Understanding viruses and viral infections by biophotonic methods

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Abstract
In the last few decades outbreaks of viral infections have often challenged the world-wide health infrastructure and caused a significant financial burden as well as human suffering despite progress in diagnostic technologies. The recent outbreaks of the Ebola virus in the African continent, the Zika virus in the American continent, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), influenza A and lately severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral infections have repeatedly highlighted the importance of technological advancement enabling a better understanding of virions. In this review, we systematically discuss different aspects of virions and how their properties and functions can be studied using different light-based technologies. We focus on virion classification, detection and interactions with the host's immune system. Further, the potential of advanced biophotonic methods, for example, Raman, infrared reflection, absorption and fluorescence spectroscopy, advanced microscopic techniques and biosensor-based approaches for diagnosing viral infections, investigating therapeutics and vaccine development are described. Although significant advancements have already been made in photonic technologies, which even enable visualizing virion-host interactions on single-cell level, the continuous evolution of viruses demands further progress in biophotonic solutions for fast, affordable and robust health monitoring devices for screening viral infections.
1 | INTRODUCTION

For decades humans have been struggling with infectious diseases leading to life-threatening severe conditions such as sepsis, which is the result of a dysregulated host response and the effects of the pathogen itself. The most recent pandemic, caused by SARS-CoV-2, has once again highlighted the need for robust technological platforms enabling continuous monitoring of the spread of infectious diseases among the population. For this purpose, fast and easy-to-apply on-site assays are required. On the other hand, more sophisticated methods capable of retrieving in-depth information on the virus are equally important to improve the understanding of viral infections. Both the aforementioned fast and high-end approaches are necessary to effectively study the proliferation of highly virulent viruses and to develop potent drugs and vaccines. Light-based technologies have proven to be very promising in the field of infectious disease diagnostics and have been explored to understand the structure and composition of the virus enabling deeper insights into virion built up. State-of-the-art microscopy techniques have the capability to observe interactions between viruses and their host’s immune cells, capture evasive mechanisms employed by the virus and trigger of different hosts’ immune responses. Further, biophotonic methods such as vibrational spectroscopy, atomic force microscopy, and so on, have enabled to apprehend the overall host response thus allowing the diagnosis of infectious diseases and detecting the causative agents [1–8].

In this review, we provide an overview on how photonics-based technologies can contribute to understand the viral infections. Key aspects are viral classification, detection, virus-host interactions and identifying potential targets for diagnostics and therapeutics and vaccine development.

2 | VIRAL CLASSIFICATION

Viruses can be classified based on morphology, chemical composition and mode of replication [9–11]. In terms of chemical composition, the nucleic acids (the genome) and the proteins are the major components of the viral particles [11]. In addition, lipids and carbohydrates in glycoprotein peplomers are found in all enveloped viruses [12, 13]. Viral nucleic acid, that is, deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), can be double-stranded.
(ds) or single-stranded (ss) [11, 12, 14]. In Figure 1 a brief overview of virion classification according to their physical and chemical properties is presented.

An important aspect of studying and classifying viruses is that they need host cells for replicating. To propagate them and for performing further analysis on them it is necessary to introduce them into suitable host cell lines. This is termed virus isolation [15]. However, highly sensitive detection methods, such as polymerase chain reaction (PCR) and immunoassays, also allow studying viruses without the need for prior isolation. Such techniques are referred to as “direct” detection methods, while approaches involving virus culture are categorized as “indirect” methods [15]. For characterizing the physical properties of viruses, such as shape, size and chemical composition a wide range of analytical methods is available. Two relevant and frequently used techniques entail mass spectrometry (MS) [16] for retrieving information on their chemical composition and electron microscopy (EM) [17] (i.e., cryo-EM and negative stain transmission EM) for elucidating their morphology. Kondylis et al. have summarized the current state of available methods for capsid characterization in their review article [18]. A key criterion in classifying viruses is the makeup of their genetic material. While cellular organisms all feature double-stranded DNA, viruses display an astonishing diversity of nucleic acids [19]. O’Carroll et al. provide a comprehensive overview article on viral nucleic acids [14]. Before the nucleic acids can be analyzed, the virus material may need to be enriched and purified, that is, using centrifugation. Subsequently, the nucleic acids can be extracted and separated using electrophoresis [20]. This allows determining their genome size and structure. Analysis of the viral proteins is another important step, as it enables, for example, identifying the receptor which the virus uses for entering the host cells [21]. Nowadays, new viruses are often classified solely relying on genome sequencing, which comes along with its challenges [22]. The interested reader can find further information on virus classification and the current issue of taxonomy using the following resources by Louten et al., Simmonds et al., Adams et al. and DeLong et al. [9, 22–24].

Light-based techniques allow studying the biochemical properties either in a labeled or label-free manner (Figure 2) [25–28]. Optical tweezers have been used to investigate dynamic processes in viral particles and to quantify in real-time the DNA packaging in bacteriophage capsids [29–31]. Furthermore, optical tweezers enable determining physical forces involved in the action of molecular motors during the mechanochemical process of dsDNA encapsulation inside a preformed bacteriophage capsid [32]. Hou et al. showed that combining epifluorescence imaging with optical trapping is used to monitor the attachment and dissociation events of single manipulated virions attached to the host cell surface [32]. Pang et al. previously employed such a technique to study individual human immunodeficiency virus 1 (HIV-1) viruses which can be optically trapped and manipulated, allowing multiparameter analysis of single virions in culture fluid under native conditions [33]. Sieben et al. showed, in combination with atomic force microscopy, optical trapping can enable monitoring the multivalent binding between virus and cell membrane [34].

3 VIRUS DETECTION

PCR-based methods are considered to be the current gold standard for virus detection [37, 38]. Due to the amplification of specific nucleic acid sequences, viruses can be identified in a sensitive and highly specific manner [38, 39]. By employing certain fluorescent dyes (or pairing them with quenchers) even quantification is possible [40]. As PCR-based methods are prone to contamination [41] and usually require a specialized laboratory as well as trained personnel, for on-site testing immunoassays are preferred, which rely on the highly specific interaction between antibodies and their antigens [42]. Immunoassays can be performed in different formats [15]. While some also need to be conducted within a laboratory, lateral flow tests are ideal for point-of-care testing since little to no sample preparation is necessary and the result can be directly read by the naked eye. However, in comparison to PCR-based methods, immunoassays are often less sensitive [15]. Despite the advantages of these widely applied methods, there still is a need for researching alternatives, both for on-site and inside the lab application, with the goal in mind to improve specificity and sensitivity, increase speed, lower the costs and gain further insights into viral infections [38]. Especially within the last 2 years, fueled by the progression of the coronavirus disease 2019 (COVID-19) pandemic, tremendous efforts have been made by researchers to explore the potential of their methods for virus diagnostics. Many highly useful reviews with different core themes (specific methodologies, sample matrices and material types) already have been published. We have summarized some of the most recent articles in Table S1.

Within this section, we will focus specifically on how biophotonic methods can contribute to detecting viral infections. Beforehand, it is helpful to become aware of the different general possibilities (see overview provided in Figure 3) to verify the presence of the virus. The most obvious strategy is to detect the virus itself directly. Here, either the whole virus or specific parts (a nucleic acid sequence or certain proteins) can be
addressed. An important prerequisite for such approaches is a suitable sample preparation strategy as the virus will be concealed in a more or less complex sample matrix. Alternatively, viral infections can also be diagnosed indirectly by focusing on the response of the hosts’ immune system. The most obvious choice here involves detecting the antibodies, that are produced as a defense mechanism, partially to neutralize the virus particles. Depending on the stage of the infection different classes of antibodies (also termed immune globulins [Ig]) such as IgG, IgA or IgM will be present (predominantly) in the blood. Further examples of biomolecules that are secreted as part of the immune response are inflammasomes [43] (protein complexes) and cytokines [44] (small proteins), for example, tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) or interferon γ (IFN-γ). Such biomarkers are as well important components for understanding viral infections [45–47]. Furthermore, it is also a feasible strategy to investigate the overall change of a sample, such as tissue, cells or a body fluid due to a viral infection instead of identifying a specific structure. In the following, we will discuss the aforementioned basic approaches along with selected examples for application.
### Key factors for virus detection

| Sample type       | Target                  | Sample preparation                  | Detection method                                      |
|-------------------|-------------------------|-------------------------------------|-------------------------------------------------------|
| Air, water, food  | Whole sample            | Centrifugation, ultracentrifugation | Spectroscopy, fluorescence, UV-Vis, IR, Terahertz     |
|                   | detect overall changes  |                                     | RAMAN, Raman, Resonance RAMAN, CARS                   |
| Leaf, plant material | Whole virus             | Filtration, ultrafiltration         | Microscopy fluorescence, IR                           |
| Cell, tissue      | Viral protein           | Immunomagnetic capture              | Raman, super resolution, electron microscopy          |
| Body fluids: blood, urine, sputum, saliva | Nucleic acid: DNA, RNA | Chip- or surface-based enrichment | Label, recognition element vs label-free               |
|                   | Antibody (host)         |                                    | Direct or indirect                                    |
|                   | Cytokines (host)        |                                    |                                                       |

**FIGURE 3** (A) Overview of different aspects relevant for detection of viral infection and possible detection methods. (B) Visualization of possible targets in samples that can be addressed to detect viral infections.

### Detection of whole virus particles

Detecting whole virus particles can be pursued with different objectives in mind. For diagnosing viral infections, the verification of the mere presence of a specific virus is usually sufficient. For many researchers, however, gaining a deeper understanding of the viruses’ structure and properties is the main focus. For the latter, microscopic techniques can deliver an important contribution. Due to the small size of virus particles, conventional optical microscopy has only limited potential, and approaches capable of higher resolution have to be applied [4]. EM provides a nanometer-scale resolution and enables direct visualization of virus particles (Figure 4). Despite not being frequently used in routine diagnostics anymore, EM techniques undoubtedly have their merits and are especially valuable in terms of elucidating virus morphology and investigating samples with unknown content [48]. Technological advancements in the field have enabled studying interactions between viruses and their hosts and
identifying functional features [17]. While initially EM was the method of choice for studying virus particles, over the last two decades the trend has shifted toward light microscopy (LM) techniques due to the tremendous improvements regarding resolution, sensitivity and also data analysis. Witte et al. and Parveen et al. give detailed information on the different emerging technologies in their comprehensive reviews [49, 50]. Another interesting possibility within this context is interferometric light microscopy (ILM) [51]. This technique can be applied for

**FIGURE 4** In cellulo focused ion beam scanning electron microscopy (FIB-SEM) and electron tomography images of an infected cell. (A) Schematic diagram showing SARS-CoV-2 infection of lung epithelial cells. (B) FIB-SEM analysis of infected Calu-3 cells (multiplicity of infection [MOI]=5) for 24 hours, two different slices through whole-cell volume, (C) 3D visualization of the infected cell (color code is shown at the bottom), (D) the rectangle in (A) and (C), cluster of double-membrane vesicles (DMV) in red (see inset in C) are highlighted. (E-J) Electron tomography images give insights into spatial coupling of SARS-CoV-2 replication and assembly site mediated by the close proximity of DMVs, vesicular-tubular compartment and golgi apparatus, (E) slice through the tomogram, (F) the same region as (A) with superimposed visualization of cellular and viral organelles (color coding as shown at the bottom), (G) 3D visualization of the organelles shown in (E). (H-J) Zoomed view of the vesicular-tubular compartment (VTC; cyan) and golgi apparatus (dark blue) with budding virion (yellow) and fully assembled virions (orange). Scale bar 200 nm. Reprinted (adapted) with permission from Ref. [54] with copyright by Elsevier and Copyright Clearance Center.
differentiating virus particles from other nanometer-sized objects and is also highly useful for determining the virus particle concentration [52, 53].

In contrast to EM and LM, where the image is generated through lenses, scanning probe microscopy (SPM) characterizes surfaces by employing a physical probe. Depending on the specific technique used, atomic resolution can be achieved [55]. SPM can be combined with spectroscopic techniques, as in atomic force microscopy-infrared (AFM-IR) spectroscopy, enabling both a high spatial resolution and access to chemical information on the sample. Dou et al. have employed AFM-IR spectroscopy for retrieving structural characteristics of herpes simplex type 1 viruses (HSV-1) and bacteriophage MS2 [4]. The two virus types can be clearly distinguished by means of the single AFM-IR spectra using partial least-squares discriminant analysis (PLS-DA; Figure 5) [4]. They further applied tip enhanced Raman scattering (TERS) to determine the secondary protein structure of the virions [4]. Due to the different penetration depths and operating principles, complementary information is provided. TERS has a penetration depth of approximately 1 nm, while for AFM-IR it is several hundreds of nanometers [56]. Both AFM-IR and TERS fall into the category of vibrational spectroscopy, yielding highly specific information on molecular structure [57]. However, TERS is based on the Raman effect, that is the inelastic scattering of photons due to changes in polarizability, whereas IR spectroscopy requires changes in the dipole moment during molecular vibrations resulting in the absorption of IR light [4].

A related technique for characterizing virus particles is mid-infrared photothermal (MIP) imaging. The IR absorption of molecules is also probed based on the photothermal effect (Figure 6A). In contrast to AFM-IR spectroscopy, MIP imaging is a contact-free approach, which minimizes the risk of damaging the sample during measurements [58].

Zhang et al. were able to record the vibrational fingerprint of single virions (Figure 6B-E) by exploiting the interferometric scattering principle in confocal

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**Figure 5** (A) Schematic representation of atomic force microscopy-infrared (AFM-IR) and tip-enhanced Raman scattering (TERS) technique for virion characterization, (B) averaged AFM-IR spectra collected from virions MS2 (red) and HSV-1 (blue), (C) The loadings plot and (D) the variant component plot of partial least square discriminant analysis model used for differentiation of AFM-IR spectra collected from MS2 and HSV-1 virions. Stars indicate spectral artifacts. Reprinted (adapted) with permission from Ref. [4] with Copyright (2020) by American Chemical Society.
configuration [58]. In contrast to TERS, no plasmonic enhancement using nanoparticles or nanostructured substrates is necessary.

While the application of plasmonic substrates can potentially introduce some issues regarding reproducibility [59], their usefulness especially in terms of Raman spectroscopic detection of viruses is unquestioned. Similar to TERS, surface-enhanced Raman scattering (SERS) takes the advantage of electromagnetic field enhancement at the surface due to plasmonic effects of the surface of nanostructured metallic particles [60, 61]. SERS enables enhancing the inherently weak Raman signal by several orders of magnitude, thus allowing the detection of small entities such as viruses in the first place [62]. A difficult aspect of investigating viruses using a plasmonic substrate comes along from the nature of the so-called “hot spots,” in which the enhancement of the Raman signal takes place most efficiently [63]. Those regions are typically confined to areas with a diameter smaller than 10 nm [64]. Accordingly, not the whole virus particle but just certain features will contribute to the Raman spectrum (Figure 7A). Zhang et al. have developed a novel substrate, providing “volumetric hot spots” addressing this specific problem [65]. Their substrate consists of hexagonally arranged hollow nanocones located at the bottom of micro-bowls (HNCMB), in which many virus particles will fit well (Figure 7B). They successfully demonstrated that these HNCMB structures enable acquiring spectra of adenovirus type 5 (Ad5) particles with significantly improved reproducibility over conventional nanoparticle arrays [65].

Yeh et al. introduced a three-dimensional (3D) structure for simultaneously enriching, purifying and detecting virus particles [66]. They constructed a micro-fluidic platform with aligned nitrogen-doped carbon nanotube (CN/CNT) arrays, decorated with gold (Au) nanoparticles. The nanotube array functions as a size-selective filtration unit, while the Au nanoparticles allow acquiring characteristic SERS fingerprint spectra from the captured virus particles [66]. Yeh et al. challenged their system by investigating nasopharyngeal swab samples from patients diagnosed with three different respiratory viruses [66]. According to a previously established Raman database and a machine learning strategy, they were able to correctly identify the different virus species. A significant advantage over currently used assays is the speed of their method. Viral capture and detection were achieved within minutes [66].

While all the aforementioned SERS-based approaches within this section operated label-free and characterized the virus particles according to their Raman spectra, the SERS technique can also be combined with specific recognition elements for identifying viruses [67]. Despite the fact that enabling label-free identification is one major strength of Raman spectroscopic detection, in some cases, it can be advantageous to rely on another mechanism for specificity and just exploit the high sensitivity of SERS. For identification of pathogens according to their specific
Raman spectrum, it is generally required to establish a representative database beforehand [68]. Issues will most likely arise when samples are investigated, which contain species or strains not included in this database [69]. Relying on a specific capture probe bypasses this problem in a convenient way. Camacho et al. developed a core-shell nanoparticle functionalized with antibodies against the Zika virus nonstructural 1 (NS1) protein [70]. Nile blue was chosen as Raman reporter, because it has an absorption maximum around 633 nm excitation wavelength, which enables additional enhancement of the Raman signal due to the resonance effect along with the plasmonic enhancement, leading to sensitive detection of the virus (Figure 8) [70].

Next to antibodies, aptamers can also be applied as recognition elements. Aptamers are single-stranded oligonucleotides with nucleic acid sequences forming a 3D structure, which can specifically bind a target molecule [71]. Gribanyov et al. used an aptamer against hemagglutinin (HA) for SERS-based detection of the influenza A virus [72]. Chen et al. also introduced an aptamer-based strategy for detection of Influenza A targeting HA (Figure 9) [73]. In contrast to the previous approach, the aptamer was labeled with Cy3. The binding of the virus particles leads to a conformational change in the aptamer structure, resulting in a decrease of the SERS signal of Cy3 [73].

SERS can be enabled using nanoparticles, nanostructured surfaces or smooth metallic surfaces deposited on a highly refractive prism, the latter can also be used for surface plasmon resonance (SPR) sensing (Figure 10) [74]. SPR belongs to the family of optical biosensors, where a recognition element is combined with a transducer enabling highly specific and sensitive detection of the analyte (Figure 10A) [75]. In the case of SPR, the metal layer on the prism is functionalized with a sensor film, that is, consisting of antibodies (Figure 10B). The binding of the analyte leads to a small change in the refractive index of the sensor film. By exciting surface plasmons in the metal layer, those changes can be detected via reflection decrease. SPR is ideal for characterizing various biomolecular interactions, obtaining binding and dissociation constants and investigating reaction kinetics [76]. Due to its high sensitivity toward binding events, SPR is also highly useful for detecting virus particles by immobilizing specific antibodies or receptors on the sensor surface (Figure 10C) [77]. In a recent review article by Singh, the role of SPR in viral diagnostics is discussed in detail [78].

Another opportunity to detect viruses based on changes of the optical constants (i.e., complex refractive index or dielectric function) is Terahertz spectroscopy (Figure 11) [79]. Here, metamaterials are designed to enable absorption of radiation in the Terahertz frequency range [80]. Those materials usually feature slit-like structures, in which the presence of virus particles leads to a change in absorption frequency. Park et al. successfully demonstrated this concept with a split ring resonator for detecting bacteriophages [81].

### 3.2 Detection of viral load

The viral load is the number of viral particles found in a patient’s sample, mostly (parts of) blood or sputum. Commonly it is expressed as the concentration of nucleic acids per ml of sample. Determination of viral loads is often...
challenging due to the small size of the virus particle [82]. Therefore, it is difficult to isolate them from the sample fluid and as described above they are often bound to nanoparticles or antibodies for isolation and quantification. In clinical surroundings, standardized tests based on PCR methods are used for viral load determination [37, 39]. Since a higher viral load correlates with the severity of the infection, many different optical approaches—like fluorescence microscopy, vibrational spectroscopy—are being explored to detect viral particles [83]. For example, HIV particles were bound to the anti-gp120 antibody, and this combination was used to demonstrate the potential of fluorescence in monitoring the viral load of HIV [84]. Du et al. further presented a fluorescence quantitation study, where the integrated fluorescence signals showed a linear dependency on the concentration of Ebola virus RNA in blood in the range of 200 aM to 8 pM (Figure 12) [85].

As aforementioned, SERS-based techniques have enabled apart from viral particle detection also viral load quantification. For example, Xiao et al. developed a lateral flow test for detecting the avian influenza virus (AIV) by employing specific antibodies conjugated to Au/Ag core-shell nanoparticles decorated with a Raman dye [86]. While the presence of AIV can already be verified by the naked eye, SERS detection enabled

**FIGURE 8** Schematic illustration of Zika monoclonal antibodies (Zika-mAb) surface-enhanced Raman scattering (SERS) nanoprobe assembly. (A) Gold shell-isolated nanoparticles (Au-SHIN; ~100 nm Au core + 4 nm silica shell thickness), (B) Au-SHIN + nile blue (NB) Raman reporter layer, (C) Au-SHIN + NB Raman reporter layer + final ~10 nm silica shell (SERS nanoprobe), (D) conjugation onto Zika NS1 monoclonal antibodies (Zika-mAb), (E) SERS immunoassay platform for detecting different concentrations of Zika NS1. The platform is irradiated with a 633 nm laser line and the surface-enhanced resonance Raman scattering (SERRS) signal from NB molecules, located at a close distance of gold nanoparticles (~4 nm), are recorded by area mappings. Brighter spots indicate the higher intensity of the NB band at 593 cm$^{-1}$. Reprinted (adapted) with permission from Ref. [70] with Copyright (2018) by American Chemical Society.
Further, the limit-of-detection (LOD) for viral particles was determined via SERS using an in vitro blood spiking experiment. The resulting LOD curve was applied to detect and differentiate Hepatitis B and C infected patients from the healthy donors. Nasir et al. used a SERS-based approach to quantify viral loads of human papilloma virus (HPV) in blood samples by investigating the extracted RNA and Santos et al. quantified the viral load of dengue virus serotype 3 (DENV-3) in plasma and blood samples using ATR FTIR spectroscopy. A SERS-sensor including virus-traps in form of nano-forests of an oblique gold-nanoneedles array (GNA’s) coated with ACE-2 receptors allowed the detection of SARS-CoV-2 from urine samples with a viral load of 80 copies/mL within 5 minutes.

Eom et al. developed a method enabling not only to detect the viruses in nasal swabs and saliva samples but also to determine possible resistance. Oseltamivir hexythiol (OHT), a derivative of the viral drug Tamiflu, shows a significantly higher binding affinity to Tamiflu-resistant viruses than to the sensitive wild type. This allowed using OHT combined with Au nanoparticles as SERS substrates. Since these SERS substrates bind specifically to resistant mutants, they showed significantly more intense signals than in sensitive viruses to which the nanoparticles do not bind. Hence, the detection of resistant influenza viruses in nasal fluid as well as in saliva was successfully realized.

We further reviewed recent advances of optical biosensors in analyzing the viral load of different viruses in biological samples stemming from humans. An optical biosensor is a compact analytical device including a biorecognition element integrated with an optical sensor. Optical biosensor systems can be an easy-to-use, rapid, portable, multiplexed and cost-effective diagnosis approach. Inci et al. and Shafiee et al. found a strong correlation between HIV viral loads and wavelength differences shifted by nanoplasmonic viral load detection platform and using a nanostructured optical photonic crystal biosensor, detection of the viral load of HIV ranging from $10^4$ to $10^8$ copies/mL can be achieved. Another optical biosensor was presented for probing viral loads in urine samples. Urinary polyomavirus BK (BKV) viral load detection was reported. Su et al. established the correlation between a dual-channel heterodyne-based SPR biosensor signal and the concentration of the BKV in urine over the range of $2 \times 10^2$ to $2 \times 10^6$ copies/mL. The particle interferometric reflectance imaging sensor (IRIS) signal is based on the
interference of the fields reflected off a layered substrate that is typically comprised of a SiO₂ layer thermally grown atop a Si surface [101]. Single particle interferometric reflectance imaging sensor (SP-IRIS) is a modality that can perform digital detection of single particles with high magnification [101]. SP-IRIS combined with affinity-based capture, size discrimination and automatic counting scheme enabled the detection of recombinant vesicular stomatitis viruses (rVSV) and Ebola virus with a concentration of 10⁴ plaque-forming units/mL (PFU/mL) under 10 minutes for the in whole blood sample [102, 103]. Although these studies only focused on the limit of detection for viral loads, they indicate enormous potential of SP-IRIS for determining the viral loads in blood.
3.3 | Detection of viral proteins

Many viruses can be identified according to their specific proteins [8]. Influenza A viruses contain hemagglutinin within their envelope and also infected cells exhibit this protein [34, 104]. Zhao et al. developed an aptamer-based sensor for detecting HA by exploiting the Förster resonance energy transfer (FRET) principle [105]. Wiriyachaiporn et al. addressed the influenza B virus nucleoprotein with a fluorescence-based lateral flow test. The protein is first captured with specific antibodies and then detection is enabled using silica nanoparticles coupled with Cy5 and specific antibodies [106]. Takemura et al. proposed another fluorescence-based detection scheme for the NS1 protein of the Zika virus. Their immunoassay offers a high sensitivity due to localized surface plasmon resonance (LSPR) enhancement of the fluorescence signal of quantum dots [107]. Yoo et al. developed a concept involving antibody functionalized magnetic nanoparticles that allows reusing an SPR chip for detection of the nucleoprotein of influenza A virus [108].

3.4 | Detection of viral nucleic acids

Nucleic acid-based detection offers a high specificity by identifying viral species according to characteristic base sequences [15]. While such approaches often fall into the category of molecular methods, the underlying principle can also be used in biophotonic detection schemes. Fluorescence in situ hybridization (FISH) is a well-known technique for visualizing the presence and distribution of specific base sequences in cell or tissue samples by employing a fluorescence-labeled complementary sequence [109]. Wang et al. used quantum dots as fluorescent labels for detecting Ectromelia virus (ECTV) in cell and tissue samples [110]. Du et al. demonstrated how Raman spectroscopy in combination with multivariate
analysis can be utilized for quantifying hepatitis B virus (HBV) DNA in serum samples [111].

### 3.5 Detection of antibodies

Biophotonic methods can also serve for detecting antibodies indicative of a viral infection [83, 112]. Nishiyama et al. proposed a fluorescence polarization immunoassay (FPIA) for detecting the anti-H5 subtype AIV antibody in serum [113]. The assay can be performed on-site using a portable microdevice [113]. Dos Santos et al. investigated the potential of the hepatitis A virus (HAV) capsid protein VP1 for detecting IgM against HAV in serum samples using SPR [114]. They found VP1 to be a promising serological marker for HAV infections [114].

### 4 VIRUS-HOST INTERACTION

Recently, the world has seen the pandemic spurred by the SARS coronavirus, which is known to modulate key host factors to evade the immune response, displaying similar pathophysiological and clinical features as sepsis [115, 116]. In this section, we will briefly provide insight into the various molecular aspects of virus-host interactions and the photonic methods employed to understand the biomolecular profile of the host’s immune response toward a viral infection.

The most challenging aspect of viral infection is that the virions continuously evolve by modifying their surface proteins and DNA or RNA expression in a unique way to evade host response [45, 117]. This modification occurs either by mutation and substitution during the natural selection process or polymerase errors [118, 119]. However, Duffy et al. state virus modification also benefit from host antiviral enzymes, spontaneous chemical reactions and environmental mutagens such as ultraviolet irradiation [120].

A review article by Putlyaeva et al. describes the application of fluorescence microscopy to study the infection cycle of the SARS-CoV-2 virus (Figure 14) [121]. Caldas et al. employed scanning electron microscopy to show SARS-CoV-2 virus interaction with the Vero cells [122]. The study shows virions adhere to the cell surface 4 hours postinfection and aggregate in cellular vacuoles [122]. Further, the cell membrane is massively remodeled by the corona virus to enable fusion of the vacuole membrane with the cell plasma membrane to ensure high transport/release of viral particles [122]. Further, Cortese et al. used
3D electron microscopy and revealed extensive alteration of cellular organelles such as the mitochondrial network and fragmentation of Golgi bodies in the SARS-CoV-2 infected human lung epithelial cells (Figure 4) [54]. They mapped spatial coordination of SARS-CoV-2 virus replication within the clusters of double-membrane vesicles giving insights into the cytopathic effects induced by the corona virus [54]. Belhaouari et al. with the aid of electron microscopy...
showed that the SARS-CoV-2 virion is capable of overcoming the membrane barrier through membrane fusion [123] and the spike protein (S) on the surface of the virus binds to the host angiotensin-converting enzyme 2 (ACE2) [124]. Yang et al. investigated the mechanism of S-glycoprotein and ACE2 receptor interaction using AFM (Figure 15) [125]. Benton et al. employed modeling of cryo-electron microscopy data to show binding of ACE2 through furin protease's cleavage of the viral transmembrane spike glycoprotein S into its subunits S1 and S2 [126]. They further illustrated that the S1 subunit undergoes structural changes leading to the exposure of the receptor-binding domain (RBD) on the surface of the molecules [126]. Poppe et al. used a high-resolution transcriptomic study to show the capability of RNA viruses to complete their lifecycle within the cytoplasm and manipulate the host cellular nuclear chromatin for its benefit [127]. They showed that the viruses fine-tune the NF-kB signaling causing epigenetic changes within the infected cells [127]. Viswanathan et al. applied X-ray diffraction and discovered that the SARS-
CoV-2 virus masks its messenger RNA (mRNA) by mimicking the host cell RNA leading to the host immune system not being able to recognize the viral mRNA [46]. Similarly, Grikscheit et al. combined live-cell microscopy to show the Ebola virus regulating actin polymerization for transport of its nucleocapsid [59]. Rut et al. used fluorogenic substrates with glutamine to determine the substrate’s preference of coronavirus particles [47]. They designed active probes to visualize viral protease SARS-CoV-2Mpro within the nasopharyngeal epithelial cells of infected COVID patients, hence showing that human cells collected ex vivo contain active SARS-CoV-2Mpro during SARS-CoV-2 infection [47].

4.1 Immune response toward virus

The innate immune system is the first line of defense during infection and is essential in sensing and detecting pathogens [60]. The innate immune cell surface has unique receptors namely, retinoic acid-inducible gene-I-like receptors (RLRs), Toll-like receptors (TLRs) and NOD-like receptors (NLRs) designed for sensing virus particles (Figure 16) [60, 61]. The fluorescence microscopy technique is a well-known method applied for sensing and exploring the receptor function in a given cell [62]. For example, Kawai et al. summarized the roles of different receptors, such as the RLRs and TLRs which are responsible for the production of proinflammatory cytokines, recognizing virus RNA and detecting virus invasion [62]. Blanco-Melo et al. showed, employing different optical methods combined with transcriptomics, that cells use the aforementioned pattern recognition receptors (PRRs) resulting in transcriptional activity to launch antiviral defenses such as interferon (IFN) regulatory factors and nuclear factor κB [63]. This triggers the adaptive immune cells which follow the antiviral response mediated via cytokine secretion [63].

Cell-based functional microarrays are often used to gain insight into virus-host interactions by in vitro immune cell-viral interactions studies [64]. For
example, the PRR of immune cells for targeted sensing of microbes is well studied using different fluorescence imaging techniques [132]. Either the immune cells are screened directly for expression of specific PRR in an ex vivo setup or indirectly by profiling the host's cytokines in the blood plasma or serum [132]. Further, LM was used to show TLR3 recognizing double-stranded viral RNA whereas TLR7 and TLR8 can sense single-stranded RNA [136]. Functional assays when combined with high-end fluorescence microscopy, allow high-throughput screening of broad-spectrum coronavirus entry inhibitors [137, 138], where, quantum-dot-based nanoparticle probes allow precisely identifying and validating inhibitors of virus-host cell receptor binding sites [139]. Guo et al. addressed the cellular response toward viral infection by employing an on-chip investigation of individual cells [140]. Within this study, they illustrated a population-based effect where viral and cellular factors' contribution is unique [140]. Triana et al. combined single-cell RNA-sequencing with multiplex RNA FISH to investigate a human organoid model of enteric virus infection [141]. The researchers showed the contribution of intestinal epithelium cells in resolving viral infection [141].

For deeper insight into cell-virion interaction more advanced fluorescence imaging methods, such as FRET, fluorescence recovery after photobleaching (FRAP) and fluorescence lifetime imaging (FLIM) are being used to visualize the virus-cell protein interactions, detection and quantification of the virus within the cell, surface receptor-
binding, and so on [142, 143]. For example, Gordon et al. investigated SARS and MERS viral-human protein interaction using 3-Å cryoelectron microscopy, immunofluorescence, functional genetic screening and proteomics combined with cell biology [144]. Using the aforementioned broad panel of photonic and computational-based techniques enables to resolve the expression of viral proteins within the cells spatially [54, 144, 145]. Further, the panel made it possible to identify mitochondrial outer-membrane protein Tom70 and SARS-CoV2 nucleocapsid protein Orf9b to be important players in interferon signaling [146]. Similarly, AFM was applied to study the mechanism of SARS-CoV-2 spike glycoprotein with ACE2 cellular receptor using an isolated receptor system and validated the results on live epithelial A549 cells [147]. AFM was also applied for probing virus-cell interactions, where R18-labeled influenza virions adsorbed to polystyrene beads were covalently tethered to the AFM tip via a bifunctional polyethylene glycol (PEG) cross-linker and binding forces were measured between viruses on beads and adherent cells grown in glass-bottom Petri dishes (Figure 17A) [34].

In yet another study hepatitis C virus-induced alterations in hepatic metabolism and virus-related cellular alteration such as lipid biosynthesis, formation and trafficking of lipid droplets leading to hepatic steatosis was visualized using coherent anti-stokes Raman scattering (CARS) and two-photon fluorescence [148]. Using the FLIM technique the fluorescence lifetime of
NAD(P)H was monitored to be the state of transfected cells (Figure 17B) and the expression of lipid droplets was studied by CARS and the localization of the virus was visualized using two-photon fluorescence (Figure 17C) [148]. The infected Huh-7 human hepatoma cells showed an increased NAD(P)H fluorescence, the increased intensity ratio of free to bound NAD(P)H, and the reduced fluorescence lifetime of bound and free NAD(P)H indicated viral protein-induced alterations in the cofactors binding and microenvironment [148]. On the other hand, super-resolution fluorescence microscopy was used to study the replication cycle of human immunodeficiency virus type 1 and to investigate the behavior of subviral structures and their interaction with cells [150]. The viral trafficking of the influenza A virus was visualized by stimulated emission depletion microscopy (STED; Figure 17D) [149]. Further, other methods such as X-ray crystallography and computational chemistry allow determining the molecular structure, simulating viral-host protein interactions, detecting potential therapeutic pockets for intervention and understanding the receptor functions [151, 152]. In-depth crystal structure analysis of the RBD of the SARS-CoV spike protein and ACE2 using X-ray diffraction data [152] and computational approaches indicated that SARS-CoV-2 binds with ACE2 more robustly compared with SARS-CoV [151, 153]. Extensive review articles, for example by Arista-Romero et al., Cortese et al., Liu et al. and Putlyaeva et al. provide insights into advancements of LM techniques for the study of viral infections [54, 121, 145, 154].

4.2 Study of host’s immune status during viral infection

The various aspects of virus-host interaction described in the above sections give an overview of the interplay between viral particles and the host’s immune system. However, information on the host’s immune status during infection is obtained by comparing the changes in the biochemical profile of the healthy and the disease state [155, 156]. An intriguing option for identifying viral infections is to apply vibrational spectroscopic techniques combined with multivariate analysis [157] because this enables the investigation of highly complex samples without the need for extensive sample preparation [135, 158]. A viral infection will result in characteristic chemical changes of the affected tissue or body fluid [118]. Not only the presence of virus particles itself will be indicative of the infection, but also the highly complex immune response of the host leads to a cascade of biochemical reactions, resulting in altered sample composition [159].

Fourier-transform infrared (FTIR) spectroscopy has proven to be a promising tool for viral infection detection in cells and body fluids [160]. FTIR was employed as a fast testing technique for COVID-19 detection, where SARS-CoV-2 RNA extracted from patients were screened [161]. Melo et al. demonstrated how FTIR spectroscopy can be employed for risk assessment of HPV infections by investigating cervical fluid samples [37]. Depending on the HPV type the oncogenic risk can be high or low [37]. Barauna et al. utilized attenuated total reflection (ATR) FTIR spectroscopy for investigating both spiked and clinical saliva samples for differentiating between SARS-CoV-2 positive and negative samples [162]. Results can be obtained within minutes, which highlights the great potential of spectroscopic techniques for on-site testing [162]. Pizarro et al. developed a non-targeted metabolomics approach (the application of FTIR spectroscopy together with multivariate data analysis) for identifying different patient subgroups suffering from an HIV infection according to plasma samples [163]. For example, they were able to discriminate between asymptomatic patients and those who had developed acquired immune deficiency syndrome (AIDS) and also successfully diagnose a co-infection with hepatitis C virus (HCV) in each of the groups [163]. The ATR FTIR was applied to detect and classify COVID-19 patients, among COVID-19 and control patient’s spectra, significant shifts were observed at protein and lipid bands especially higher total phospholipid in COVID-19 patients compared with controls (Figure 18A) [162]. In this study, saliva was studied for on-site detection of SARS-COV-2 infection [162]. It was possible to detect IR spectral changes at low copy numbers (down to 1582 copies/mL) [162]. Significant virus spectral bands were tentatively associated with nucleic acids and RNA [162]. A multivariate analysis was used to discriminate the complex milieu of a saliva sample as shown in Figure 18B. Marker IR peaks could be linked to the response of the host organism to the virus infection [162]. The review article by Santos et al. discusses further applications of ATR FTIR spectroscopy for virus identification [164].

Raman spectroscopy is another promising option for investigating the sum parameters of complex samples [1–3, 155, 166–174]. For detecting HBV and HCV infections serum samples are well suited [5, 175, 176]. Several studies exist using either Raman spectroscopy [155, 175, 177–182] or SERS [176, 183] for discriminating between healthy and infected patients. From Raman spectra of serum samples also a differentiation between typhoid and dengue fever can be achieved [184]. Moreover, Mahmood et al. were able to identify dengue-infected patients through Raman spectra acquired from plasma samples [173]. Similarly, Raman spectroscopy was applied to study...
in vitro adenovirus infection of human embryonic kidney 293 cells [185], herpes virus infection of Vero cells [186], Epstein-Barr virus infection in glial cells [187] and COVID-19 detection based on biochemical profile of patients’ serum sample [188]. Raman spectroscopy-based investigations of activated lymphocytes’ chemical profile during viral infection in patients significantly differed compared with the healthy donors [2, 189]. The analysis of saliva samples as shown in Figure 18C indicated clear differences in the Raman spectral profile of COVID positive and negative subjects [165]. Investigation of serum samples from patients with confirmed SARS-CoV-2 disease, patients with flu-like symptoms (suspected cases) and healthy controls could be distinguished based on Raman spectroscopic profiles [165, 190, 191]. Validation of the results with an independent data set revealed correct classification of all samples though the majority of the patients showed non-severe symptoms and about 20% were asymptomatic [191]. Further, Tabish et al. discuss extensively the potential of another variant of Raman spectroscopy, namely CARS, for the detection of COVID-19 infection and SARS-CoV-2 particles [192].

SERS can be applied for screening the SARS-CoV-2 virus in the saliva samples from sick, recovered and uninfected subjects [8, 190, 193, 194]. Clear differences in the mean spectra were found, which could not be assigned to the saliva but the virus itself [165]. There were only very slight spectral differences between Covid
positive and recovered patients, as the antibodies can still be detected in saliva up to 3 months after the end of the infection [165]. By detecting these biomarkers, statements can be made about an infection without directly detecting the virus [165].

Carlomagno et al. focused as well on saliva for detecting SARS-CoV-2 infections using SERS, without the need for invasive sample collection procedures [165]. Lim et al. addressed the issue of identifying newly emerging viruses using SERS [195]. They investigated cells infected by influenza viruses exploiting that cells will express the envelope proteins of the different virus types [196]. Stanborough et al. used a thiolated aptamer that binds selectively and specifically to nanoparticle surface and the target analyte which leads to change in SERS peak intensity allowing to probe the spike protein of SARS-CoV-2 virus (Figure 18D) [8]. The unsupervised statistical analysis method principle component analysis (PCA) was applied to observe spectral shifts upon protein binding and significant changes were observed in the SERS spectra [8]. Several SERS-based methods have been proposed and tested using purified SARS-CoV-2 proteins and patient samples [190, 193, 194]. The detection of SARS-CoV-2 spike protein in untreated saliva samples down to a concentration of 6.07 fg/mL was achieved using a SERS-based biosensor [197]. It has been estimated that SERS enables the detection of asymptomatic carriers 2 days earlier than rRT-PCR [197].

The combination of SERS nanotags and a lateral flow immunochromatographic assay in one biosensor for the detection of the host's anti-SARS-CoV-2 IgG and IgM antibodies in serum has shown to effectively differentiate SARS-CoV-2 negative from positive samples with high accuracy even when the serum was diluted up to 10⁶ times [193].

A novel approach using biosensors was shown to aid in rapid detection of SARS-CoV-2 infection where the sensor surface was modified for antibody-based detection of SARS-CoV-2 spike proteins present in the nasopharyngeal swab samples collected from the patients [198]. Taha et al. have elaborately described various sensors designed for the detection of corona virus diseases [199]. MicroRNA profiling studies of plasma show the possibility to identify viral infection in patients [200, 201]. Tribolet et al. highlight the use of microRNA-based point-of-care testing for screening infectious diseases [202]. Another method being widely used for biomarker screening in the field of diagnostics is mass spectrometry, which for example enables the detection of SARS-CoV-2 viral antigens [203] and nucleoprotein peptides in nasopharyngeal swab samples from COVID-19 patients [204]. Exhaled breath screening is another option for detecting viral infection-related volatile biomarkers [205]. In recent years fiberoptic endoscopic devices have been approved for medical use and are commercially available [206]. The use of fiberoptic tracheal intubation with fiberoptic bronchoscopy has been tested in SARS-CoV patients with severe disease and is supposed to be faster and able to reduce the risk for virus transmission to healthcare workers due to the larger distance to the patient possible [206]. However, the design and results of this study are partially seen very critical [207, 208].

5 | PHOTONIC SOLUTIONS FOR LONGITUDINAL MONITORING OF VACCINE EFFICACY AGAINST COVID-19

When viewed from the point of the history of vaccine development, the COVID-19 pandemic certainly displays an indisputable landmark, as even optimistic estimates for the development duration of an effective vaccination agent hypothesized at least 1 year after the onset of the pandemic. Therefore, it is notable that the US Food and Drug Administration (FDA) already issued the first emergency use authorization for the Pfizer-BioNTech COVID-19 vaccine on 11 December 2020, delineating the starting signal of a global vaccination campaign in many countries. Although, numerous clinical trials including various vaccines developed and authorized in the meantime, there is still a lack of answers to relevant questions, such as the timepoint for re-administration of the vaccine maintaining an individually efficient immune response or its efficiency against new SARS-CoV-2 mutants [209]. Monitoring the body's immune response to both current and prior infection is therefore mandatory and can be accomplished by serological testing, also known as antibody testing [210, 211]. It mainly targets the detection and quantification of pathogen-specific immunoglobulins expressed by B cells, namely IgM, IgA and IgG, which are also referred to as neutralizing antibodies [211, 212]. The former two types of antibodies are produced within the first 6 to 10 days after SARS-CoV-2 infection, while their concentration starts to decline rapidly after 4 to 5 weeks [213]. In contrast, IgG appears later reaching a peak level at 3 to 4 weeks and persisting for a long time even after the infection has been cleared [214, 215]. Well-established tools for serological testing include chemiluminescent assays (CLIA) [216], enzyme-linked immunosorbent assays (ELISA) [217] and lateral flow immunoassays (LIFA) [218, 219]. While CLIA and ELISA reliably provide quantitative information for antibody profiling, both techniques suffer from a time-consuming assay handling procedure as well as inevitably high costs for expensive instruments and consumables. Lateral flow assays (LFAs) display a low-cost and rapid alternative, as
they are based on disposable paper-based test strips [220]. Still, in their current implementation as test kits with a binary outcome (positive/negative), they are lacking quantitative information on a patient’s anti-SARS-CoV-2 antibody levels [220]. To overcome the above-mentioned limitations of existing assays photonics researchers have significantly contributed to provide integrated diagnostic platforms for low-cost, rapid and accurate antibody assessment. Liu et al. has published one of the pioneering works in this regard, where he developed a two-channel SERS-based LFIA biosensor for the simultaneous detection of IgM/IgG [193]. The testing principle relies on the immune-reaction of SARS-CoV-2 spike protein-modified SiO\(_2@Ag\) SERS tags with abundant anti-SARS-CoV-2 IgM/IgG species, which can specifically bind to their respective antibodies dispensed on the test line of the strip [193]. Probing both test lines with a 785 nm laser allows for the acquisition of SERS spectra and concomitantly the concentration estimation of the biomolecules [193]. A performance comparison between the SERS-LFIA and a commercially available colloid gold strip kit using a clinical serum from a patient with COVID-19 revealed an 800 times higher sensitivity of the former [193]. Zhao et al. proposed an immunoassay employing photonic resonator absorption microscopy (PRAM) [210]. Here, a photonic crystal (PC) biosensor coated with the SARS-CoV-2 spike protein interacts with antibody-functionalized gold nanoparticles serving as binding targets for IgG molecules (Figure 18A) [210]. When Au particles bind to the PC surface, the locally reflected resonant LED light intensity is substantially reduced [210]. Sampling the reflected light with a simple CCD camera enables the acquisition of peak intensity value (PIV) images with dark pixels due to bound nanoparticles [210]. The single-step PRAM-based immunoassay allowed for the detection of human COVID-19 IgG within 15 minutes at a detection limit of 100 pg/mL [210]. Another promising photonics-based diagnostic platform has been demonstrated utilizing disposable photonic ring resonator sensor chips [221]. Upon functionalization of the resonator structure with antibodies, the subsequent binding of target molecules in a microfluidic flow is detected by a shift of the corresponding resonance wavelength [221].

**Figure 19** Detection scheme for human COVID-19 IgG via a PRAM-based immunoassay (A). Bound IgG-Au nanoparticles can be quantified by digital counting in PIV images (bottom). Reproduced from Ref. [210] with the permission of Elsevier. Side view on a disposable chip with photonic integrated circuits (PICs) allowing for sensitively detection of protein markers due to shifts in the resonance wavelength under microfluidic flow (B, top). The below graph depicts the shift-related amount of anti-SARS-CoV-2 antibodies over time in samples of four subjects after having received doses of vaccine. Reprinted (adapted) from Ref. [221] with permission of MDPI. A low-cost and portable LIT device for quantitative read-out of Au particles in commercial LFAs (C, left panel) together with bright-field and thermo-photonic amplitude images of test cassettes developed at various IgG concentrations (C, right panel). Reprinted (adapted) from Ref. [220] with permission of IEEE. Workflow of a quantum dot barcode immunoassay for COVID-19 diagnostics (D, left panel). The quantification of targeted antibodies is realized in a miniaturized smartphone imaging system (D, right panel). Reprinted (adapted) from Ref. [222] with permissions of American Chemical Society
magnitude of shifting is directly proportional to the number of molecules bound to the resonator surface, allowing for direct quantification. Chips were tailored to either detect SARS-CoV 2 spike protein or human COVID-19 Ig’s together with additional supplied bovine serum albumin serving as an internal reference for correct shift estimations [221]. With their platform, the authors investigated serum samples of recently vaccinated test subjects over a time course of 70 days (Figure 19B) [221]. Despite the reported LOD of just 4.2 μg/mL for the spike protein, the observed concentration patterns were in good agreement with data from clinical trials [221]. To conclude this section, two recent reports on fully integrated diagnostic devices are especially recommended to the reader. Thapa et al. designed a handheld thermo-photonic device for the quantitative read-out of target analytes in common LFAs [220]. The underlying principle is the exploitation of lock-in thermography (LIT) signals generated by the Au nanoparticles immobilized on the test line of the strip [220]. The system was tested with a commercial test cassette for IgG detection and test solutions of various IgG concentrations (Figure 19C) [220]. The concomitantly acquired signal from a fixed reference LFA with known IgG concentration serves as internal standard considering power fluctuations of the intensity-modulated 808 nm excitation laser [220]. The signal detection via a simple smartphone infrared camera dramatically reduces price, size and test duration compared with equally performing ELISA systems. Zhang et al. presented another smartphone based serological assay device [222]. Here, the target molecules are captured by antigen-coated, spectrally barcoded quantum dot microbeads followed by binding of fluorophore-conjugated secondary antibodies as depicted in Figure 19D [222]. Interrogation of the resulting sandwich structure by laser excitation enables both the identification of individuals beads as well as the number of target molecules [222].

Quantum dots were prepared for the simultaneous detection of anti-spike-1 IgG and anti-nucleocapsid IgG in the serum of COVID-19 patients over time [110]. The overall assay sensitivity of 90% and specificity of 100% outperform commonly available LFAs. The automated readout of the quantum dot barcode assay is linked to a data dashboard enabling for real-time surveillance of patients by clinicians or even entire patient cohorts by public health agencies [110]. Though hampered by the course of the pandemic itself, photonic science has been a flourishing field of research contributions in many different ways to win the fight against the COVID-19 virus. Further insights in recent developments including advances in disinfection technologies are provided by a very comprehensible review, which is highly recommended to the reader [223]. Each of the studies presented within this section demonstrates fast, robust and low-cost solutions for dynamic antibody testing directly transferable to high-throughput diagnostics. Advancing and translating these lab-based prototypes for longitudinal serological analysis into the medical market together with the simultaneous development of smart data libraries will empower physicians and clinical scientists to gain deeper insights into the pathophysiological courses and epidemiological processes connected to harmful diseases and facilitate the development of effective and secure vaccines.

6 | SUMMARY AND CONCLUSION

The wide range of application examples and the multiplicity of different approaches for understanding viral infections by means of biophotonic methods reflect their great value and versatility. Depending on the requirements either highly specific assays by implementing recognition elements can be designed or the spectroscopic fingerprint alone can enable label-free detection of the target. For the latter building a database beforehand in order to establish a chemometric model is necessary. The size and quality of such a database will significantly impact the results regarding the identification of unknown samples. The main advantage of such approaches is that no costs for antibodies or labels will arise. Acquiring Raman or IR spectra and their automated evaluation can usually be achieved within minutes. Over the last years, many handheld or portable instruments have been developed enabling application in the field. Besides, many of the published studies were performed with or included real patient samples. For becoming a viable alternative to the currently applied PCR tests and lateral flow tests, however, many important criteria have to be met in parallel. It is certainly a big challenge to design fast, cost-efficient, sensitive, reliable and on-site applicable assays for detecting viral infections. The recent COVID-19 pandemic, highlighting the urgent need for alternative testing methods, has resulted in increased efforts in the research community for reaching this ambitious goal and highly innovative approaches were presented.

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Haodong Shen finished his Bachelor in Beijing institute of Technology, Beijing, China in 2014 and then obtained his Masters in Optics & Photonics in 2016 at University of Michigan, Ann Arbor, MI. Afterward he joined the group of Prof. Jürgen Popp as a PhD student at Leibniz Institute of Photonic Technologies (IPHT) in Jena, Germany. His studies are mainly on rapid Raman spectroscopic classification and identification of bacterial biofilms and microcolonies on agar plate. Photonic as noninvasive methods in investigating biofilm are also his interest.

Christina Wichmann obtained her Masters in Microbiology in 2016 at Friedrich-Schiller-University in Jena. Then she joined Leibniz Institute of Photonic Technologies in the biophotonics group to investigate bacteria by means of Raman spectroscopy. Her main focus was the influence of biotic and abiotic parameters toward bacteria and their Raman spectra. She is currently working as a researcher at Institute for Physical Chemistry in Jena about Raman spectroscopic detection of bacteria in clinical wastewater. In future research she wants to keep the focus on vibrational spectroscopy of pathogen bacteria in medical and environmental samples.

Christoph Krafft studied physics at the University Oldenburg (Germany) and received his PhD in biophysics from the Humboldt University Berlin in 1998 (Germany). He went for postgraduate studies to the University of Missouri Kansas City (USA) and University Trieste (Italy). He led the research group “Molecular Endospectroscopy” at the Dresden University of Technology (Germany) where he finished his habilitation. Since 2008 he leads a research group at Leibniz IPHT in Jena. His research is focused on Raman-based and infrared-based studies of biomolecules, tissues and cells for bioanalytical and medical diagnostic applications.

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