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Review

How Nanophotonic Label-Free Biosensors Can Contribute to Rapid and Massive Diagnostics of Respiratory Virus Infections: COVID-19 Case

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Cite This: https://dx.doi.org/10.1021/acssensors.0c01180

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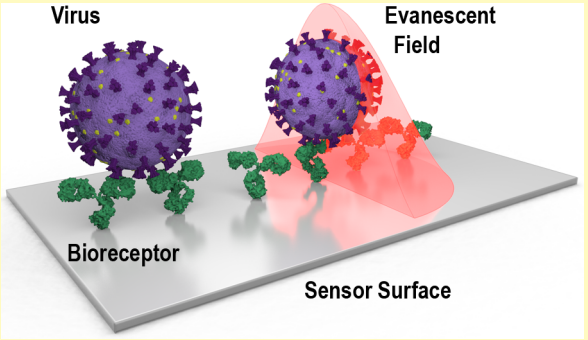
ABSTRACT: The global sanitary crisis caused by the emergence of the respiratory virus SARS-CoV-2 and the COVID-19 outbreak has revealed the urgent need for rapid, accurate, and affordable diagnostic tests to broadly and massively monitor the population in order to properly manage and control the spread of the pandemic. Current diagnostic techniques essentially rely on polymerase chain reaction (PCR) tests, which provide the required sensitivity and specificity. However, its relatively long time-to-result, including sample transport to a specialized laboratory, delays massive detection. Rapid lateral flow tests (both antigen and serological tests) are a remarkable alternative for rapid point-of-care diagnostics, but they exhibit critical limitations as they do not always achieve the required sensitivity for reliable diagnostics and surveillance. Next-generation diagnostic tools capable of overcoming all the above limitations are in demand, and optical biosensors are an excellent option to surpass such critical issues. Label-free nanophotonic biosensors offer high sensitivity and operational robustness with an enormous potential for integration in compact autonomous devices to be delivered out-of-the-lab at the point-of-care (POC). Taking the current COVID-19 pandemic as a critical case scenario, we provide an overview of the diagnostic techniques for respiratory viruses and analyze how nanophotonic biosensors can contribute to improving such diagnostics. We review the ongoing published work using this biosensor technology for intact virus detection, nucleic acid detection or serological tests, and the key factors for bringing nanophotonic POC biosensors to accurate and effective COVID-19 diagnosis on the short term.



KEYWORDS: *point-of-care diagnostics, optical biosensors, nanoplasmonics, silicon photonics, virus detection, coronavirus, covid-19, respiratory virus, label-free detection*

Viral respiratory tract infections (RTI) are one of the leading causes of hospitalization in developed countries and a common cause of death.[1] In developing countries, the concern is even greater due to the shortfall in healthcare (vaccines, efficient diagnostics, clinical resources, etc.), nutrition, and hygienic measures.[2] Since the discovery of influenza in the early 1930s, tens of common viruses have been identified, and in the past decades, new emerging viruses have appeared with increasing frequency, gradually warning the scientific and social community toward improving identification, surveillance, and control to prevent and stop them from causing epidemics. After the critical outbreaks of SARS (Severe Acute Respiratory Syndrome) in 2003, H1N1 flu (swine flu) in 2009, and MERS (Middle East Respiratory Syndrome) in 2012, it has been in 2019−2020 when the world has been globally dealing with the most critical health situation in years due to the SARS-CoV-2 (a coronavirus closely related to MERS-CoV and SARS-CoV) causing the COVID-19 outbreak. COVID-19 has become a global pandemic in less than four months since its appearance in late December 2019 in Wuhan (China). In such a short period since the appearance of the first suspicious cases and further confirmation, the virus has massively spread worldwide, affecting all countries and territories, leaving to date (July 2020) more than 14 million infected people and nearly 600,000 deaths. The surprisingly fast propagation of SARS-CoV-2 has been due to factors such as its contact and airborne routes of transmission (i.e., transfer from contaminated fomites to mouth, nose, and eyes, and also via aerosols generated and remaining in suspension when sneezing, coughing, breathing, and talking), its high infection rate, long incubation period from exposure to onset of symptoms,

Received: June 11, 2020
Accepted: August 7, 2020
Published: August 7, 2020

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https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

contagious time (three-week period from onset of symptoms), the variety of symptoms (fatigue, fever, cough, dyspnea···) and, more importantly, the lack of symptomatology, which hinders a rapid identification, facilitating its imperceptible spread. The significance of this crisis has also sadly revealed the lack of global readiness from governments, public organizations, and communities to face and manage both the social and health consequences of the outburst. The scientific community is now globally challenged to provide solutions in three main areas: (1) prevention, through the development of efficient vaccines that provide immunity and protection to the population; (2) therapeutics, through the development of specific drugs and therapies to treat and cure the disease; and (3) diagnostics, through the implementation of massive, affordable, and reliable detection tests.

Once the emergence has been recognized and identified, it is crucial to initiate actions to stop the virus transmission and prevent the spread. Besides social distancing and confinement, the strategy must also involve careful tracing and massive testing of the community, which has dramatically revealed the need for efficient, readily accessible diagnostics methods that specifically confirm the presence of the virus as soon as possible. Given the health and social emergency, mass-produced, reliable, and very sensitive diagnostics to promptly identify the pathogen are then mandatory and a paramount priority to improve patient management, infection control, and more efficient epidemiological studies.

In this review, we provide an overview of current and new diagnostic strategies that can be implemented for SARS-CoV-2 detection. We mainly focus on label-free nanophotonic biosensors as a potential technology for rapid and efficient virus infection diagnostics. The main working principles and characteristics of nanophotonic biosensors are briefly described together with examples of their recent applications for respiratory virus detection.

DIAGNOSTIC STRATEGIES FOR RESPIRATORY VIRUS INFECTION

Viral culture has been for a long time the traditional gold standard diagnostics technique for RTI, as it provides high specificity, moderate sensitivity, and quantitative information. However, it is time-consuming (5 to 20 days) and requires experienced staff for results interpretation. Current diagnostic methods for respiratory tract infections are divided into three distinct approaches:[3,4]

(1) Detection of the viral genome through nucleic acid amplification tests (NAATs), mainly polymerase chain reaction (PCR) tests in its different variants;
(2) Direct detection and identification of the intact virus (or fragments) through the recognition of viral antigens (structural proteins of the virus);
(3) Indirect detection of antibodies produced by the infected host during the course of the infection, known as serological testing.

Strategies 1 and 2 usually require a nasopharyngeal sample (nasal swabs), whose viral load is generally high, and they are useful while the infection is active; whereas strategy 3 requires a blood sample and it can only be applied from day 4 or 5 after the infection.

Nucleic Acid Amplification Tests (NAATs). NAATs based on PCR in its different variants (reverse-transcriptase PCR, RT-PCR, real-time quantitative PCR, qPCR, etc.) for the amplification and detection of specific sequences of the viral nucleic acids, are currently well established in routine analysis and indeed have drastically redefined the diagnostics protocol and efficiency. The relatively fast access to the genomic sequence of the pathogens, even in newly emerged viruses, such as the SARS-CoV-2, enables the rapid design and production of highly specific PCR kits for its ready and sensitive detection, and even offering quantitative values of the viral load (typically between 100 and 1000 copies/mL). This has been evidenced with COVID-19, when within the first months after the outbreak, many PCR kits were commercialized and distributed, offering excellent sensitivity and specificity, turning them into the gold standard diagnostics technique for SARS-CoV-2 infection. Although the analysis is relatively fast (i.e., less than 2 h), the sample-to-result time can be considerably longer (i.e., from 4−6 h to a few days), mainly due to sample transportation and treatments that must be performed in centralized laboratories. The initial limitations of PCR techniques related to reduced multiplexing capabilities have also improved significantly. Many companies offer either detection kits for the three most common respiratory viruses (influenza A/B and the respiratory syncytial virus, RSV), or more sophisticated platforms which offer cartridges for the detection of panels of 6, 12, or even 20 respiratory pathogens.[5,6] In all cases, the performance of such testing platforms renders sensitivity and specificity values around 95−100% and could be adapted to include the novel coronavirus.[6] However, most of them are not compatible with point-of-care testing, do not offer quantitative information, are necessarily linked to their commercial instrument, and are relatively costly.[7]

Current efforts are mostly directed to point-of-care (POC) decentralization, to accelerate and promote the analysis outside the lab, close to the sample collection,[8] and already a couple of them have received express authorization to be used for qualitative detection of SARS-CoV-2, such as the Accula test from Mesa Biotech Inc. or the ID Now test from Abbott Diagnostics. The development in recent years of new NAAT-based techniques that circumvent the use of thermocyclers, required for up and down temperature cycles in PCR, have boosted these new designs, easing the portability and eventually the transfer into POC devices.[9,10] This is the case, for instance, of Loop-Mediated Isothermal Amplification (LAMP) or Rolling Circle Amplification (RCA) techniques, which also rely on DNA amplification, but are designed to operate at constant temperature (60−65 or 37 °C, respectively).[11−14] Both have been implemented for the detection of several pathogens, including viruses such SARS-CoV,[15,16] H5 avian influenza virus,[17] and also SARS-CoV-2.[18] Besides, microarray-based methods, enabling multiplexed assays and with a high potential for POC testing, have been designed and applied for different virus detection (influenza, RSV, generic CoV, MERS-CoV, etc.).[19,20] Finally, CRISPR-based techniques are also emerging as novel diagnostic tools, using the RNA-targeting Cas13 enzyme for highly specific pathogen detection, which is being intensively studied and applied as well for COVID-19 diagnostics.[21−23]

Antigen-Directed Virus Detection. Antigen-directed diagnostics involve the detection of the virus via recognition elements (commonly specific antibodies) that capture exposed proteins in the structure (viral antigens), such as the S (spike) protein in coronaviruses, or the HA protein (hemagglutinin glycoprotein) in influenza virus. Conventional approaches, such as the enzyme-linked immunosorbent assays (ELISA) or immunofluorescence staining, are used to identify the virus

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

and provide qualitative or semiquantitative results with adequate sensitivity. Although very specific, they require highly skilled personnel for optimal accuracy, and they are time- and labor-consuming.[3,24−26] A few of them are also commercialized either for the detection of a panel of respiratory viruses with its benchtop instrument, such as mariPOC tests (via sandwich immunoassay run in automatized mode), or for individual plate-based kits, although they are not commonly used for routine diagnosis.

Instead, rapid antigen diagnostic tests (RADTs) based on immunochromatography (IC) or lateral flow assays (LFA) have expanded widely, adapting the immunoassay concept to disposable, inexpensive, fast (around 15 to 60 min turnaround time), qualitative (yes/no), and point-of-care testing. The RADTs are cellulose-membrane strip tests, commonly using a sandwich assay. The sample containing the virus (or viral antigens) is placed in the sample pad area, which flows by capillarity to differentiated areas: first, a pad containing specific labeled (fluorescent tag, gold nanoparticles, etc.) antibodies against the antigens, which captures the analyte (if present). When the sample gets to the detection line, the analyte−antibody complex is captured by other immobilized specific antibodies, forming a sandwich complex and revealing the presence of the viral antigen in the sample. These tests are commercially available for most common respiratory viruses such as influenza A and B or the RSV.[6] Some RADTs have also been urgently developed and launched for COVID-19 diagnostics.[18] For example, Coris BioConcept has launched a dipstick rapid test (COVID-19 Ag Respi-Strip) with a claimed specificity of around 98% but a sensitivity of about 60%; SD Biosensor, with the Standard Q COVID-19 Ag test, reaches a sensitivity of 84%. Despite the advantages for POC massive testing, these tests have critical issues, such as the very limited sensitivity (especially in adults)[27] and a poor PPV (positive predictive value), which is more evidently revealed when they are compared with nucleic acid tests, whose levels of accuracy and sensitivity are very high. The low reliability of these tests might be in part due to the limited quality of biological material employed for their development (both antibodies and antigens, used for either the antibody production or the assay development and validation), which essentially requires time for optimization, and is especially manifest with a new, unknown emerging virus.

Serological Assays. Serology assays are based on the indirect identification of the virus infection, through the specific detection of the antibodies generated by the infected host during the immune response. Usually, this process starts a few days after infection, and once the virus has been cleared from the organism, the antibodies remain in the bloodstream, generally for several years (although it depends on the disease and the pathogen). This approach is useful for diagnostics (particularly in retrospective diagnosis), but it is especially attractive for surveillance and to perform epidemiological studies,[28,29] to estimate the prevalence, and to monitor the levels of antibodies over time, which help assess the duration of the acquired protection or immunity. Antibodies generated by the body often target key antigenic determinants in the pathogen, for example, the structural proteins of the virus. Thus, the detection mechanism of this strategy involves the analysis of blood (or serum) from the patient and the antibody detection through their specific capture by those viral antigens.[30−32]

There are several enzyme immunoassay (ELISA) kits available for the detection of serum antibodies to most common respiratory viruses, such as adenoviruses, influenza A and B viruses, parainfluenza, or RSV. These kits can provide more reliable and quantifiable data and are meant to be used in central laboratories with appropriate readers. However, the most straightforward and most widely commercialized assays rely on LFA tests, analogous to those for virus rapid detection in test-strip format, with the viral antigens being adsorbed on the paper-based pads. Most of them provide information on the type of antibody detected (IgG and/or IgM), which may help differentiate different stages of the infection (e.g., acute phase or past infection). Due to the emergency situation, COVID-19 serology studies are currently intensively performed worldwide for both diagnostics and pandemic monitoring purposes,[33−35] and these tests are under development and commercialization. Both microplate format immunoassays (ELISA) and rapid LFA tests have been the main focus,[8,18,36] although not with the desired outcome in the case of rapid tests. Most of the commercialized tests lack minimum sensitivity and reproducibility to be reliable and accurate, partially due to the lack of rigorous quality and accuracy controls before commercialization. Besides, LFA tests only provide qualitative information (yes/no to the presence of antibodies). As the COVID-19 disease is not well-known yet, the immune response according to the severity (asymptomatic, mild, or severe outcome) or the duration of the acquired immunity, if any, requires a highly reliable, sensitive, and ideally quantitative strategy, which current LFA-based techniques are not providing.

Optical Biosensors as Potential Integrated Diagnostic Approach. For respiratory virus infections, especially of emergent viruses, which we are facing more often, a more integral diagnostic approach that provides fast and quantitative information is highly needed. Having a unique single platform that can group all three diagnostic strategies discussed above, combining the best features of all of them, represents an unmet need that currently available technologies cannot provide. Developing, implementing, and commercializing a device which can deliver the specificity and accuracy of nucleic acid-based detection, the convenience of fast detection of the virus directly in the extracted sample, or the detection and quantification of antibody titer directly in collected serum or blood, at the point-of-care, with enough reproducibility, accuracy, and sensitivity, is a very challenging task. Optical biosensors are at the forefront in this singular race, as they provide outstanding characteristics as excellent levels of sensitivity, robustness, and immunity to electromagnetic interferences, miniaturization and integration capabilities, and portability, among others.[37,38] Therefore, optical biosensors are excellent candidates to move the analysis from centralized laboratories to the point-of-care.[39,40] Optical biosensors measure variations of the optical properties of the propagated light (i.e., absorption, polarization, intensity, wavelength, dispersion, or refractive index) when the interaction between the receptor (antibody, nucleic acid sequences, enzymes, proteins, etc.) and the target analyte takes place. Among them, the ones founded on the evanescent field sensing principle, such as the photonic and plasmonic biosensors,[41−43] have already demonstrated their enormous potential as sensing tools in a myriad of environmental, industrial, pharmaceutical, and, especially, clinical scenarios.[44−46] Evanescent wave optical biosensors operate in a label-free configuration (no fluorescent, colorimetric, enzymatic tag is needed), and their level of sensitivity allows them to get rid of further amplification steps that prolong and complicate the assay. They have the potential to increase the multiplexing capability, facilitating the

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

simultaneous detection of several targets, by having different sensing channels within the same chip. As they rely on the monitoring of changes occurring on the surface of the biosensor chip, they can also be literally adapted to any kind of target (for example, detection of the virus, nucleic acid sequences, or antibodies; see Figure 1), as long as carefully selected receptors are immobilized on the surface, to ensure the sensitivity and specificity requirements for a given application.

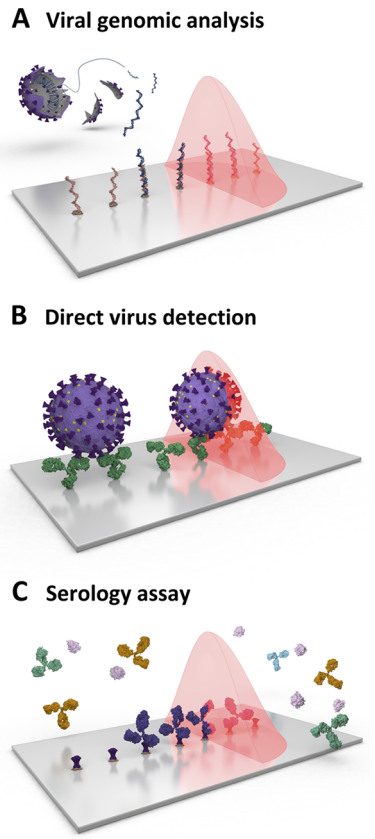

Figure 1. Biosensing strategies for virus infection diagnosis: (A) detection of viral genomic material (i.e., DNA or RNA) by direct hybridization to a DNA probe; (B) detection of intact virus entities by antigen-specific recognition of antibodies; (C) detection of human antibodies against the virus antigens (serology assay).

Both plasmonic sensors, based on metallic transducers (thin gold films or nanostructures), and silicon photonics, fabricated by conventional microelectronics technology, are in continuous progress to move from working laboratory prototypes to compact, integrated versions that can be deployed outside the lab, near to the patient. These devices have already been successfully applied for the diagnosis of pathogens, both bacteria and viruses, demonstrating their potential for this diagnostic area.[47,48] As discussed in the following sections, the technology sensitivity and scalability, the selection of the receptors to target the virus, and the appropriate biofunctionalization strategy on the sensor surface are crucial aspects to consider for implementing successful diagnostic biosensors.

NANOPHOTONIC BIOSENSORS: WORKING PRINCIPLES AND TECHNOLOGIES

Most common nanophotonic transducers rely on nanoplasmonics or silicon photonics technologies. The underlying physics of these sensors have been widely studied and described previously and can be explained on the basis of the evanescent field sensing principle.[40,42,49,50] In optics, an evanescent wave is formed when light traveling in a medium undergoes total internal reflection (TIR), generating a near-field enhancement at the boundaries that penetrates in the surrounding dielectric medium with a vanishing intensity. The evanescent field is extremely sensitive to changes in the refractive index (RI) of the medium, which are translated in more drastic changes of light properties such as the intensity, phase, resonance momentum, or polarization. It is important to note that the intensity of the evanescent field decays exponentially; therefore, the sensing probe is restricted to the immediate vicinity of the transducer surface and expanding up to a few tens of nanometers depending on the transducer. In other words, when a biomolecular interaction or binding occurs at the sensor surface within the evanescent field, it can be directly monitored by interrogating wavelength displacements, light intensity, or phase variations, among others, in a real-time and label-free format with impressive sensitivity.

Nanoplasmonic Biosensors. The Surface Plasmon Resonance (SPR) system is the landmark of label-free nanophotonic biosensors. The SPR biosensor generally employs a nanometer-thin layer (40−50 nm) of gold as a transducer. An incident light beam excites coherent oscillations of the metal conduction band electrons (i.e., surface plasmon polariton) that propagates along the interface metal-dielectric, generating an evanescent field that can extend up to a few hundreds of nanometers (10−300 nm) into the surrounding medium. When specific biorecognition elements (e.g., antibodies or DNA strands) are immobilized onto the gold surface, the selective capture and binding of the target molecule induce a change of the RI and in the light properties, which can be monitored and is directly proportional to the concentration of the analyte in the sample (Figure 2a). The RI limit of detection of SPR biosensors typically reaches 10^{-5}−10^{-6} refractive index units (RIU), which commonly relates to detection limits in the low nM or even pM level in surface analyte detection. This analytical technique is considered mature nowadays, and its potential has been vastly demonstrated by a myriad of applications in molecular biology for the study of biomolecular interactions, in pharmaceutics for the affinity and kinetics evaluation of drug candidates, and in environmental and biomedical diagnostics for the detection and quantification of specific substances (i.e., small molecules, protein, nucleic acids, or pathogens) in different types of samples (e.g., food, water, human bodily fluids, etc.).[51−53] In the past two decades, amid the rise of nanotechnology, plasmonic biosensors have evolved to incorporate more sophisticated nanostructures that can enhance the sensing performance and improve miniaturization and integration capabilities. Nanopatterned surfaces, such as arrays of nanoholes, nanodisks, or nanorods, and more complex geometries like nanostars, nanodimers, or oligomer assemblies, have emerged as interesting alternatives to conventional SPR for the development of ultrasensitive label-free biosensors as point-of-care integrated devices (Figure 2b).[39,43] Instead of propagating SPR, these plasmonic nanostructures exhibit a localized resonance (i.e., localized surface plasmon resonance, LSPR) that is characterized by the higher confinement of the evanescent field, with penetration depths around 10−50 nm, and by the spectral tunability of the resonance. Theoretically, the LSPR provides higher detection sensitivity, especially for relatively small targets, given that the biomolecular interaction occurs within the entire depth of the evanescent field. The evidence of

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ACS Sens. XXXX, XXX, XXX−XXX

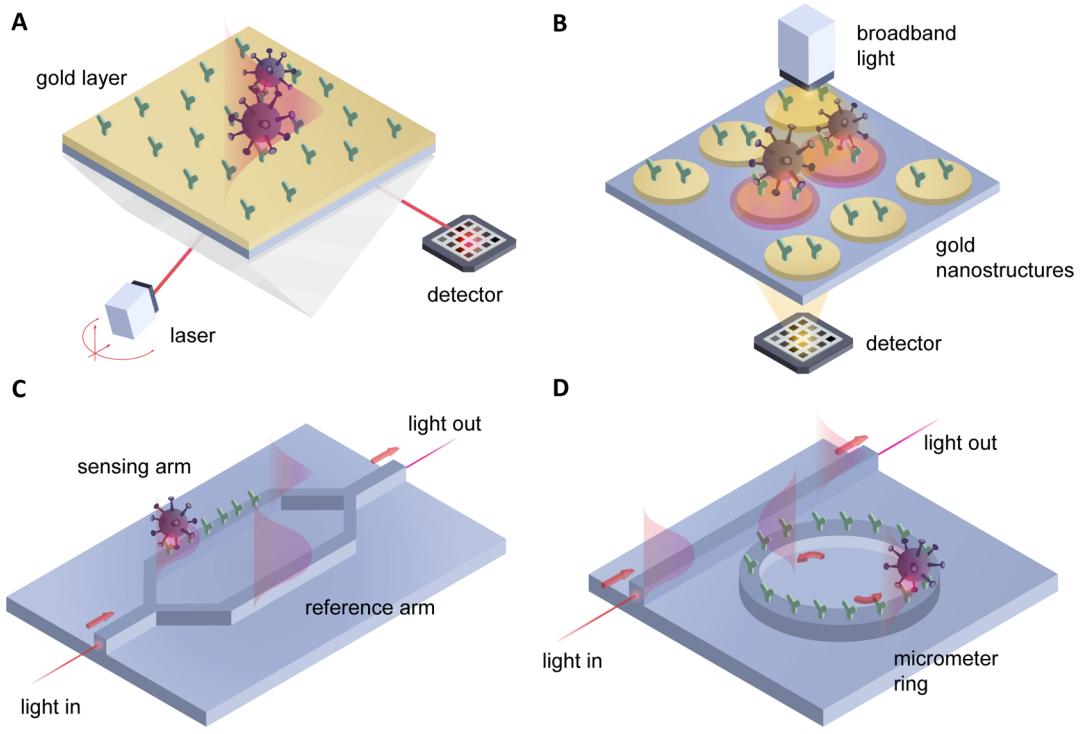

Figure 2. Schematic illustrations of the main label-free nanophotonic biosensor technologies: (A) Surface Plasmon Resonance (SPR) biosensor in Kretschmann configuration; (B) Localized Surface Plasmon Resonance (LSPR) biosensor based on gold nanodisks; (C) Mach−Zehnder interferometric (MZI) biosensor; and (D) ring resonator biosensor.

this has been especially shown in Surface-Enhanced Raman Spectroscopy (SERS) sensors, where the LSPR near-field is exploited to largely intensify the Raman effect, providing outstanding sensitivities that can even achieve single-molecule detection.[54] On the other hand, perhaps the most appealing features of nanoplasmonic sensors rely on the capabilities for spectral resonance design and more straightforward optical configuration possibilities. While propagating SPR generally requires complex light coupling schemes (e.g., prism-based Kretschmann configuration), several LSPR sensing platforms can work under normal light illumination, thereby simplifying miniaturization and integration in small, portable, and user-friendly devices. This capability also facilitates the development of two-dimensional sensing microarrays for multiplexed analysis. Besides, the design and modeling of the nanostructured architecture enable the rational definition of the spectral peak position at convenient wavelengths, so allowing for the use of affordable and widely available light sources, like light-emitting diodes (LEDs).[43]

Nanoplasmonic biosensor technologies, both SPR and LSPR based, are nowadays available and commercialized worldwide. In the case of SPR, large companies like GE Healthcare (Biacore), Horiba, or Bruker, among others, deliver high-quality benchtop systems that are routinely used in research laboratories or the pharmaceutic industry. A few manufacturers also commercialize LSPR-based instruments, such as Nicoya Life Sciences or LSPR AG. However, despite the advanced technological and analytical capabilities offered by these instruments, their adoption in the clinical diagnostics field for a given application is still very limited. This can be related to the need for a robust, reproducible sensor surface biofunctionalization protocol, which usually must be optimized for each analyte, and the difficulties of analyzing complex samples like biological fluids. Furthermore, the achievement of truly POC nanoplasmonic biosensors needs to incorporate and improve microfluidic systems that minimize or automatize sample processing, and user-friendly readouts that can be directly interpreted by nonspecialized users.

Silicon Photonics Biosensors. Despite the predominance of nanoplasmonic biosensors as a reference in label-free optical sensing, silicon photonics technologies have arisen as leading platforms in terms of sensitivity and integration capabilities. Silicon photonics biosensors are based on integrated optical waveguides fabricated on Si, Si_3N_4, SiON, or SiO_2 materials. Light travels along these microscopic waveguides under a TIR regime, generating an evanescent electromagnetic field with penetration depths between 100 and 900 nm that is used as biosensing probe. The vast knowledge and robustness of silicon-based technologies, including conventional microelectronics fabrication and characterization procedures, together with the low power consumption and the capability to incorporate all the functions (chemical, optical, microfluidics, and electronics) in one single platform, make these systems ideal for lab-on-a-chip implementation and use as point-of-care diagnostics.[40,41]

Most common Si photonics sensors are interferometers, ring resonators, and photonic crystals, although other technologies have also been developed, such as grating-coupled waveguides, silicon wires, slot waveguides, or optomechanical sensors, among others.[41] Interferometric biosensors, like the Mach−Zehnder (MZ), Young, or bimodal waveguide (BiMW) interferometers, stand out for their broad dynamic range and exceptional sensitivity, reaching detection limits in the range of 10^{-7}−10^{-8} RIU.[44,50] Figure 2c illustrates the working principle of a MZ interferometric sensor. Briefly, an input waveguide is split into two arms (sensing and reference arms) that, after certain distance, are recombined into a single output. Biomolecular interactions occurring at the sensing arm within the evanescent field induce a phase difference with the light

https://dx.doi.org/10.1021/acssensors.0c01180
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traveling along the reference arm. When recombined, the generated interference provides the direct, label-free, and real-time signal directly proportional to the analyte concentration. A variety of interferometric biosensors with different designs have been developed and applied for clinical diagnosis purposes, showing detection of small molecules,[44] protein biomarkers or glycolipids,[55] nucleic acids in the aM−fM range,[56] or just a few pathogenic entities (e.g., 4−10 bacteria/mL).[57]

Ring resonators, meanwhile, have attracted attention due to their unique potential for integration into high-throughput arrays for multiplexed analysis,[38,58] with highly efficient and scalable fabrication. Ring resonator transducers consist of circular waveguides that generate whispering-gallery modes (WGM) upon light coupling to the loop, enhancing the evanescent field intensity (Figure 2d). Detection is performed by interrogating the WGM spectral resonance position. The multiplexing capabilities of ring-resonator arrays have been demonstrated for the simultaneous detection of panels of relevant diagnostic biomarkers, like cancer-related proteins or microRNAs.[38]

Finally, photonic crystals also hold promising potential for point-of-care biosensors due to their cost-effective fabrication and high-throughput possibilities.[59,60] Photonic crystals are well-defined nanostructures with periodically repeated layers of different refractive index. The lattice structure generates photonic bandgaps, highly dependent on the dielectric refractive index. These sensors have also been applied for biological assays achieving moderate sensitivities, compared to their silicon photonics counterparts (in the nM−pM range), but they would greatly benefit from an easy and low-cost mass production of disposable photonic sensor chips.

Silicon photonic technologies have also started recently to hit the biosensors market with some successful systems like the ring resonator-based platform supplied from Genalyte. However, they suffer from the same shortcomings as nanoplasmonic technologies in terms of surface biofunctionalization and integration in automated and user-friendly lab-on-a-chip devices for their implementation in the clinical field. Extended discussion in this regard is provided in the following sections of this review.

BIORECEPTORS AND SENSOR SURFACE BIOFUNCTIONALIZATION

The performance and, especially, the sensitivity and selectivity of surface-based nanophotonic transducers will ultimately depend on the most appropriate biorecognition element that binds specifically the target analyte, and on the conditions for the coating of this receptor. As for any application to be developed, bioreceptors are selected according the approach for detection of the target. In the case of viral infection diagnosis, considering the three main strategies described before, these would be DNA capture probes for nucleic acid detection, specific receptors like antibodies for antigen-directed virus capture, or viral antigens for serological tests. However, many variants and innovative bioreceptor elements have been developed to enhance the sensing performances (see Table 1). It is important to note though that the access to high-quality and well-characterized biological reagents, especially antigens and antibodies, can be a significant problem, in particular at the start of an outbreak from a new pathogen, like the SARS-CoV-2. This inherently delays the development of reliable sensor technologies as well as other diagnostic approaches, such as the RADTs, as discussed before.

Table 1. Bioreceptors Applied for Virus Diagnosis

genomic detection		
receptor	advantages	limitations
DNA probes	Stability and specificity	Not sensitive enough in some scenarios
	Easy to produce and attach to the surface	
Tagged stem-loop probes	Lower limits of detection	Need for labels, chemical modifications
		Additional steps
Amplification + DNA probes	Lower limits of detection	Additional steps
		Cost of the enzyme, reagents
intact virus detection		
receptor	advantages	limitations
Antibodies	Robust and well established	Animal requirement
	Wide range of ligands	High production costs
	High binding affinities and selectivity	Activity decrease in long-term storage
		Low reusability
Recombinant antibody fragments	No animal requirement in the production	Need to know the sequence, or find binding regions through phage display
	Less costly to produce than the whole antibodies (microorganism bioreactors)	Storage and reusability
Aptamers	Possibility of engineering	SELEX procedure is long and complex
	Less costly than antibodies	
	Stability and long-term storage	
	Reusability	
Glycans	Less cost in production	Limited to a few viruses
	Increase selectivity in combination with other receptors	Need of prior studies of glycan affinities to viruses
serological assay		
receptor	advantages	limitations
Viral lysates	Easy to produce	Not homogeneous receptor layers
	No need to know the viral antigens or genome sequence	Cross-reactivity due to incorporation of host proteins in virions
Recombinant viral antigens	Homogeneous receptor layer	Need to identify the gene sequence
	Relatively easy to produce	Cross-reactivity with strains of the same family
Antigenic domains of viral proteins	Less cross-reactivity with viruses of the same family	Need to know sequence, structure, and identify the domain

For the case of genomic hybridization assays, oligonucleotide capture probes are designed and synthesized with the complementary sequence to the specific viral target region. Primarily, the specificity will depend on the sequence selection of the probe and, second, on the hybridization conditions, mainly buffer composition and temperature. Aspects to be considered when designing the probe will be the pair length and the nucleotide sequence, because they will affect the binding energy and stability of the complex. In addition, especially in RNA detection, secondary structures around the targeted zone might hinder the binding to the probe, and then, it is essential to select regions where these folds are not formed.[61] For some cases, where the biosensor sensitivity is not enough, the use of tagged hairpin probes or the hybridization of additional labeled sequences has been prevalent as amplification steps. For instance, stem-loop oligos tagged with signal amplification

https://dx.doi.org/10.1021/acssensors.0c01180
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compounds (e.g., SERS reporters) have been used to improve the detection limit in the analysis of different human viruses.[62]

For antigen-directed virus detection, most common bioreceptors are monoclonal antibodies, which can be produced to target specific viral antigens and have been extensively used in biosensor development. In the past decades, smaller recombinant antibody fragments and engineered variants have become advantageous alternatives.[63] These peptides can be selected by phage display, which allows screening of clone libraries of recombinant phages expressing on their surface the binding regions of antibodies and proteins. The selected ones can be identified and produced synthetically or expressed in bacteria, with fewer requirements than the production of antibodies in hybridomas or mammal cells. As an alternative, aptamers, which are short DNA/RNA sequences that also bind with high affinity and specificity to target molecules, have also been proposed for the detection of viral antigens, showing sensitivities comparable to monoclonal antibodies.[64−66] Main advantages are related to the simpler production and possibility to introduce any desired functionality for surface immobilization. However, the selection procedure is not yet well established to be massively commercialized and employed with sufficient robustness. At present, many aptamers have been isolated for the recognition of human viruses,[67,68] and particularly for respiratory viruses; most of them have been designed to detect distinct strains of influenza.[69−71] Finally, cell receptors have also been proposed for direct virus detection, as the case of glycans from the host cells. Surface receptor glycans have been, in fact, widely used in conjunction with biosensors for the detection of different strains of influenza.[72−74] Moreover, the combination of glycoprotein receptors and antibodies has improved the specificity of the sensor to certain viral strains.[75]

For serological assays, the bioreceptors essentially consist of viral antigens, the same that cause the host immunogenic reaction.[76,77] The first serological tests employed whole virus lysates for the recognition of the host antibodies, resulting in test variability and false positives due to the presence of host proteins in the virions.[78] Since then, advances in cloning, genetic engineering, and the establishment of robust expression systems make possible the use of recombinant viral antigens instead. Once the genome of different viral strains is available, recombinant proteins are produced at large scale in bioreactors, facilitating the production of homogeneous reactive layers. By using cloned proteins, large amounts of purified antigens can be produced and isolated with less cost in addition to reducing the risk of infection.[79] In some cases, the whole viral protein is not necessary since only a part of the protein causes most of the immunogenic reaction in the individuals. Then, the production of this small region (which can be a short peptide) may be enough, simplifying the process. Compared to the whole recombinant protein, these shorter peptides are designed to include nonconservative regions, so they have fewer cross-reactions with sera raised against similar viral infections,[80,81] thus increasing the specificity. These short peptides are habitually conjugated to larger proteins, known as carrier proteins, to increase stability and facilitate surface immobilization.[82,83]

Besides the bioreceptor selection and its quality, a critical factor for the biosensor performance is the appropriate surface functionalization and its immobilization, taking into account orientation, stability, reproducibility, and prevention of nonspecific adsorption of matrix components of the biological samples. The bioreceptor immobilization to the transducer surface (e.g., gold or silicon derivatives) can be done in different ways: by direct chemical or physical adsorption, by covalent linking to a chemical matrix, and through affinity binding to certain molecules. Physical adsorption through electrostatic and hydrophobic interactions is not recommended, as it can lead to biomolecule denaturation, and very low stability and reproducibility. Certain biomolecules, however, can be directly chemisorbed to the surface, for example, via thiol (-SH) binding to gold.[84] This is widely employed for oligonucleotide probe functionalization (DNA probes or aptamers). It can also be used with proteins by the cysteine residues, although in some cases, it can lead to the wrong orientation of the molecules.[85]

Covalent immobilization involves the chemical modification of the transducer surface to generate a functional chemical matrix (e.g., self-assembled monolayers, SAMs), where biomolecules can be covalently bound. Compared to the previous approaches, it offers some advantages: a more uniform and controlled surface coverage, possibility for long-term storage, and sensor reusability. The SAMs are created by distinct compounds depending on the transducer material, e.g., alkanethiol molecules for gold (which involves very well established and reproducible procedures) and alkosysilanes for Si-based sensors.[85,86] The silanization procedure, although also widely used, is relatively complex, and many factors influence its reproducibility, such as the silane concentration, solvents employed, reaction times, or temperature. The molecules that comprise either type of SAMs can be engineered to have different lengths and terminal groups that allow the grafting of the probes. The most common SAMs terminations are amine (NH_2), thiol (SH), carboxyl (COOH), and epoxy (COC) functionalities that can be cross-linked with native or engineered functional groups in the biomolecules. Given the broad range of linkers used depending on terminal groups, we invite the reader to check other reviews and chapters for a more in-depth classification of compounds, protocols, advantages, and drawbacks.[85,87−89] Finally, affinity-based immobilization strategies are also commonly employed for bioreceptor immobilization, with streptavidin−biotin coupling the more employed.[90] This strategy involves streptavidin coverage of the surface by adsorption or covalent linkage and the addition of the biotinylated receptor. This approach requires the labeling of the receptor with biotin groups but provides a highly stable and specific bond. Another affinity-based immobilization strategy widely used, especially for antibodies, involves the use of Protein A or G, which binds with high affinity to the Fc region of antibodies. This methodology provides an efficient strategy to orientate the antibodies to the outer medium, leaving more exposed the regions responsible for the specific binding.[91]

Lastly, there are two other critical aspects to consider: (i) ensuring adequate bioreceptor density, minimizing possible steric hindrance effects, and (ii) avoiding nonspecific interactions on the sensor surface. In the first case, it is necessary to study the effect of combining mixed SAMs (with reactive and nonreactive) groups, which help space out the distribution of receptors (particularly important if they are large molecules, like are bacteria or viruses) or also spacers that move away the receptor from the sensor surface (i.e., increasing their accessibility). On the other hand, nonspecific interactions, often referred to as "fouling", are a critical issue when analyzing clinical samples such as saliva, sputum, urine, serum, or blood, as they can induce misleading signals (i.e., false positives resultant of binding to the sensor surface and change in the refractive index) and might also hinder the recognition of low-

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

Table 2. Nanophotonic Biosensors Applied for Respiratory Virus Diagnosis

genomic detection						
biosensor technique	virus	receptor	target	sample	sensitivity	ref
LSPR	SARS-CoV-2	DNA probe	RNA	Buffer	0.2 pM	98
Microring Resonators	Influenza A and B, hCoV-OC43/229E, RSV A and B	DNA probe	RNA	Nasopharyngeal samples	25 copies/reaction	99
SPR	Influenza A and B, H1N1, RSV, parainfluenza, adenovirus, and SARS-CoV	DNA probe	RNA	Buffer	1−5 nM	100
SERS	High-pathogenic avian influenza (HPAI)	Raman-tag hairpin DNA probe	RNA	Buffer	2−3 aM	62

intact virus detection						
biosensor technique	virus	receptor	target	sample	sensitivity	ref
SPR	Avian influenza H6N1	Antibodies	H6N1 virion	Buffer	5×10^6 EID_{50}/mL	103
SPR	Avian influenza H7N9	Antibodies	HA protein	Buffer	200 copies/mL	104
SPR	Enterovirus 71	Antibodies	VP1 protein	Culture media	67 virus particles/mL	105
SPR	Avian influenza H5N1	Aptamer	HA protein	Poultry swab samples	0.128 HAU/mL	70
SPR	Avian influenza H5Nx	Aptamer	HA protein	Buffer	200 EID_{50}/mL	69
Waveguide interferometer	High-pathogenic avian influenza (HPAI)	Antibodies	HA protein	Buffer	0.0005 HAU/mL	106
Silica Inverse Opal	Avian influenza H1N1	Antibodies	HA protein	Buffer	10^3 PFU/mL	107
Photonic Crystal	Avian influenza H1N1	Antibodies	HA protein	Saliva samples	1 ng/mL	108
Mach−Zehnder	Avian influenza H1N1	Antibodies	HA protein	Buffer	10^{10} virus particles	109

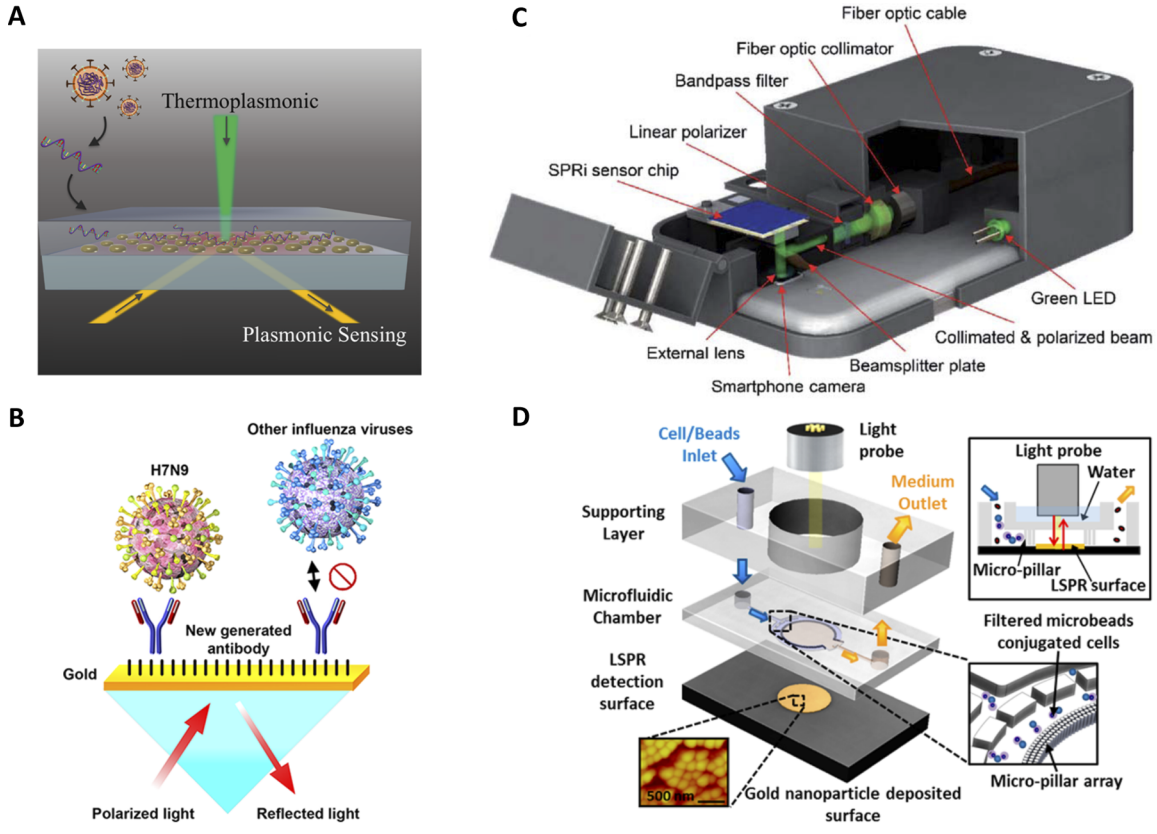

Figure 3. (A) Example of nanophotonic biosensor applied for COVID-19 diagnosis via genomic detection of SARS-CoV-2. Reprinted (adapted) with permission from ref 98. Copyright (2020) American Chemical Society. (B) Example of nanophotonic biosensor applied for direct detection of intact viruses (influenza). Reprinted (adapted) with permission from ref 104. Copyright (2018) American Chemical Society. (C) Example of smartphone-integrated optical biosensor. Reprinted (adapted) with permission from ref 130. Copyright (2017) Elsevier. (D) Example of microfluidics-integrated optical biosensor. Reprinted (adapted) with permission from ref 134. Copyright (2014). American Chemical Society. Please refer to original articles for reprint.

concentration analytes.[92] There is extended research devoted to study the factors influencing these interactions and to develop strategies to counteract them. The antifouling strategies involve the use of compounds that prevent the adsorption of other compounds while preserving the affinity and sensitivity of the recognition elements. These coatings have been able to avoid the fouling attachment by the creation of hydration layers and electrostatic and steric repulsions.[93] Materials that have shown

https://dx.doi.org/10.1021/acssensors.0c01180
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excellent antifouling properties include polyethylene glycol (PEG), zwitterionic polymers, polysaccharides such as dextran, and proteins like bovine serum albumin (BSA).[94]

NANOPHOTONIC BIOSENSORS FOR VIRUS DETECTION

The potential and versatility of label-free nanophotonic biosensors for medical diagnostics have been widely demonstrated, including virus detection and identification, especially for the human immunodeficiency virus (HIV) and the hepatitis B virus.[47,94−97] Hereby, we provide a brief review of the latest developments in nanophotonic biosensors for respiratory virus infection diagnosis based on the three main assay strategies described above.

Nanophotonic Biosensors for Viral Genomic Analysis. Detection of specific genomic sequences with label-free photonic biosensors is usually carried out through rapid hybridization assays. If the biosensor is sensitive enough, direct detection from the sample might be possible, without preamplification steps (PCR-free), although it still requires extraction and fragmentation procedures to be performed before the biosensing assay.

Due to the intricate bioassay design and procedure, only a few biosensors have been developed for the genomic assay of respiratory viruses (see Table 2). At the time of writing this review, at least one nanoplasmonic biosensor has been reported for the detection of the SARS-CoV-2 virus. Qiu et al. have developed a dual-mode nanoplasmonic system, consisting of two-dimensional gold nanoislands, that combines plasmonic photothermal effect and LSPR sensing transduction (Figure 3a).[98] The sensor is functionalized with a DNA probe of a complementary sequence to the RdRp RNA gene of the COVID-19 virus. The specific nucleic acid hybridization, enhanced by the thermoplasmonic heat generated at the nanoislands, is detected as a LSPR phase shift that can be monitored in real-time. They achieved a limit of detection around 0.2 pM for the target RNA sequence, which could be translated into approximately 2×10^4 copies of the SARS-CoV-2 virus, showing good potential for COVID-19 diagnostics.

Previous studies have also addressed the genomic analysis of viral infections. Koo et al., for example, developed a label-free biosensor using silicon microring resonators for the diagnosis of respiratory viral infections. They performed an isothermal RNA amplification coupled to the hybridization biosensing assay for the detection, all within 20 min.[99] The biosensor was applied for the analysis of nasopharyngeal samples from patients infected with influenza A and B, human coronavirus OC43/229E, and RSV A and B, respectively. The limit of detection was found close to 2.5×10^1 copies/reaction and clinical sensitivities between 85% and 100%, although specificity was low for some viruses (40−60% for hCoV) probably due to the lack of enough samples. This biosensor exhibited a reliability comparable to that of conventional RT-PCR, reducing the analysis time to a few minutes and avoiding additional fluorescence or electrophoresis-based detection methods. With a similar aim, Shi et al. reported an SPR biosensor for multiplexed RNA detection of several respiratory viruses: influenza A and B, H1N1, RSV, parainfluenza virus, adenovirus, and SARS coronavirus simultaneously.[100] However, a previous PCR amplification of the target genetic material plus a secondary streptavidin-amplification step after hybridization were required. Overall, sensitivities achieved were in the range of 1−5 nM. Other nanoplasmonic approaches like SERS have also been applied for respiratory virus genomic analysis. For example, Pang et al. developed a SERS sensor combined with a molecular sentinel for the detection of the avian influenza virus. They reached detection limits around 2−3 aM, allowing a PCR-free analysis method.[62]

As major assets compared to standard RT-PCR technique for viral genomic analysis, these nanophotonic biosensors account with the direct and label-free capabilities that reduce total turnaround analysis time from several hours (4 to 6) to less than 30 min. Potential applications of these technologies for the COVID-19 pandemic and possible future outbreaks could signify the realization of truly reliable massive testing and screening. By targeting appropriate gene sequences and providing ultrasensitive analysis, the genomic biosensing assays can provide highly accurate and specific detection of the virus, even when viral load is low. Furthermore, by establishing multiplexed genomic analyses, it could be possible to identify different types of viruses or strains in one single assay.

Nanophotonic Biosensors for Antigen-Directed Virus Detection. The first works for intact virus detection with label-free optical biosensors were reported by Schofield and Dimmock around 25 years ago.[101] They employed an SPR biosensor to determine the affinities of different antibodies and Fab fragments for influenza A viruses by monitoring the capture of whole virions. Since then, many researchers have been developing new biosensor assays for rapid virus detection and quantification with plasmonic and photonic technologies (see Table 2). The vast majority of the work in respiratory virus diagnosis targets influenza viruses and the different subtypes.[102] In recent publications, for example, Zhao et al. reported an optical SPR fiber sensor for the detection of avian influenza virus.[103] With a compact and low-cost system, they were able to detect around 5×10^5 EID_{50} in 100 μL sample within 10 min. Chang et al. also employed an SPR biosensor for avian influenza detection with a newly generated antibody (Figure 3b).[104] They reached a detection limit of around 200 copies/mL, which resulted in 20-fold improved sensitivity compared to analogous ELISA tests, and better than conventional RT-PCR and rapid diagnostic tests. Likewise, Prabowo et al. proposed a portable SPR biosensor for the rapid quantification of enterovirus antibodies targeting the VP1 membrane protein.[105] The SPR system showed a detection limit of 67 virus particles per milliliter. Besides antibodies, aptamers have also been applied for avian influenza virus detection. Bai et al. developed an SPR aptasensor with detection sensitivities around 10^5.[70] A more exhaustive study was reported by Nguyen et al., including the selection of aptamer pairs to develop a sandwich-type SPR assay for avian influenza virus detection.[69] In this assay, they reached sensitivities around 200 EID_{50}/mL.

Apart from plasmonics, other nanophotonic sensors have also been applied for direct virus detection. Xu et al. developed a waveguide-based interferometric biosensor for the direct detection of influenza viruses.[106] They tested different monoclonal and polyclonal antibodies targeting the HA protein of the membrane of influenza viruses. Their biosensor immunoassay reached outstanding sensitivities, with a detection limit of 0.0005 HAU. Lee et al. fabricated a label-free optical sensor based on three-dimensional inverse opal silica nanostructures.[107] Their application for direct avian influenza virus detection by antibody capturing rendered sensitivities in the range of 10^3−10^5 PFUs (plaque forming units). Endo et al. developed a flexible two-dimensional photonic crystal biosensor employing antibodies targeting avian influenza virus HA protein.[108] They reached a limit of detection of 1 ng/mL for

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

direct measurement in human saliva samples. Finally, Sakamoto et al. employed a Mach−Zehnder interferometer for avian influenza diagnostics.[109] They only tested the technology for the detection of 10^{10} virus particles that showed a clear signal response in comparison with the negative control.

The availability of sensitive and reliable intact virus detection tests for SARS-CoV-2 could transform the outbreak status, management, and progression. The implementation of biosensors for rapid screening of the population and immediate detection of COVID-19 would allow more efficient patient isolation and spread control. Nanophotonic biosensors have demonstrated superior sensitivity and robustness compared to LFA tests, plus they offer quantitative results in approximately the same turnaround assay times. Ongoing research is focusing on developing and applying these valuable technologies for direct and rapid diagnostics, and especially for the point-of-care testing of coronavirus infection. A good example is one of the first and largest research projects funded by the European Commission to tackle the COVID-19 pandemics (i.e., CoNVat project), which will employ a silicon interferometric sensor for intact virus detection in nasopharyngeal and saliva samples.[110] Besides, a few reports have been uploaded in non-peer-reviewed repositories showing proof-of-concept studies. For example, Ahmadivand et al. have developed a plasmonic metasurface for sensing the S protein at the femtomolar level.[111] On the other hand, Zhang et al. employed silver nanorod SERS sensors for ultrafast detection of viral antigens (RBD). The technology has been applied for water monitoring but could also be adapted for diagnostics.[112]

Nanophotonic Biosensors for Serology and Immunology Assays. Although not considered a primary diagnosis strategy, serology and immunology assays have a strong relevance in an infection disease prevalence study and the design and assessment of vaccines and other therapeutics. Optical label-free biosensors offer a unique technique to facilitate, accelerate, and improve the routine pharmaceutical and biomedical analysis.

Serology biosensing assays are relatively easy to implement, as they essentially consist of direct quantification of antibodies through binding to their corresponding antigen immobilized on the sensor surface. Scarcely any articles have been recently published for the development and validation of such serological biosensors for respiratory viruses.[113,114] However, the emergence of SARS-CoV-2 and the urgent need for massive testing has promoted the application of nanophotonic sensors for COVID-19 serology with diagnosis and monitoring purposes. Very recently, Shaw et al. have published a clinical evaluation study of a nanoplasmonic technology for quantitative detection of SARS-CoV-2 antibodies in different disease stages.[115] Other examples can be found in repositories. Abdelhadi et al. have also developed a SPR bioassay for SARS-CoV-2 antibody testing,[116] and Kulp et al. demonstrate the utility of SPR biosensors for rapid antibody neutralization assays, studying recognition of the ACE2 receptor.[117]

On the other hand, many research works in vaccine development and evaluation employ plasmonic and photonic biosensors for characterization and evaluation procedures. For example, Fuentes et al. characterized the affinity and kinetics of antibodies against RSV generated during vaccine development, a key step in preclinical evaluation.[118] Marsh et al. employed a SPR system to assess the susceptibility of RSV to antifusion protein antibodies,[119] and Gauger et al. also used it for evaluation of live attenuated influenza A virus vaccine.[120] On the other hand, Zhang et al. developed a novel arrayed imaging reflectometry platform for the high-throughput study of the serologic antigenicity of influenza viruses.[121]

It is important to note as well that nanophotonic biosensors have shown exceptional utility in different virology and immunology studies. Nilsson et al., for instance, published an SPR biosensor for quantification of influenza virus HA protein based on an inhibition assay.[122] They immobilized the HA proteins for different virus subtypes on the sensor surface and quantified the levels of free specific antibodies in the sample, after incubation with the infected sample. The limit of detection was around 0.5 μg/mL, but they were able to identify the different avian influenza virus subtypes and strains in serum samples. Negri et al. used label-free SERS sensors for the detection of viral nucleoprotein binding to an anti-influenza aptamer.[71] Although they did not reach a high sensitivity (1 μg/mL), they could demonstrate specific detection of the influenza nucleoprotein challenged to other respiratory viruses and biological samples. On the other hand, Vidic et al. used an SPR biosensor to identify and characterize a novel complex between the nucleoprotein and the RNA polymerase of the influenza A virus.[123] This molecular complex has a role in the final steps of transcription and replication of the virus, so it could be a target for therapeutics. Xiong et al. applied biolayer interferometry technology to measure the binding affinity of human and influenza viruses to their cell receptors.[124]

In the coronavirus area, some studies have also been performed with optical biosensors. After the last coronavirus outbreaks (SARS and MERS), researchers have continued studying and characterizing the virus structures and searching for potential drugs and therapeutic agents. Huang et al., for example, studied the evolution of the receptor-binding domain (RBD) of the Spike protein of β-coronavirus and its interaction with cell receptors, employing an SPR platform to characterize the affinity and kinetics of the interaction.[125] Likewise, Park et al. evaluated different polyphenols as coronavirus protease inhibitors with an SPR biosensor.[126] They could select one of these molecules as a promising candidate for developing anticoronaviral agents.

The use of plasmonic and photonic biosensors in serology and immunology studies has undoubtedly benefited the pharmaceutical industry and biomedical research. It could soon replace long and tedious ELISA, offering a faster, simpler, and even cheaper technique. For the COVID-19 case, serological analyses have become crucial not only as a complementary diagnosis but also for pandemics management and epidemiology studies. It is now essential to perform massive tests for the whole population to understand the incidence and mortality of the disease, and the effects on human health and immunity. For the development and evaluation of new vaccines or other therapies (e.g., hyperimmune serum treatments), it is crucial to accurately study the generation and kinetics of sera antibodies, and optimally in a quantitative and highly specific way. For that, label-free nanophotonic biosensors are promising and realistic solutions.

FUTURE PERSPECTIVES

Nanophotonic biosensors have demonstrated their detection performance and capabilities for clinical diagnostics, including discrete microorganisms such as micrometer-sized bacteria and small viruses on the nanometer scale. The detection of pathogens that tackle the drawbacks of current methodologies is possible with the high levels of sensitivity this technology can

https://dx.doi.org/10.1021/acssensors.0c01180
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reach. They can be used for the detection of antibodies in serum, the detection of DNA and RNA fragments with excellent performance, avoiding amplification, and even for the detection of whole viruses, which shows the versatility to implement such tools in the diagnosis of respiratory viruses such as SARS-CoV-2.

However, there is still much effort to be done, especially on two fronts. First, to systematically perform rigorous clinical validation of each individually optimized assay to fully assess the reliability and robustness of the detection strategy. This is not always provided as it involves the evaluation of a high number of real samples to determine specificity and sensitivity statistics parameters. Second and most importantly, we need a strong push to move the technology from laboratory prototypes, commonly installed in a controlled and robust environment, to decentralized settings. This step involves the design of all-integrated, autonomous, compact devices, incorporating optical elements that facilitate both the reduction in size and in cost, while retaining the performance achieved in laboratory versions, to further pursue viable commercialization. In this sense, highly advanced smartphone technology offers excellent and promising platforms to design point-of-care versions (Figure 3c).[127−130] For example, an optical interface based on complementary metal-oxide semiconductor (CMOS) technology in the phone cameras for readout, in combination with customized software implemented via smartphone applications, can be used for different optical detection approaches, from labeled approaches (like colorimetry and fluorescence) to label-free detection, through imaging readout or spectrum monitoring.[131] Additional components such as external light sources, like affordable LEDs or broadband lights, collimators, or polarizers, can be included in an adapted miniaturized design, involving also compatible and suitable customized polymer shell or housing to merge with the cell phone. More examples are appearing trying to combine smartphone technology with plasmonic biosensors,[132,133] although the performance is still undermined compared with robust laboratory versions and they have been tested with model biosensing systems or with limited analytical characterization.

Microfluidics also play a crucial role to provide disposable, stable over time, easy-to-manipulate cartridges incorporating the sensor chip with the specific biofunctionality for each detection assay.[135−137] In the case of airborne respiratory viruses, like SARS-CoV-2, it will be essential to integrate such a cartridge with additional sections that include sample preprocessing steps before the final detection.[138] Some of these steps might include extraction of the viral genome from the clinical samples,[139] separation of plasma, or serum from blood[134,140−142] whose direct measurement is exceptionally challenging with optical biosensors, or even more appealing, oral samples pre-treatment (nasopharyngeal swabs, saliva, sputum, etc.).[143] In the case of oral samples, viral load is expected to be high, and minimum manipulation would facilitate the prevention of healthcare staff infection as well as further expansion of the device use outside of biosafety settings (e.g., primary care centers). There are plenty of examples of microfluidics that perform filtration, extraction, and centrifugation for preconcentration, and more complex processes such as nucleic acid amplification. However, they are commonly coupled colorimetric or electrochemical detections and it is rarely reported for label-free optical biosensors. Some interesting examples have already been proposed merging microfluidics integration and nanophotonic biosensors (Figure 3d). For example, an individual polymeric cartridge with an interferometric photonic chip that can be directly inserted in the POC device, which has been validated for the pump-free direct detection of tuberculosis in urine samples.[55] Using automated pneumatic-valve based microfluidics[144−146] or digital microfluidics[147,148] instead of conventional microchannel-based fluidic chips represents an emerging trend to be used with silicon photonics given its potential to be integrated in miniaturized design and its versatility to transport and mix very small volumes with high precision, thus being very attractive for multiplexed automated analysis. However, scarce examples are found in the literature[149] and not tested further with biosensors. Despite the excellent biosensing performance of nanophotonic biosensors, there is still a long road ahead in several technological aspects to achieve fully operative silicon and plasmonic devices for clinical diagnosis. The current emergency situation, which has revealed the shortage of really effective diagnostic techniques, may represent an inflection point to push further the research and investment in optical biosensors toward such levels of integration necessary to successfully hit the market.

SUMMARY AND CONCLUSIONS

The outbreak of COVID-19 has dramatically exposed the urgent need to upgrade the clinical diagnostics to implement new technologies for POC testing with sufficient accuracy and reliability. Nanophotonic biosensors have demonstrated in academic settings the capabilities for such endeavor, offering a unique potential to combine rapid virus detection and identification, genomic analysis, and even serological assays in one integrated platform. Key factors for the successful implementation of label-free nanophotonic biosensors in virus infection diagnosis are essentially the selection of appropriate and high-quality bioreceptors, which confer enough sensitivity and selectivity, together with a robust surface biofunctionalization. The rational design and optimization of label-free nanophotonic biosensors can provide direct detection of intact virus entities or specific genomic identification directly from a human sample with outstanding sensitivities (few copies per milliliter). Further, the simplicity and efficiency for routine and fast serological analysis could significantly contribute to epidemiology studies or vaccine development. Henceforth, research in nanophotonic technologies for diagnostic biosensors should move a step forward to prove their capabilities in the clinical field and provide fully operative point-of-care devices for rapid, accurate, and massive infection diagnosis.

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https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

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Funding

Financial support is acknowledged from CoNVat project from the H2020 Research and Innovation Programme of the European Commission (H2020-SC1-PHE-CORONAVIRUS-2020, Project No. 101003544).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.C.C. and A.A. acknowledge financial support from European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement (No. 754510 and No. 754397, respectively). The ICN2 is funded by the CERCA programme/Generalitat de Catalunya. The ICN2 is supported by the Severo Ochoa Centres of Excellence programme, funded by AEI (grant no. SEV-2017-0706).

■ ABBREVIATIONS

COVID-19, coronavirus disease 2019; SARS, severe acute respiratory syndrome; POC, point-of-care; SPR, surface plasmon resonance; LSPR, localized surface plasmon resonance; TIR, total internal reflection; HA, hemagglutinin; RSV, respiratory syncytial virus

■ VOCABULARY

Biosensor: an analytical device that integrates a biological receptor (e.g., antibody, DNA probe, enzyme) in close contact with a transducer (optical, electrochemical or mechanical). Specific recognition events between the bioreceptor and the analyte of interest produces physicochemical changes in the transducer that are transformed into readable and quantifiable signals; Nanophotonics: area of research that studies the behavior of light and light-matter interactions at the nanoscale. It includes research in physics, optical and electrical engineering, and materials sciences, and it can be applied to multiple fields, such as biology and biomedicine, energy, or telecommunications; Microfluidics: area of research that focuses on the behavior and manipulation of fluids at the micrometer scale. Microfluidic systems enable precise control and distribution of fluids at very low volumes (from μL to fL); Surface biofunctionalization: a series of procedures, usually based on (bio)chemical processes, to incorporate or anchor a biological element to a surface to provide specific functionality, e.g. analyte recognition or capture; Virus: submicroscopic biological entity that infects and replicates inside living cells of an organism, often with pathogenic consequences; Antibody: also known as immunoglobulin is a blood-circulating protein produced by the immune system of an organism to recognize and neutralize specific antigenic molecules or pathogens; Serology: analysis and study of the blood serum composition for diagnostic purposes, especially with regard to the identification of immune system response (i.e., antibody production) to pathogenic agents or other antigenic substances

■ REFERENCES

(1) Troeger, C.; Blacker, B.; Khalil, I. A.; Rao, P. C.; Cao, J.; Zimsen, S. R. M.; Albertson, S. B.; Deshpande, A.; Farag, T.; Abebe, Z.; et al. Estimates of the Global, Regional, and National Morbidity, Mortality, and Aetiologies of Lower Respiratory Infections in 195 Countries, 1990−2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* **2018**, *18* (11), 1191−1210.

(2) Hossain, M. Z.; Bambrick, H.; Wraith, D.; Tong, S.; Khan, A. F.; Hore, S. K.; Hu, W. Sociodemographic, Climatic Variability and Lower Respiratory Tract Infections: A Systematic Literature Review. *Int. J. Biometeorol.* **2019**, *63*, 209−219.

(3) Ginocchio, C. C.; McAdam, A. J. Current Best Practices for Respiratory Virus Testing. *J. Clin. Microbiol.* **2011**, *49*, 44−48.

(4) Pinsky, B. A.; Hayden, R. T. Cost-Effective Respiratory Virus Testing. *J. Clin. Microbiol.* **2019**, *57* (9), 1 DOI: 10.1128/JCM.00373-19.

(5) Zhang, N.; Wang, L.; Deng, X.; Liang, R.; Su, M.; He, C.; Hu, L.; Su, Y.; Ren, J.; Yu, F.; et al. Recent Advances in the Detection of Respiratory Virus Infection in Humans. *J. Med. Virol.* **2020**, *92* (4), 408−417.

(6) Basile, K.; Kok, J.; Dwyer, D. E. Point-of-Care Diagnostics for Respiratory Viral Infections. *Expert Rev. Mol. Diagn.* **2018**, *18* (1), 75−83.

(7) Caliendo, A. M. Multiplex PCR and Emerging Technologies for the Detection of Respiratory Pathogens. *Clin. Infect. Dis.* **2011**, *52*, S326.

(8) Sheridan, C. Fast, Portable Tests Come Online to Curb Coronavirus Pandemic. *Nat. Biotechnol.* **2020**, 1 DOI: 10.1038/d41587-020-00015-x.

(9) Kaur, N.; Toley, B. J. Paper-Based Nucleic Acid Amplification Tests for Point-of-Care Diagnostics. *Analyst* **2018**, *143* (10), 2213−2234.

(10) Shen, M.; Zhou, Y.; Ye, J.; Abdullah AL-Maskri, A. A.; Kang, Y.; Zeng, S.; Cai, S. Recent Advances and Perspectives of Nucleic Acid Detection for Coronavirus. *J. Pharm. Anal.* **2020**, *10*, 97−101.

(11) Keikha, M. LAMP Method as One of the Best Candidates for Replacing with PCR Method. *Malaysian J. Med. Sci.* **2018**, *25* (1), 121−123.

(12) Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. Loop-Mediated Isothermal Amplification of DNA. *Nucleic Acids Res.* **2000**, *28* (12), 63e−63.

(13) Parida, M.; Sannarangaiah, S.; Dash, P. K.; Rao, P. V. L.; Morita, K. Loop Mediated Isothermal Amplification (LAMP): A New Generation of Innovative Gene Amplification Technique; Perspectives in Clinical Diagnosis of Infectious Diseases. *Rev. Med. Virol.* **2008**, *18* (6), 407−421.

(14) Ali, M. M.; Li, F.; Zhang, Z.; Zhang, K.; Kang, D. K.; Ankrum, J. A.; Le, X. C.; Zhao, W. Rolling Circle Amplification: A Versatile Tool for Chemical Biology, Materials Science and Medicine. *Chem. Soc. Rev.* **2014**, *43*, 3324−3341.

(15) Thai, H. T. C.; Le, M. Q.; Vuong, C. D.; Parida, M.; Minekawa, H.; Notomi, T.; Hasebe, F.; Morita, K. Development and Evaluation of a Novel Loop-Mediated Isothermal Amplification Method for Rapid Detection of Severe Acute Respiratory Syndrome Coronavirus. *J. Clin. Microbiol.* **2004**, *42* (5), 1956−1961.

(16) Wang, B.; Potter, S. J.; Lin, Y.; Cunningham, A. L.; Dwyer, D. E.; Su, Y.; Ma, X.; Hou, Y.; Saksena, N. K. Rapid and Sensitive Detection of Severe Acute Respiratory Syndrome Coronavirus by Rolling Circle Amplification. *J. Clin. Microbiol.* **2005**, *43* (5), 2339−2344.

(17) Imai, M.; Ninomiya, A.; Minekawa, H.; Notomi, T.; Ishizaki, T.; Van Tu, P.; Tien, N. T. K.; Tashiro, M.; Odagiri, T. Rapid Diagnosis of H5N1 Avian Influenza Virus Infection by Newly Developed Influenza H5 Hemagglutinin Gene-Specific Loop-Mediated Isothermal Amplification Method. *J. Virol. Methods* **2007**, *141* (2), 173−180.
(18) Carter, L. J.; Garner, L. V.; Smoot, W.; Li, Y.; Zhou, Q.; Catherine, J.; Sasso, J. M.; Gregg, A. C.; Soares, D. J.; Beskid, R.; et al. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent. Sci.* **2020**, *6*, 591−605.
(19) De Souza Luna, L. K.; Heiser, V.; Regamey, N.; Panning, M.; Drexler, J. F.; Mulangu, S.; Poon, L.; Baumgarte, S.; Haijema, B. J.; Kaiser, L.; et al. Generic Detection of Coronaviruses and Differentiation at the Prototype Strain Level by Reverse Transcription-PCR and Nonfluorescent Low-Density Microarray. *J. Clin. Microbiol.* **2007**, *45* (3), 1049−1052.
(20) Hardick, J.; Metzgar, D.; Risen, L.; Myers, C.; Balansay, M.; Malcom, T.; Rothman, R.; Gaydos, C. Initial Performance Evaluation of a Spotted Array Mobile Analysis Platform (MAP) for the Detection of Influenza A/B, RSV, and MERS Coronavirus. *Diagn. Microbiol. Infect. Dis.* **2018**, *91* (3), 245−247.
(21) Gootenberg, J. S.; Abudayyeh, O. O.; Lee, J. W.; Essletzbichler, P.; Dy, A. J.; Joung, J.; Verdine, V.; Donghia, N.; Daringer, N. M.; Freije, C. A.; et al. Nucleic Acid Detection with CRISPR-Cas13a/C2c2. *Science (Washington, DC, U. S.)* **2017**, *356* (6336), 438−442.
(22) Freije, C. A.; Myhrvold, C.; Boehm, C. K.; Lin, A. E.; Welch, N. L.; Carter, A.; Metsky, H. C.; Luo, C. Y.; Abudayyeh, O. O.; Gootenberg, J. S.; et al. Programmable Inhibition and Detection of RNA Viruses Using Cas13. *Mol. Cell* **2019**, *76* (5), 826−837.
(23) Zhang, F.; Abudayyeh, O. O.; Gootenberg, J. S. *A Protocol for Detection of COVID-19 Using CRISPR Diagnostics*; https://www.broadinstitute.org/files/publications/special/COVID-19%20detection%20(updated).pdf.
(24) Sadeghi, C. D.; Aebi, C.; Gorgievski-Hrisoho, M.; Mühlemann, K.; Barbani, M. T. Twelve Years' Detection of Respiratory Viruses by Immunofluorescence in Hospitalised Children: Impact of the Introduction of a New Respiratory Picornavirus Assay. *BMC Infect. Dis.* **2011**, *11*, 1 DOI: 10.1186/1471-2334-11-41.
(25) Shafik, C. F.; Mohareb, E. W.; Youssef, F. G. Comparison of Direct Fluorescence Assay and Real-Time RT-PCR as Diagnostics for Respiratory Syncytial Virus in Young Children. *J. Trop. Med.* **2011**, *2011*, 1.
(26) Boonham, N.; Kreuze, J.; Winter, S.; van der Vlugt, R.; Bergervoet, J.; Tomlinson, J.; Mumford, R. Methods in Virus Diagnostics: From ELISA to next Generation Sequencing. *Virus Res.* **2014**, *186*, 20−31.
(27) Chartrand, C.; Tremblay, N.; Renaud, C.; Papenburg, J. Diagnostic Accuracy of Rapid Antigen Detection Tests for Respiratory Syncytial Virus Infection: Systematic Review and Meta-Analysis. *J. Clin. Microbiol.* **2015**, *53* (12), 3738−3749.
(28) Broberg, E.; Nicoll, A.; Amato-Gauci, A. Seroprevalence to Influenza A(H1N1) 2009 Virus—Where Are We? *Clin. Vaccine Immunol.* **2011**, *18* (8), 1205−1212.
(29) Leung, G. M.; Lim, W. W.; Ho, L.-M.; Lam, T.-H.; Ghani, A. C.; Donnelly, C. A.; Fraser, C.; Riley, S.; Ferguson, N. M.; Anderson, R. M.; et al. Seroprevalence of IgG Antibodies to SARS-Coronavirus in Asymptomatic or Subclinical Population Groups. *Epidemiol. Infect.* **2006**, *134* (2), 211−221.
(30) Takahashi, M.; Kusakai, S.; Mizuo, H.; Suzuki, K.; Fujimura, K.; Masuko, K.; Sugai, Y.; Aikawa, T.; Nishizawa, T.; Okamoto, H. Simultaneous Detection of Immunoglobulin A (IgA) and IgM Antibodies against Hepatitis E Virus (HEV) Is Highly Specific for Diagnosis of Acute HEV Infection. *J. Clin. Microbiol.* **2005**, *43* (1), 49−56.
(31) Hsueh, P.-R.; Huang, L.-M.; Chen, P.-J.; Kao, C.-L.; Yang, P.-C. Chronological Evolution of IgM, IgA, IgG and Neutralisation Antibodies after Infection with SARS-Associated Coronavirus. *Clin. Microbiol. Infect.* **2004**, *10* (12), 1062−1066.
(32) Woo, P. C. Y.; Lau, S. K. P.; Wong, B. H. L.; Chan, K.; Chu, C.; Tsoi, H.; Huang, Y.; Peiris, J. S. M.; Yuen, K. Longitudinal Profile of Immunoglobulin G (IgG), IgM, and IgA Antibodies against the Severe Acute Respiratory Syndrome (SARS) Coronavirus Nucleocapsid Protein in Patients with Pneumonia Due to the SARS Coronavirus. *Clin. Diagn. Lab. Immunol.* **2004**, *11* (4), 665−668.
(33) Long, Q.-X.; Liu, B.-Z.; Deng, H.-J.; Wu, G.-C.; Deng, K.; Chen, Y.-K.; Liao, P.; Qiu, J.-F.; Lin, Y.; Cai, X.-F. Antibody Responses to SARS-CoV-2 in Patients with COVID-19. *Nat. Med.* **2020**, *26*, 845.
(34) Zhao, J.; Yuan, Q.; Wang, H.; Liu, W.; Liao, X.; Su, Y.; Wang, X.; Yuan, J.; Li, T.; Li, J. Antibody Responses to SARS-CoV-2 in Patients of Novel Coronavirus Disease 2019. *Clin. Infect. Dis.* **2020**, 1 DOI: 10.1093/cid/ciaa344.
(35) Dortet, L.; Emeraud, C.; Vauloup-Fellous, C.; Khecharem, M.; Ronat, J.-B.; Fortineau, N.; Roque-Afonso, A.-M.; Naas, T. Rapid Determination of SARS-CoV-2 Antibodies Using a Bedside, Point-of-Care, Serological Test. *SSRN Electron. J.* **2020**, 1 DOI: 10.2139/ssrn.3582814.
(36) *FindDx*; https://www.finddx.org/covid-19/sarscov2-eval-immuno/.
(37) Fracchiolla, N.; Artuso, S.; Cortelezzi, A. Biosensors in Clinical Practice: Focus on Oncohematology. *Sensors* **2013**, *13* (5), 6423−6447.
(38) Steglich, P.; Hülsemann, M.; Dietzel, B.; Mai, A. Optical Biosensors Based on Silicon-On-Insulator Ring Resonators: A Review. *Molecules* **2019**, *24* (3), 519.
(39) Zanchetta, G.; Lanfranco, R.; Giavazzi, F.; Bellini, T.; Buscaglia, M. Emerging Applications of Label-Free Optical Biosensors. *Nanophotonics* **2017**, *6* (4), 627−645.
(40) Ciminelli, C.; Dell'Olio, F.; Conteduca, D.; Armenise, M. N. Silicon Photonic Biosensors. *IET Optoelectron.* **2019**, *13* (2), 48−54.
(41) Luan, E.; Shoman, H.; Ratner, D.; Cheung, K.; Chrostowski, L. Silicon Photonic Biosensors Using Label-Free Detection. *Sensors* **2018**, *18* (10), 3519.
(42) Han, X.; Liu, K.; Sun, C. Plasmonics for Biosensing. *Materials* **2019**, *12* (9), 1411.
(43) Lopez, G. A.; Estevez, M. C.; Soler, M.; Lechuga, L. M. Recent Advances in Nanoplasmonic Biosensors: Applications and Lab-on-a-Chip Integration. *Nanophotonics* **2017**, *6* (1), 123−136.
(44) Chocarro-Ruiz, B.; Fernández-Gavela, A.; Herranz, S.; Lechuga, L. M. Nanophotonic Label-Free Biosensors for Environmental Monitoring. *Curr. Opin. Biotechnol.* **2017**, *45*, 175−183.
(45) Mauriz, E.; Dey, P.; Lechuga, L. M. Advances in Nanoplasmonic Biosensors for Clinical Applications. *Analyst* **2019**, *144* (24), 7105−7129.
(46) Huertas, C. S.; Calvo-Lozano, O.; Mitchell, A.; Lechuga, L. M. Advanced Evanescent-Wave Optical Biosensors for the Detection of Nucleic Acids: An Analytic Perspective. *Front. Chem.* **2019**, *7*, 1−25.
(47) Saylan, Y.; Erdem, Ö.; Ünal, S.; Denizli, A. An Alternative Medical Diagnosis Method: Biosensors for Virus Detection. *Biosensors* **2019**, *9* (2), 65.
(48) Daaboul, G. G.; Lopez, C. A.; Yurt, A.; Goldberg, B. B.; Connor, J. H.; Ünlü, M. S. Label-Free Optical Biosensors for Virus Detection and Characterization. *IEEE J. Sel. Top. Quantum Electron.* **2012**, *18* (4), 1422−1433.
(49) Soler, M.; Huertas, C. S.; Lechuga, L. M. Label-Free Plasmonic Biosensors for Point-of-Care Diagnostics: A Review. *Expert Rev. Mol. Diagn.* **2019**, *19*, 71−81.
(50) Fernández Gavela, A.; Grajales García, D.; Ramirez, J.; Lechuga, L. Last Advances in Silicon-Based Optical Biosensors. *Sensors* **2016**, *16* (3), 285.
(51) Soler, M.; Huertas, C. S.; Lechuga, L. M. Label-Free Plasmonic Biosensors for Point-of-Care Diagnostics: A Review. *Expert Rev. Mol. Diagn.* **2019**, *19* (1), 71−81.
(52) Masson, J.-F. Surface Plasmon Resonance Clinical Biosensors for Medical Diagnostics. *ACS Sensors* **2017**, *2* (1), 16−30.
(53) Hill, R. T. Plasmonic Biosensors. *Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology* **2015**, *7* (2), 152−168.
(54) Li, M.; Cushing Ab, S. K.; Wu, N. Plasmon-Enhanced Optical Sensors: A Review. *Analyst* **2015**, *140*, 386.
(55) Ramirez-Priego, P.; Martens, D.; Elamin, A. A.; Soetaert, P.; Van Roy, W.; Vos, R.; Anton, B.; Bockstaele, R.; Becker, H.; Singh, M.; et al.

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

Label-Free and Real-Time Detection of Tuberculosis in Human Urine Samples Using a Nanophotonic Point-of-Care Platform. *ACS Sensors* **2018**, *3* (10), 2079−2086.
(56) Huertas, C. S.; Fariña, D.; Lechuga, L. M. Direct and Label-Free Quantification of Micro-RNA-181a at Attomolar Level in Complex Media Using a Nanophotonic Biosensor. *ACS Sensors* **2016**, *1* (6), 748−756.
(57) Maldonado, J.; Estévez, M. C.; Fernández-Gavela, A.; González-López, J. J.; González-Guerrero, A. B.; Lechuga, L. M. Label-Free Detection of Nosocomial Bacteria Using a Nanophotonic Interferometric Biosensor. *Analyst* **2020**, *145* (2), 497−506.
(58) Bogaerts, W.; De Heyn, P.; Van Vaerenbergh, T.; De Vos, K.; Kumar Selvaraja, S.; Claes, T.; Dumon, P.; Bienstman, P.; Van Thourhout, D.; Baets, R. Silicon Microring Resonators. *Laser Photon. Rev.* **2012**, *6* (1), 47−73.
(59) Threm, D.; Nazirizadeh, Y.; Gerken, M. Photonic Crystal Biosensors towards On-Chip Integration. *J. Biophotonics* **2012**, *5* (8−9), 601−616.
(60) Zhuo, Y.; Cunningham, B. Label-Free Biosensor Imaging on Photonic Crystal Surfaces. *Sensors* **2015**, *15* (9), 21613−21635.
(61) Tedeschi, L.; Mercatanti, A.; Domenici, C.; Citti, L. Design, Preparation and Testing of Suitable Probe-Receptors for RNA Biosensing. *Bioelectrochemistry* **2005**, *67*, 171−179.
(62) Pang, Y.; Wang, J.; Xiao, R.; Wang, S. SERS Molecular Sentinel for the RNA Genetic Marker of PB1-F2 Protein in Highly Pathogenic Avian Influenza (HPAI) Virus. *Biosens. Bioelectron.* **2014**, *61*, 460−465.
(63) Holliger, P.; Hudson, P. J. Engineered Antibody Fragments and the Rise of Single Domains. *Nat. Biotechnol.* **2005**, *23*, 1126.
(64) Pan, Q.; Luo, F.; Liu, M.; Zhang, X. L. Oligonucleotide Aptamers: Promising and Powerful Diagnostic and Therapeutic Tools for Infectious Diseases. *J. Infect.* **2018**, *77*, 83−98.
(65) Wang, R.; Zhao, J.; Jiang, T.; Kwon, Y. M.; Lu, H.; Jiao, P.; Liao, M.; Li, Y. Selection and Characterization of DNA Aptamers for Use in Detection of Avian Influenza Virus H5N1. *J. Virol. Methods* **2013**, *189* (2), 362−369.
(66) Kiilerich-Pedersen, K.; Daprà, J.; Cherré, S.; Rozlosnik, N. High Sensitivity Point-of-Care Device for Direct Virus Diagnostics. *Biosens. Bioelectron.* **2013**, *49*, 374−379.
(67) Kumar, P. Monitoring Intact Viruses Using Aptamers. *Biosensors* **2016**, *6* (3), 40.
(68) Zou, X.; Wu, J.; Gu, J.; Shen, L.; Mao, L. Application of Aptamers in Virus Detection and Antiviral Therapy. *Front. Microbiol.* **2019**, *10*, 1 DOI: 10.3389/fmicb.2019.01462.
(69) Nguyen, V. T.; Seo, H. B.; Kim, B. C.; Kim, S. K.; Song, C. S.; Gu, M. B. Highly Sensitive Sandwich-Type SPR Based Detection of Whole H5Nx Viruses Using a Pair of Aptamers. *Biosens. Bioelectron.* **2016**, *86*, 293−300.
(70) Bai, H.; Wang, R.; Hargis, B.; Lu, H.; Li, Y. A SPR Aptasensor for Detection of Avian Influenza Virus H5N1. *Sensors* **2012**, *12* (9), 12506−12518.
(71) Negri, P.; Chen, G.; Kage, A.; Nitsche, A.; Naumann, D.; Xu, B.; Dluhy, R. A. Direct Optical Detection of Viral Nucleoprotein Binding to an Anti-Influenza Aptamer. *Anal. Chem.* **2012**, *84* (13), 5501−5508.
(72) Kale, R. R.; Mukundan, H.; Price, D. N.; Harris, J. F.; Lewallen, D. M.; Swanson, B. I.; Schmidt, J. G.; Iyer, S. S. Detection of Intact Influenza Viruses Using Biotinylated Biantennary S-Sialosides. *J. Am. Chem. Soc.* **2008**, *130*, 8169−8171.
(73) Hideshima, S.; Hayashi, H.; Hinou, H.; Nambuya, S.; Kuroiwa, S.; Nakanishi, T.; Momma, T.; Nishimura, S. I.; Sakoda, Y.; Osaka, T. Glycan-Immobilized Dual-Channel Field Effect Transistor Biosensor for the Rapid Identification of Pandemic Influenza Viral Particles. *Sci. Rep.* **2019**, *9*, 1−10.
(74) Hushegyi, A.; Pihíková, D.; Bertok, T.; Adam, V.; Kizek, R.; Tkac, J. Ultrasensitive Detection of Influenza Viruses with a Glycan-Based Impedimetric Biosensor. *Biosens. Bioelectron.* **2016**, *79*, 644−649.
(75) Sayhi, M.; Ouerghi, O.; Belgacem, K.; Arbi, M.; Tepeli, Y.; Ghram, A.; Anik, Ü.; Österlund, L.; Laouini, D.; Diouani, M. F. Electrochemical Detection of Influenza Virus H9N2 Based on Both Immunomagnetic Extraction and Gold Catalysis Using an Immobilization-Free Screen Printed Carbon Microelectrode. *Biosens. Bioelectron.* **2018**, *107*, 170−177.
(76) Jahanshahi, P.; Zalnezhad, E.; Sekaran, S. D.; Adikan, F. R. M. Rapid Immunoglobulin M-Based Dengue Diagnostic Test Using Surface Plasmon Resonance Biosensor. *Sci. Rep.* **2015**, *4*, 1−7.
(77) Petrosova, A.; Konry, T.; Cosnier, S.; Trakht, I.; Lutwama, J.; Rwaguma, E.; Chepurnov, A.; Mühlberger, E.; Lobel, L.; Marks, R. S. Development of a Highly Sensitive, Field Operable Biosensor for Serological Studies of Ebola Virus in Central Africa. *Sens. Actuators, B* **2007**, *122* (2), 578−586.
(78) Ng, V L Serological Diagnosis with Recombinant Peptides/Proteins. *Clin. Chem.* **1991**, *37*, 1667−1668.
(79) Balamurugan, V.; Venkatesan, G.; Sen, A.; Annamalai, L.; Bhanuprakash, V.; Singh, R. K. Recombinant Protein-Based Viral Disease Diagnostics in Veterinary Medicine. *Expert Rev. Mol. Diagn.* **2010**, *10* (6), 731−753.
(80) Cabral-Miranda, G.; Cardoso, A. R.; Ferreira, L. C. S.; Sales, M. G. F.; Bachmann, M. F. Biosensor-Based Selective Detection of Zika Virus Specific Antibodies in Infected Individuals. *Biosens. Bioelectron.* **2018**, *113*, 101−107.
(81) Zhao, N.; Grund, C.; Beer, M.; Harder, T. C. Engineered Recombinant Protein Products of the Avian Paramyxovirus Type-1 Nucleocapsid and Phosphoprotein Genes for Serological Diagnosis. *Virol. J.* **2018**, *15* (1), 8.
(82) Kumbhat, S.; Sharma, K.; Gehlot, R.; Solanki, A.; Joshi, V. Surface Plasmon Resonance Based Immunosensor for Serological Diagnosis of Dengue Virus Infection. *J. Pharm. Biomed. Anal.* **2010**, *52* (2), 255−259.
(83) Vaisocherová, H.; Mrkvová, K.; Piliarik, M.; Jinoch, P.; Šteinbachová, M.; Homola, J. Surface Plasmon Resonance Biosensor for Direct Detection of Antibody against Epstein-Barr Virus. *Biosens. Bioelectron.* **2007**, *22* (6), 1020−1026.
(84) Rashid, J. I. A.; Yusof, N. A. The Strategies of DNA Immobilization and Hybridization Detection Mechanism in the Construction of Electrochemical DNA Sensor: A Review. *Sensing and Bio-Sensing Research* **2017**, *16*, 19−31.
(85) Oliverio, M.; Perotto, S.; Messina, G. C.; Lovato, L.; De Angelis, F. Chemical Functionalization of Plasmonic Surface Biosensors: A Tutorial Review on Issues, Strategies, and Costs. *ACS Appl. Mater. Interfaces* **2017**, *9* (35), 29394−29411.
(86) Bañuls, M. J.; Puchades, R.; Maquieira, Á. Chemical Surface Modifications for the Development of Silicon-Based Label-Free Integrated Optical (IO) Biosensors: A Review. *Anal. Chim. Acta* **2013**, *777*, 1−16.
(87) Escorihuela, J.; Bañuls, M. J.; Castelló, J. G.; Toccafondo, V.; García-Rupérez, J.; Puchades, R.; Maquieira, Á. Chemical Silicon Surface Modification and Bioreceptor Attachment to Develop Competitive Integrated Photonic Biosensors. *Anal. Bioanal. Chem.* **2012**, *404* (10), 2831−2840.
(88) Asal, M.; Özen, Ö.; Şahinler, M.; Baysal, H. T.; Polatoğlu, Ì An Overview of Biomolecules, Immobilization Methods and Support Materials of Biosensors. *Sensor Review*. Emerald Group Publishing Ltd. May 2019; pp 377−386.
(89) Dugas, V.; Elaissari, A.; Chevalier, Y. Surface Sensitization Techniques and Recognition Receptors Immobilization on Biosensors and Microarrays. In *Recognition Receptors in Biosensors*; Springer: New York, 2010; pp 47−134.
(90) Smith, C. L.; Milea, J. S.; Nguyen, G. H. Immobilization of Nucleic Acids Using Biotin-Strept(Avidin) Systens. *Topics in Current Chemistry* **2006**, *261*, 63−90.
(91) Liu, Y.; Yu, J. Oriented Immobilization of Proteins on Solid Supports for Use in Biosensors and Biochips: A Review. *Microchim. Acta* **2016**, *183*, 1−19.
(92) Vaisocherová, H.; Brynda, E.; Homola, J. Functionalizable Low-Fouling Coatings for Label-Free Biosensing in Complex Biological Media: Advances and Applications. *Anal. Bioanal. Chem.* **2015**, *407* (14), 3927−3953.

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

(93) Liu, B.; Liu, X.; Shi, S.; Huang, R.; Su, R.; Qi, W.; He, Z. Design and Mechanisms of Antifouling Materials for Surface Plasmon Resonance Sensors. *Acta Biomater.* **2016**, *40*, 100−118.
(94) Inci, F.; Tokel, O.; Wang, S.; Gurkan, U. A.; Tasoglu, S.; Kuritzkes, D. R.; Demirci, U. Nanoplasmonic Quantitative Detection of Intact Viruses from Unprocessed Whole Blood. *ACS Nano* **2013**, *7* (6), 4733−4745.
(95) Lifson, M. A.; Ozen, M. O.; Inci, F.; Wang, S. Q.; Inan, H.; Baday, M.; Henrich, T. J.; Demirci, U. Advances in Biosensing Strategies for HIV-1 Detection, Diagnosis, and Therapeutic Monitoring. *Adv. Drug Delivery Rev.* **2016**, *103*, 90−104.
(96) Inci, F.; Filippini, C.; Baday, M.; Ozen, M. O.; Calamak, S.; Durmus, N. G.; Wang, S. Q.; Hanhauser, E.; Hobbs, K. S.; Juillard, F.; et al. Multitarget, Quantitative Nanoplasmonic Electrical Field-Enhanced Resonating Device (NE2RD) for Diagnostics. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112* (32), E4354−E4363.
(97) Leonardi, A. A.; Lo Faro, M. J.; Petralia, S.; Fazio, B.; Musumeci, P.; Conoci, S.; Irrera, A.; Priolo, F. Ultrasensitive Label- and PCR-Free Genome Detection Based on Cooperative Hybridization of Silicon Nanowires Optical Biosensors. *ACS Sensors* **2018**, *3* (9), 1690−1697.
(98) 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*, 5268.
(99) Koo, B.; Jin, C. E.; Lee, T. Y.; Lee, J. H.; Park, M. K.; Sung, H.; Park, S. Y.; Lee, H. J.; Kim, S. M.; Kim, J. Y.; et al. An Isothermal, Label-Free, and Rapid One-Step RNA Amplification/Detection Assay for Diagnosis of Respiratory Viral Infections. *Biosens. Bioelectron.* **2017**, *90*, 187−194.
(100) Shi, L.; Sun, Q.; He, J.; Xu, H.; Liu, C.; Zhao, C.; Xu, Y.; Wu, C.; Xiang, J.; Gu, D.; et al. Development of SPR Biosensor for Simultaneous Detection of Multiplex Respiratory Viruses. *Bio-Med. Mater. Eng.* **2015**, *26*, S2207−S2216.
(101) Schofield, D. J.; Dimmock, N. J. Determination of Affinities of a Panel of IgGs and Fabs for Whole Enveloped (Influenza A) Virions Using Surface Plasmon Resonance. *J. Virol. Methods* **1996**, *62* (1), 33−42.
(102) Ravina; Dalal, A.; Mohan, H.; Prasad, M.; Pundir, C.S. Detection Methods for Influenza A H1N1 Virus with Special Reference to Biosensors: A Review. *Biosci. Rep.* **2020**, 1 DOI: 10.1042/BSR20193852.
(103) Zhao, X.; Tsao, Y. C.; Lee, F. J.; Tsai, W. H.; Wang, C. H.; Chuang, T. L.; Wu, M. S.; Lin, C. W. Optical Fiber Sensor Based on Surface Plasmon Resonance for Rapid Detection of Avian Influenza Virus Subtype H6: Initial Studies. *J. Virol. Methods* **2016**, *233*, 15−22.
(104) Chang, Y. F.; Wang, W. H.; Hong, Y. W.; Yuan, R. Y.; Chen, K. H.; Huang, Y. W.; Lu, P. L.; Chen, Y. H.; Chen, Y. M. A.; Su, L. C.; et al. Simple Strategy for Rapid and Sensitive Detection of Avian Influenza A H7N9 Virus Based on Intensity-Modulated SPR Biosensor and New Generated Antibody. *Anal. Chem.* **2018**, *90* (3), 1861−1869.
(105) Prabowo, B. A.; Wang, R. Y. L.; Secario, M. K.; Ou, P. T.; Alom, A.; Liu, J. J.; Liu, K. C. Rapid Detection and Quantification of Enterovirus 71 by a Portable Surface Plasmon Resonance Biosensor. *Biosens. Bioelectron.* **2017**, *92*, 186−191.
(106) Xu, J.; Suarez, D.; Gottfried, D. S. Detection of Avian Influenza Virus Using an Interferometric Biosensor. *Anal. Bioanal. Chem.* **2007**, *389* (4), 1193−1199.
(107) Lee, W.; Kang, T.; Kim, S.-H.; Jeong, J. An Antibody-Immobilized Silica Inverse Opal Nanostructure for Label-Free Optical Biosensors. *Sensors* **2018**, *18* (1), 307.
(108) Endo, T.; Ozawa, S.; Okuda, N.; Yanagida, Y.; Tanaka, S.; Hatsuzawa, T. Reflectometric Detection of Influenza Virus in Human Saliva Using Nanoimprint Lithography-Based Flexible Two-Dimensional Photonic Crystal Biosensor. *Sens. Actuators, B* **2010**, *148* (1), 269−276.
(109) Sakamoto, H.; Minpou, Y.; Sawai, T.; Enami, Y.; Suye, S.-I. A Novel Optical Biosensing System Using Mach-Zehnder-Type Optical Waveguide for Influenza Virus Detection. *Appl. Biochem. Biotechnol.* **2016**, *178* (4), 687−694.
(110) Combating 2019-nCoV: Advanced Nanobiosensing platforms for POC global diagnostics and surveillance | CoNVat Project | H2020 | CORDIS | European Commission; https://cordis.europa.eu/project/id/101003544.
(111) Ahmadivand, A.; Gerislioglu, B.; Ramezani, Z.; Kaushik, A.; Manickam, P.; Ghoreishi, S. A. Femtomolar-Level Detection of SARS-CoV-2 Spike Proteins Using Toroidal Plasmonic Metasensors. *arXiv:2006.08536 (physics)*, 15 Jun 2020.
(112) Zhang, D.; Zhang, X.; Ma, R.; Deng, S.; Wang, X.; Zhang, X.; Huang, X.; Liu, Y.; Li, G.; Qu, J.; et al. Ultra-Fast and Onsite Interrogation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in Environmental Specimens via Surface Enhanced Raman Scattering (SERS). *medRxiv*, May 6, 2020. DOI: 10.1101/2020.05.02.20086876.
(113) Zhang, H.; Miller, B. L. Immunosensor-Based Label-Free and Multiplex Detection of Influenza Viruses: State of the Art. *Biosens. Bioelectron.* **2019**, *141*, 111476.
(114) Wong, C. L.; Chua, M.; Mittman, H.; Choo, L. X.; Lim, H. Q.; Olivo, M. A Phase-Intensity Surface Plasmon Resonance Biosensor for Avian Influenza A (H5N1) Detection. *Sensors* **2017**, *17* (10), 2363.
(115) Shaw, A. M.; Hyde, C.; Merrick, B.; James-Pemberton, P.; Squires, B. K.; Olkhov, R. V.; Batra, R.; Patel, A.; Bisnauthsing, K.; Nebbia, G. Real-World Evaluation of a Novel Technology for Quantitative Simultaneous Antibody Detection against Multiple SARS-CoV-2 Antigens in a Cohort of Patients Presenting with COVID-19 Syndrome. *Analyst* **2020**, *145*, 5638.
(116) Abdelhadi, D.; Benjamin, C.; Maryam Hojjat, J.; Vincent, T.; Julien, C.; Keisean, S.; Simon, F.; Ludovic, S. L.; Denis, B.; Joelle, N. P. A Rapid and Quantitative Serum Test for SARS-CoV-2 Antibodies with Portable Surface Plasmon Resonance Sensing. *chemRxiv*. 15 April 2020.
(117) Kulp, D. W.; Walker, S.; Chokkalingam, N.; Reuschel, E. L.; Purwar, M.; Xu, Z.; Gary, E. Y.; Kim, K. Y.; Schultheis, K.; Walters, J.; et al. SARS-CoV-2 Assays to Detect Functional Antibody Responses That Block ACE2 Recognition in Vaccinated Animals and Infected Patients. *bioRxiv*, 2020.06.17.158527.
(118) Fuentes, S.; Klenow, L.; Golding, H.; Khurana, S. Preclinical Evaluation of Bacterially Produced RSV-G Protein Vaccine: Strong Protection against RSV Challenge in Cotton Rat Model. *Sci. Rep.* **2017**, *7* (1), 1−13.
(119) Marsh, R.; Connor, A.; Gias, E.; Toms, G. L. Increased Susceptibility of Human Respiratory Syncytial Virus to Neutralization by Anti-Fusion Protein Antibodies on Adaptation to Replication in Cell Culture. *J. Med. Virol.* **2007**, *79* (6), 829−837.
(120) Gauger, P. C.; Loving, C. L.; Khurana, S.; Lorusso, A.; Perez, D. R.; Kehrli, M. E.; Roth, J. A.; Golding, H.; Vincent, A. L. Live Attenuated Influenza A Virus Vaccine Protects against A(H1N1)-Pdm09 Heterologous Challenge without Vaccine Associated Enhanced Respiratory Disease. *Virology* **2014**, *471*−*473*, 93−104.
(121) Zhang, H.; Henry, C.; Anderson, C. S.; Nogales, A.; Dediego, M. L.; Bucukovski, J.; Martinez-Sobrido, L.; Wilson, P. C.; Topham, D. J.; Miller, B. L. Crowd on a Chip: Label-Free Human Monoclonal Antibody Arrays for Serotyping Influenza. *Anal. Chem.* **2018**, *90* (15), 9583−9590.
(122) Estmer Nilsson, C.; Abbas, S.; Bennemo, M.; Larsson, A.; Hämäläinen, M. D.; Frostell-Karlsson, Å. A Novel Assay for Influenza Virus Quantification Using Surface Plasmon Resonance. *Vaccine* **2010**, *28* (3), 759−766.
(123) Vidic, J.; Noiray, M.; Bagchi, A.; Slama-Schwok, A. Identification of a Novel Complex between the Nucleoprotein and PA(1−27) of Influenza A Virus Polymerase. *Biochemistry* **2016**, *55* (31), 4259−4262.
(124) Xiong, X.; Martin, S. R.; Haire, L. F.; Wharton, S. A.; Daniels, R. S.; Bennett, M. S.; McCauley, J. W.; Collins, P. J.; Walker, P. A.; Skehel, J. J.; et al. Receptor Binding by an H7N9 Influenza Virus from Humans. *Nature* **2013**, *499* (7459), 496−499.
(125) Huang, C.; Qi, J.; Lu, G.; Wang, Q.; Yuan, Y.; Wu, Y.; Zhang, Y.; Yan, J.; Gao, G. F. Putative Receptor Binding Domain of Bat-Derived Coronavirus HKU9 Spike Protein: Evolution of Betacoronavirus Receptor Binding Motifs. *Biochemistry* **2016**, *55* (43), 5977−5988.

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX

(126) Park, J. Y.; Yuk, H. J.; Ryu, H. W.; Lim, S. H.; Kim, K. S.; Park, K. H.; Ryu, Y. B.; Lee, W. S. Evaluation of Polyphenols from Broussonetia Papyrifera as Coronavirus Protease Inhibitors. *J. Enzyme Inhib. Med. Chem.* **2017**, *32* (1), 504−512.
(127) Geng, Z.; Zhang, X.; Fan, Z.; Lv, X.; Su, Y.; Chen, H. Recent Progress in Optical Biosensors Based on Smartphone Platforms. *Sensors* **2017**, *17* (11), 2449.
(128) Huang, X.; Xu, D.; Chen, J.; Liu, J.; Li, Y.; Song, J.; Ma, X.; Guo, J. Smartphone-Based Analytical Biosensors. *Analyst* **2018**, *143* (22), 5339−5351.
(129) Kim, D. W.; Jeong, K. Y.; Yoon, H. C. *Smartphone-Based Medical Diagnostics with Microfluidic Devices*; Academic Press, 2020; Chapter 7, pp 103−128.
(130) Guner, H.; Ozgur, E.; Kokturk, G.; Celik, M.; Esen, E.; Topal, A. E.; Ayas, S.; Uludag, Y.; Elbuken, C.; Dana, A. A Smartphone Based Surface Plasmon Resonance Imaging (SPRi) Platform for on-Site Biodetection. *Sens. Actuators, B* **2017**, *239*, 571−577.
(131) McGonigle, A.; Wilkes, T.; Pering, T.; Willmott, J.; Cook, J.; Mims, F.; Parisi, A. Smartphone Spectrometers. *Sensors* **2018**, *18* (2), 223.
(132) Lertvachirapaiboon, C.; Baba, A.; Shinbo, K.; Kato, K. A Smartphone-Based Surface Plasmon Resonance Platform. *Anal. Methods* **2018**, *10* (39), 4732−4740.
(133) Fan, Z.; Geng, Z.; Fang, W.; Lv, X.; Su, Y.; Wang, S.; Chen, H. Smartphone Biosensor System with Multi-Testing Unit Based on Localized Surface Plasmon Resonance Integrated with Microfluidics Chip. *Sensors* **2020**, *20* (2), 446.
(134) Oh, B. R.; Huang, N. T.; Chen, W.; Seo, J. H.; Chen, P.; Cornell, T. T.; Shanley, T. P.; Fu, J.; Kurabayashi, K. Integrated Nanoplasmonic Sensing for Cellular Functional Immunoanalysis Using Human Blood. *ACS Nano* **2014**, *8* (3), 2667−2676.
(135) Choi, S.; Goryll, M.; Sin, L. Y. M.; Wong, P. K.; Chae, J. Microfluidic-Based Biosensors toward Point-of-Care Detection of Nucleic Acids and Proteins. *Microfluid. Nanofluid.* **2011**, *10* (2), 231−247.
(136) Bates, K. E.; Lu, H. Optics-Integrated Microfluidic Platforms for Biomolecular Analyses. *Biophys. J.* **2016**, *110* (8), 1684−1697.
(137) Sonker, M.; Sahore, V.; Woolley, A. T. Recent Advances in Microfluidic Sample Preparation and Separation Techniques for Molecular Biomarker Analysis: A Critical Review. *Anal. Chim. Acta* **2017**, *986*, 1−11.
(138) Cui, F.; Rhee, M.; Singh, A.; Tripathi, A. Microfluidic Sample Preparation for Medical Diagnostics. *Annu. Rev. Biomed. Eng.* **2015**, *17* (1), 267−286.
(139) Jin, C. E.; Lee, T. Y.; Koo, B.; Sung, H.; Kim, S. H.; Shin, Y. Rapid Virus Diagnostic System Using Bio-Optical Sensor and Microfluidic Sample Processing. *Sens. Actuators, B* **2018**, *255*, 2399−2406.
(140) Mielczarek, W. S.; Obaje, E. A.; Bachmann, T. T.; Kersaudy-Kerhoas, M. Microfluidic Blood Plasma Separation for Medical Diagnostics: Is It Worth It? *Lab Chip* **2016**, *16* (18), 3441−3448.
(141) Kuo, J.-N.; Lin, B.-Y. Microfluidic Blood-Plasma Separation Chip Using Channel Size Filtration Effect. *Microsyst. Technol.* **2018**, *24* (4), 2063−2070.
(142) Fan, R.; Vermesh, O.; Srivastava, A.; Yen, B. K. H.; Qin, L.; Ahmad, H.; Kwong, G. A.; Liu, C. C.; Gould, J.; Hood, L.; et al. Integrated Barcode Chips for Rapid, Multiplexed Analysis of Proteins in Microliter Quantities of Blood. *Nat. Biotechnol.* **2008**, *26* (12), 1373−1378.
(143) Herr, A. E.; Hatch, A. V.; Giannobile, W. V.; Throckmorton, D. J.; Tran, H. M.; Brennan, J. S.; Singh, A. K. Integrated Microfluidic Platform for Oral Diagnostics. *Ann. N. Y. Acad. Sci.* **2007**, *1098* (1), 362−374.
(144) Cai, H.; Parks, J. W.; Wall, T. A.; Stott, M. A.; Stambaugh, A.; Alfson, K.; Griffiths, A.; Mathies, R. A.; Carrion, R.; Patterson, J. L.; et al. Optofluidic Analysis System for Amplification-Free, Direct Detection of Ebola Infection. *Sci. Rep.* **2015**, *5* (1), 14494.
(145) Szydzik, C.; Gavela, A. F.; Herranz, S.; Roccisano, J.; Knoerzer, M.; Thurgood, P.; Khoshmanesh, K.; Mitchell, A.; Lechuga, L. M. An Automated Optofluidic Biosensor Platform Combining Interferometric Sensors and Injection Moulded Microfluidics. *Lab Chip* **2017**, *17* (16), 2793−2804.
(146) Schudel, B. R.; Choi, C. J.; Cunningham, B. T.; Kenis, P. J. A. Microfluidic Chip for Combinatorial Mixing and Screening of Assays. *Lab Chip* **2009**, *9* (12), 1676.
(147) Choi, K.; Ng, A. H. C.; Fobel, R.; Wheeler, A. R. Digital Microfluidics. *Annu. Rev. Anal. Chem.* **2012**, *5* (1), 413−440.
(148) Luka, G.; Ahmadi, A.; Najjaran, H.; Alocilja, E.; DeRosa, M.; Wolthers, K.; Malki, A.; Aziz, H.; Althani, A.; Hoorfar, M. Microfluidics Integrated Biosensors: A Leading Technology towards Lab-on-a-Chip and Sensing Applications. *Sensors* **2015**, *15* (12), 30011−30031.
(149) Lerma Arce, C.; Witters, D.; Puers, R.; Lammertyn, J.; Bienstman, P. Silicon Photonic Sensors Incorporated in a Digital Microfluidic System. *Anal. Bioanal. Chem.* **2012**, *404* (10), 2887−2894.

https://dx.doi.org/10.1021/acssensors.0c01180
ACS Sens. XXXX, XXX, XXX−XXX