedance spectroscopy in microfluidic devices. Lab Chip 2007, 7 (6), 746–755.

(49) Zhang, Z.; Ma, P.; Ahmed, R.; Wang, J.; Akin, D.; Soto, F.; Liu, B. F.; Li, P.; Demirci, U. Advanced Point-of-Care Testing Technologies for Human Acute Respiratory Virus Detection. Adv. Mater. 2022, 34 (1), 2103646.