Single-virus analysis through chip-based optical detection

“It is ... a terrific time to assess the state-of-the art of single-virus detection on integrated chips for microscale bioanalysis.”

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Viral infectious diseases remain constant threats to human well being and one of the main causes of death across the globe [1]. Therefore, developing better approaches to both detecting and analyzing viruses continues to be a very active area of research with clear and immediate applications in disease diagnosis and treatment. In order to be competitive compared with existing diagnostic solutions, new virus detection systems need to fulfill several requirements: provide a limit of detection at the low end of clinically relevant viral loads to enable early diagnosis (typically around 10^3 viral nucleic acids per ml); a dynamic detection range large enough to produce quantitative results across all stages of the infection (typically six orders of magnitude) and the ability to carry out differentiated and simultaneous detection of different virus targets (multiplexing, typically 5–20×) [2]. In addition to these necessary technical capabilities, other factors - notably testing time, cost, ease of use and experimental complexity - play into the suitability of a detection platform for a particular environment and application.

Digitally detecting single viruses or virus components, such as nucleic acids, is very attractive for several reasons. First, if single-particle detection is possible, a system's limit of detection is based simply on the amount of time for which the assay can be operated (i.e., the available amount of test sample) rather than the detection sensitivity. Second,

because single-particle detection resolution allows for direct counting of targets, viral loads can be accurately measured over the course of an infection. Finally, single-particle resolution removes the need to perform statistical averaging over heterogeneities across a sample and enables additional levels of analysis. This is particularly relevant for fundamental research on viral populations and infections.

Optical detection remains the dominant approach to virus analysis, including visual inspection of cell cultures, fluorescence-based readout and indirect detection via refractive index changes and other mechanisms. Many optical techniques lend themselves well to chip-scale integration using micro- and nano-phonic approaches adapted from more traditional optoelectronics devices or specifically designed for bioanalysis. In parallel, there has been much effort to implement biological sample handling on a miniaturized scale. These microfluidic labs-on-a-chip can reduce many of the ancillary desirable aspects of virus detection such as small sample volumes, low-cost fabrication and rapid testing [3]. It comes as no surprise that microfluidics and integrated optics have recently merged in the field of optofluidics which promises novel, more powerful platforms for biomarker analysis in general and virus detection in particular [4–6]. It is, therefore, a terrific time to assess the state-of-the art of single-virus detection on integrated chips for microscale bioanalysis.

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In-flow versus surface-bound detection

Over the past few years, two main approaches to optofluidic bioanalysis have developed. The majority of bioanalysis devices rely on some form of specific binding between a biological target and a functionalized surface that can then be transduced into optical readout. The transduction mechanism is typically a change in the power or direction of an optical beam that interacts with the surface. Many clever ways of taking advantage of a refractive index change upon target binding have been developed as these lend themselves extremely well to creating large signal enhancement using narrow spectral resonance shifts. Such resonant structures include microrings, 1D nanophotonic crystals, microspheres, microtoroids and plasmonic nanohole arrays [4,5]. Virus detection assays based on such surface-bound optical techniques can be quite sensitive although they do not usually reach the single-virus particle limit, except in extreme cases requiring extraordinary fabrication and calibration precision. The single-particle analysis level has recently been reached using widefield imaging based methods, where the optical readout path is not integrated on the microchip, but incorporated with standard, even portable, imaging systems such as smartphone cameras [7,8]. Despite reaching the single-virus particle sensitivity limit, these approaches are subject to the same limitations as other surface-bound methods. These include insufficient limit of detection due to limited transport of target particles to the detection surface, limited dynamic range (typically three orders of magnitude) and surface deterioration. Moreover, when the optical readout is integrated with the analysis chip, the interaction with the targets is typically implemented via the evanescent tail of an optical mode propagating in a solid waveguide and therefore inherently inefficient. Finally, surface-bound virus detection is typically carried out without the use of fluorescent markers to identify the targets due to problems with rapid photobleaching and the necessity of a second specific binding step. Consequently, nonspecific binding and signals are of great concern for this type of assay.

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In-flow-based detection is an attractive alternative to the currently more widespread surface sensors. Detecting molecular biomarkers or whole bioparticles in a stream of gas or liquid overcomes many of the short-

comings described above. Unwanted binding to and fouling of surfaces are much smaller concerns, and if the target particle is only exposed to an optical beam for a short time, the problem of photobleaching of a fluorescent label is eliminated. The ability to use fluorescent markers allows for relatively large signals and excellent target specificity. Moreover, diffusion-limited transport to a detection surface is no longer an issue.

One example for microscale implementation of in-flow sensing is virus detection using optofluidic ring resonators [9]. An optofluidic ring resonator is a glass capillary through which analytes can flow and whose walls can sustain whispering gallery modes. These ring resonator frequencies can be monitored with an adjacent optical fiber and indicate target binding or proximity to the capillary surface via a spectral shift. In this somewhat intermediate approach that still relies on target transport to a solid–liquid interface, low limits of detection and large – albeit highly sublinear – dynamic range for bacteriophage detection were demonstrated. A complete step toward flow-based detection can be taken using liquid-core optical waveguides in which light is confined to the same microfluidic channel as the biological targets. Liquid-core waveguides can be constructed with various approaches with the current state-of-the-art being based on antiresonant reflecting optical waveguides (ARROWs) [10,11]. Alternating dielectric layers surrounding a nonsolid core can enable low-loss light propagation by choosing the thicknesses appropriate for a Fabry–Perot reflector or – if periodic – a Bragg stack. ARROWs can also be made with a solid core when the core material is patterned as a standard ridge waveguide. Both solid- and hollow-core ARROWs can then be interfaced lithographically to form 2D photonic networks that can enable a bioanalysis protocol that amounts to the molecular equivalent of flow cytometry in large capillaries. However, because light can be confined in optical modes of only a few microns width, intersecting ARROWs create excitation/detection volumes for optical measurements on the order of femtoliters, sufficient for reaching single-particle fluorescence detection. With this sensitivity in hand, digital detection of single biomarkers such as viral nucleic acids and whole virions is possible. Recently, strain-specific detection of nucleic acids from Zaire Ebola virus from clinical samples has been demonstrated in optofluidic ARROW chips [12]. The digital counting approach enabled specific virus detection down to 0.2 pfu/ml with a dynamic range of 13 orders of magnitude, covering the entire clinical concentration range. This demonstration pointed to an amplification-free chip-based virus analysis technique, which eliminates the need for using PCR with reverse transcription, thermal cycling and use of enzymes to achieve low limits of detection. Moreover, ARROWs

were recently shown to enable a new multiplexing technique based on wavelength division multiplexing [13]. By using a multimode interferometer waveguide section to intersect the fluidic channel, color-dependent spatial excitation patterns are generated that lead to multiplex signals in the time domain as a fluorescent target flows past this excitation region. With this approach, multiple targets labeled with different (combinations of) dyes can be distinguished by the number and spacing of the peaks in the detected signal. This technique was shown to discriminate between three influenza subtypes, again with single-virus sensitivity. Thus, the ARROW-based platform has now met all the fundamental requirements for clinical virus detection using single-particle sensing.

Hybrid integration

The physical optical sensing mechanism is only one aspect of a bioanalysis system, and other considerations need to be made in order to miniaturize and optimize both sample preparation and readout components. Ideally, a complete test starts with raw biological sample and ends with an optical readout. Additional functionality can also be envisioned such as incorporating electronic single-particle level sensing.

The ARROW-based optofluidic system is particularly well suited for such hybrid integration strategies. The planar optical layout based on intersecting solid-core and target-carrying liquid-core waveguides leaves the third dimension open for vertical integration of other functionalities. Specifically, this enables connection of a microfluidic sample processing layer made and optimized using traditional microfluidic device materials such as PDMS and glass as opposed to a silicon substrate that is preferable for the sensing section of the device [14,15]. Using such a hybrid approach, numerous key components of sample processing have been implemented. These include bead-based solid-phase extraction of nucleic acids to provide target specificity, size-based particle filtering, mixing, fluorescent labeling, analyte distribution and preconcentration of target material [12,16]. ARROW chips also lend themselves well to incorporating solid-state nanopores to provide dual-mode electrical and optical single-particle detection sensitivity. Here, the change in ionic current through a nanoscale opening upon translocation of a particle is used as the signaling mechanism [17]. When integrated with optofluidic sensors using focused ion beam mill-

ing, nanopores act as smart gates that control the entry of single particles into the detection channel while providing an electrical signal with which the particles can be further analyzed. Correlated electrical and optical analysis of single H1N1 virus particles as well as fluorescently labeled DNA were demonstrated on a nanopore-ARROW chip [18,19]. Finally, liquid-core waveguide based microchips also lend themselves well to incorporation of functional photonic elements of the bioanalysis process. Optofluidic laser sources, spectral filters and interferometers have all been demonstrated with the additional benefit of being tunable by the choice of the liquid material filling the waveguide core [5,20].

Conclusion

Detection of single viruses has progressed from a topic of fundamental research interest to a viable alternative for applications in medical diagnostics. The benefits of single-particle sensitivity include direct quantification of viral loads and the elimination of a complex amplification process. Optofluidic approaches in which both analyte handling and optical sensing are carried out on the chip scale and with micro- and nano-photonic elements are ideally suited for implementing single-virus detection. Optical sensing can be carried out using detection of targets bound to a surface or in flow as they move along a liquid-core waveguide channel. Parallel imaging of single viruses on surfaces has recently been demonstrated and is compatible with cellphone based camera technology.

Flow-based optofluidic devices have demonstrated the capability of fulfilling all quantitative requirements for clinical virus detection. Their capabilities have also been extended to multimodal detection and multistage sample processing using hybrid integration. Therefore, a fully integrated microsystem for front-to-back amplification-free virus analysis is within reach.

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