# Rapid, ultrasensitive detection of microorganisms based on interferometry and lab-on-a-chip nanotechnology

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## ABSTRACT

Future viral outbreaks are a major threat to societal and economic development throughout the world. A rapid, sensitive, and easy-to-use test for viral infections is essential to prevent and to control such viral pandemics. Furthermore, a compact, portable device is potentially very useful in remote or developing regions without easy access to sophisticated laboratory facilities. We have developed a rapid, ultrasensitive sensor that could be used in a handheld device to detect various viruses and measure their concentration. The essential innovation in this technique is the combination of an integrated optical interferometric sensor with antibody-antigen recognition approaches to yield a very sensitive, very rapid test for virus detection. The sensor is able to spot the herpes virus at concentrations of just 850 particles per milliliter under physiological conditions. The sensitivity of the sensor approaches detection of a single virus particle, yielding a sensor of unprecedented sensitivity with wide applications for viral diagnostics. The sensor's detection principle can be extended to any biological target such as bacteria, cells and proteins and for which there are specific antibodies. The nature of the sensor enables multiplexed detection of several analytes at the same time.

**Keywords:** microorganisms; rapid detection; integrated optics; biosensors; interferometry; microfluidics; nanotechnology; lab-on-a-chip; point-of-care diagnostics; portable device.

## 1. INTRODUCTION

Viruses are among the most important causes of human disease and are an increasing concern as agents for biological warfare and terrorism. In addition viral outbreaks can rapidly spread worldwide to become pandemics with devastating effect on human populations and economies. Rapid, selective, and sensitive detection of viral pathogens is essential in preventing and controlling such outbreaks. The methods currently used for virus detection such as polymerase chain reaction (PCR) based testing[1], branched-chain DNA tests[2] and enzyme-linked immunosorbent assay (ELISA)[3] all have some major drawbacks. They are time consuming and require a high degree of sample manipulation. These technologies are not suited for rapid responses to viral outbreaks. Therefore there is an ongoing interest in the development of a new generation of sensors that can not only outperform existing technology in terms of sensitivity and specificity, but are also small (portable) and affordable, enabling rapid tests for point-of-care detection. Different virus detection techniques, mostly based on nanotechnological principles, have been reported, including nanowire based sensors[4], micromechanical sensors[5], rupture event scanning techniques[6], surface-enhanced Raman spectroscopy method[7], etc. Although these techniques have demonstrated the feasibility for virus detection, further investigation is required to show their utility in clinical settings and for the development of point-of-care systems.

Here, we report an alternative detection method based on an integrated optical sensor that combines the different advantages of existing techniques in a single device. The device is extremely sensitive, fast, and requires a minimal pre-treatment of the sample. The sensor principle is based on a Young interferometer (YI) and requires no labeling of analyte molecules.[8] The sensitivity of $10^{-8}$ refractive index units, corresponding to approximately a protein mass coverage of 20 fg/mm$^2$, is among the most sensitive sensors reported, being ~ 2 orders of magnitude higher than other non-labeling sensor techniques, such as surface plasmon resonance.[9] Moreover, this sensor is simple, easy-to-use and compact,

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offering the possibility for development of portable point-of-care instruments. As such, this sensor is an excellent candidate for fast and on-site virus detection. The multichannel character of the sensor allows sensing of several different viruses and/or other analytes such as bacteria, cells and biomarkers simultaneously.

## 2. MULTICHANNEL YOUNG INTERFEROMETER

### 2.1 Sensor configuration

The high sensitivity, specificity and stability are some of the main attractive features of the integrated optical (IO) based sensors. The IO readout systems are robust and small, allowing for miniaturization where many elements of the device can be integrated in a single chip which occupies a relatively small area. These features offer the prospect for development of multi-target sensing systems. Furthermore, the potential of parallelization implies an increase of the analysis throughput, a reduction in the consumption of biomaterials and cost reduction.

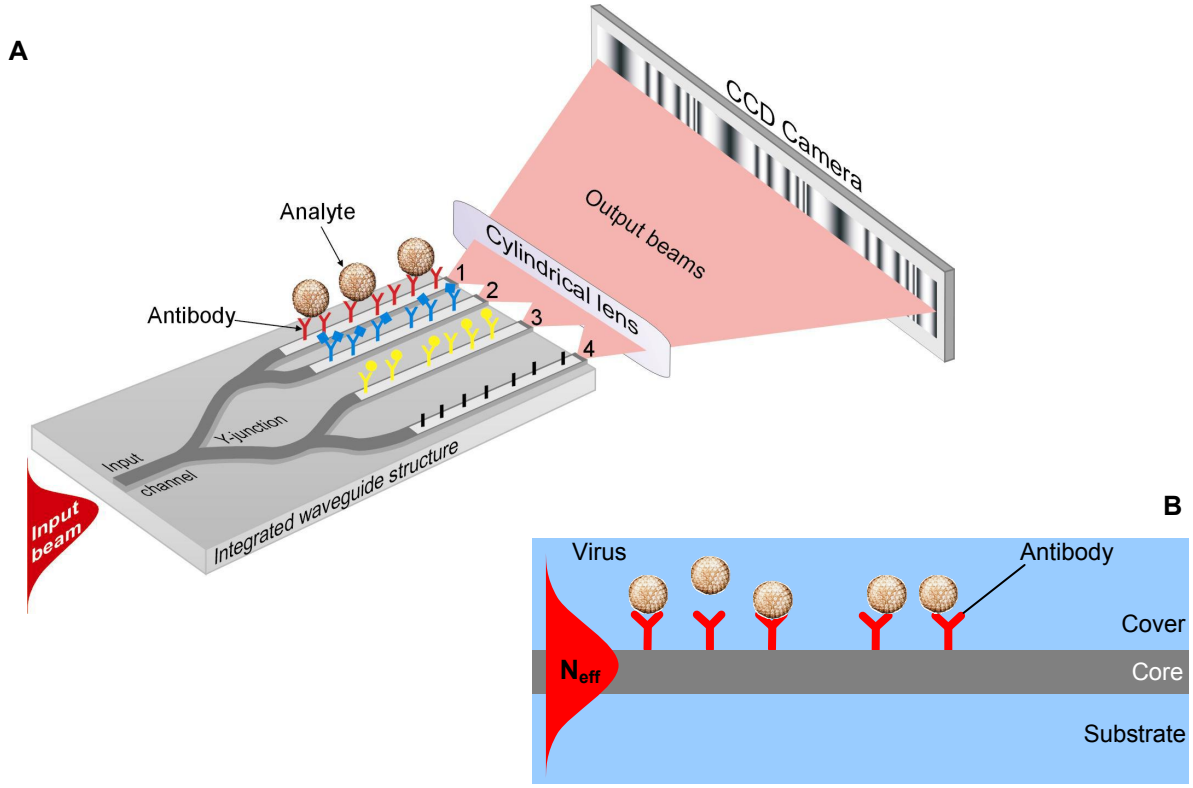


**Fig. 1. Schematic representation of the YI sensor and evanescent field detection.** (A) Schematic of the four-channel integrated optical YI sensor (not on scale): 1, 2 and 3 indicate the measuring channels and 4 is the reference channel. [Reproduced with permission from [12]. Copyright 2007 American Chemical Society]. (B) Schematic of the binding process between analyte (virus) and antibody molecules taking place at the core-cover interface of a three-layer waveguide structure within the evanescent region of a guided mode.

IO readout platforms based on interference phenomena such as the Mach-Zehnder interferometer (MZI)[10] and the Young Interferometer (YI)[8] are well known. Expanding these extremely sensitive sensors to a multichannel device, as has been already demonstrated using surface plasmon resonance techniques[11], enables monitoring of multiple binding processes simultaneously and independently from each other by immobilizing different antibodies to different channels. For an integrated YI no optical phase feedback is required to achieve high sensitivity as in the case of a phase-modulated MZI,

resulting in a simpler chip design. Another attractive aspect is that no extra readout hardware is required for development of a multichannel device based on a YI.

The principle of the YI sensor is schematically shown in Figure 1.A. Monochromatic light from a laser source is coupled to an optical channel waveguide and is guided into four parallel optical channels by means of Y-junctions. These four channels include one reference channel and up to three different measuring channels that can be used to monitor different analytes by coating the channels with appropriate antibodies. Upon exiting from these four waveguide channels, the light generates an interference pattern on a screen. The core-cover interface of the sensor waveguide structure is coated with an antibody receptor layer, which can selectively and specifically bind to analytes such as viruses and proteins that are present in the cover medium, see Figure 1.B. When the binding of an analyte occurs, the substitution of solvent by the analyte will result in a change of the refractive index at the core-cover interface. Therefore the effective refractive index of a guided mode, which probes the core-cover interface during the propagation through the waveguide will be changed within the interaction length. The resulting phase change can be determined by measuring the change in the interference pattern. Analysis of the interference pattern thus sensitively yields information on the amount of adsorbed analytes on different channels.

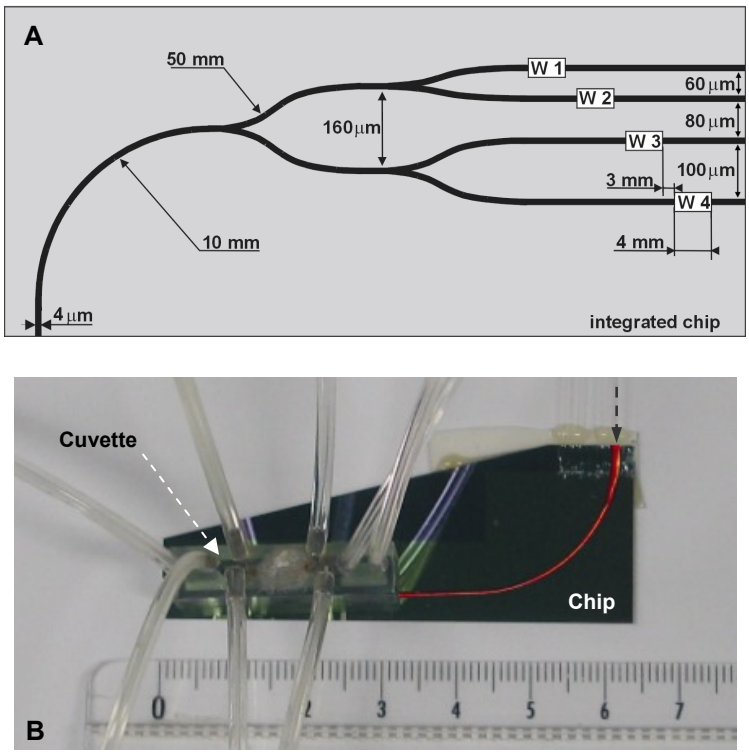


**Fig. 2. Layout of the four-channel YI integrated optical chip.** (A) Top-view of the four-channel integrated optical Young interferometer realized in SiON technology; W1, W2, W3, and W4, are the sensing windows on channel 1, 2, 3, and 4, respectively. The dimensions of the chip are 63 × 24 $mm^2$. [Reproduced with permission from [8]. Copyright 2003 Optical Society of America]. (B) Photograph of the sensor chip and the flow-through cuvette. The chip contains 11 four-channel YI sensors. The light is coupled into the chip by means of an optical fiber, indicated by the black arrow. The light is transported to the sensing area by means of a curved channel waveguide indicated in red. Ruler indicates the dimensions in centimeters.

The four-channel integrated optical Young interferometer chip has been realized as an optical circuit consisting of three Y-junctions positioned in such a way that the two output channels of the first Y-junction serve as input channels for the next two ones, as shown in Figure 2.A. The distance between the mutually parallel output branches of the first Y-

junction was chosen as 160 μm; the distances between the four output channels are $d_{12}$ = 60, $d_{23}$ = 80 and $d_{34}$ = 100 μm guaranteeing that all six spatial frequencies in the interference pattern are different. These distances are small compared to the chip endface to CCD camera distance, but are sufficiently large to exclude mode overlap of adjacent channels. The Y-junctions have been designed with S-bend structures having a bend radius of 50 mm, which allows negligible bend losses. The bend in the input waveguide (bend radius of 10 mm) was implemented for reducing the intensity of the slab light at the channel outputs. The channel waveguide structure has been realized in silicon oxy-nitride (SiON) technology as developed at the MESA+ Institute for Nanotechnology[8]. The ridge of the channel waveguide structure and the channel width have been chosen respectively as 1 nm and 4 μm for single-mode behavior for TE polarized light with a vacuum wavelength of 647 nm. A sensing window was realized on top of each output channel. A flow-through cuvette (31 x 7 $mm^2$) made of Perspex was gently pressed on the chip such that sampling liquids can flow independently through each sensing window. Sampling liquids were flowed (flow rate = 0.003 ml/min) by means of a peristaltic pump (Reglo 100, Ismatec S.A., Glattbrugg, Switzerland) that has four separate pumping channels, each of them functioning independently from one another. Figure 2.B shows a picture of the YI optical chip. In the four-channel Young interferometer, monochromatic laser light of an Ar laser (Coherent) was coupled into the input channel waveguide by means of a fiber-to-chip connection. The four output divergent beams are overlapping each other at the front side of a CCD camera (Teli CS-3440; 756 x 575 pixels), which was placed at a distance of about 60 mm from the chip endface and parallel to the endface plane to record the interference pattern. A cylindrical lens was positioned in between the chip endface and the CCD array for collimating the divergent output beams in the direction perpendicular to the sensor chip upper surface in order to collect all the light emitted from the output channels. The recorded sum interference pattern was digitized to 12-bit information, and analyzed by dedicated software in which a 2D Fast Fourier transform (FFT) algorithm was implemented. The algorithm consists of selecting six different peaks, which correspond to six pair of channels, in the amplitude of the Fast Fourier-transformed interference pattern. The phase value corresponding to each pair of channels was extracted at the phase part of the FFT at the given spatial frequencies.

### 2.2 Virus detection

In order to demonstrate the feasibility of using the YI sensor for virus detection, the interaction between herpes simplex virus type 1 (HSV-1) virus particles (Virusys Corporation, Sykesville, MD, United States) and anti-HSV-1 glycoprotein G monoclonal antibody (α-HSV-1 gG, Virusys Corporation) was monitored[12]. To promote the binding of α-HSV-1 gG and enhance proper orientation for further analyte binding, protein A (pA; Sigma-Aldrich, St. Louis, MO, United States) was initially immobilized on the sensor surface. Specificity of such an interaction was explored by coating two measuring channels of the sensor with α-HSV-1 gG and anti-human serum albumin (α-HSA; Sigma-Aldrich, St. Louis, MO, United States), respectively. Next, human serum albumin (HSA; Sigma-Aldrich, St. Louis, MO, United States) and HSV-1 solutions prepared in PBS were subsequently flowed in these channels. PBS was continuously flowed in the reference channel. Using such a sensing scheme, common effects such as temperature changes between channels due to the flow of different sample solutions can be rapidly compensated. A sensor response only to α-HSA – HSA and α-HSV-1 gG – HSV-1 interactions was observed while cross reactivity was insignificant. The results achieved clearly demonstrated the feasibility to specifically detect the capture of virus particles using an IO Young interferometer sensor. Furthermore, we explored the sensitivity and dynamic range of the sensor by measuring concentrations varying from ~ $10^3$ to ~$10^7$ particles/ml, classified as being "very low" (see Figure 3.A) to "very high" in terms of the viral load[13]. The phase changes measured for these concentrations demonstrated a dynamic range of 4 orders of magnitude. The clear linear relation between the sensor signal and the viral concentration enables virus concentration prediction in a given sample solution for a calibrated sensor. Knowing the phase change caused by the binding of a single virus particle ($1.1 \times 10^{-4}$ fringes), the measured virus concentrations correspond to the binding of $7 \times 10^2$ and $7 \times 10^6$ virus particles on the sensor surface, respectively. It was estimated that, given the detection limit of the sensor ($1 \times 10^{-4}$ fringes[8]), the detection of a single virus particle binding could be possible, yielding a sensor of unprecedented sensitivity. Although a complete virus binding curve can be obtained in about 1 hour, the concentration of the applied virus suspension can be estimated by measuring the response of the sensor in the first 5 minutes. The linear relation that was found between the virus concentration and the measured slope during the first 5 minutes (see Figure 3.B) allows for rapid virus detection, being relevant for screening purposes, e.g. in airports, but also to prevent the spread of viral infections.

To demonstrate the use of this type of sensor for clinical applications, complex samples such a virus suspended in serum were measured. The response of the sensor upon application of $10^5$ HSV-1 particles/ml in human serum in the measuring channel, which was previously coated with α-HSV-1 gG, indicated that, despite of the presence of many serum proteins,

the virus particle binding can be clearly detected[12]. The control experiment in which no antibody was immobilized in the measuring channel, and where no response was measured, indicated the specificity of HSV-1 – α-HSV-1 gG interaction even in complex samples such as serum.

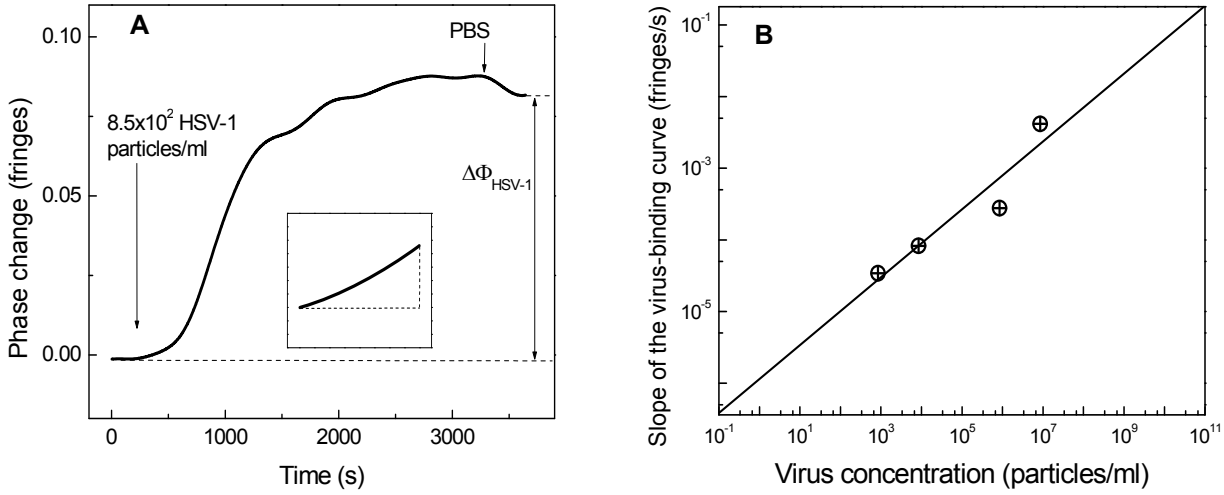


**Fig. 3. Measurement of the lowest virus concentration and fast estimation of virus concentrations.** (A) Phase change due to the binding of HSV-1 particles to a α-HSV-1 gG layer ($\Delta\Phi_{HSV-1}$) as a function of time; HSV-1 concentration was 8.5 x $10^2$ particles/ml. Note the exceptionally good signal-to-noise ratio. The inset shows the slope of the virus-binding curve in the first 5 minutes after application of the virus solution (see B). (B) Slope of the virus-binding curve measured in 5 minutes after application of the virus solution in the measuring channel (see A) vs. the virus concentration. Solid line is a linear fit of the experimental data (⊕).

The sensor was also successfully tested with other types of viruses, as well as bacteria, cells and biomarkers, being present in complex samples such as serum and milk, and in all cases a clear linear relation between the sensor signal and the specific analyte concentration was found over a dynamic range of > 4 orders of magnitude.

### 2.3 Towards a portable device

The integration of microfluidics with the optical chip offers several advantages such as reduction of the mixing between sample solutions, resulting in a short-time response, and requirement of small sample volumes. Additionally, a compact sensing system can be realized. Figure 4 shows a hybrid sensing platform obtained by bonding a glass microfluidic system to an IO four-channel YI chip[14]. The microfluidic system was structured in such a way that after bonding to the IO chip, each microfluidic channel addresses one sensing window in the four-channel YI sensor. The monitoring of α-HSA – HSA immunoreaction showed that the implementation of the microfluidics reduces the response time of the sensor from 100 s, as achieved with a bulky cuvette, to 5 s. In addition, small sample volumes (~ μL) were required[15,16,17].

Ostendum is currently developing a new, proprietary biosensor device consisting of a portable readout unit and a lab-on-a-chip (LOC) system that can be used in the field to quickly and easily detect viruses, bacteria, cells, biomarkers, etc., from human and animal body fluid and plant samples. The LOC system will be based on a newly developed chip layout. Different components of the set-up will be integrated into a portable readout unit, which could be battery-operated to enable stand-alone operation.

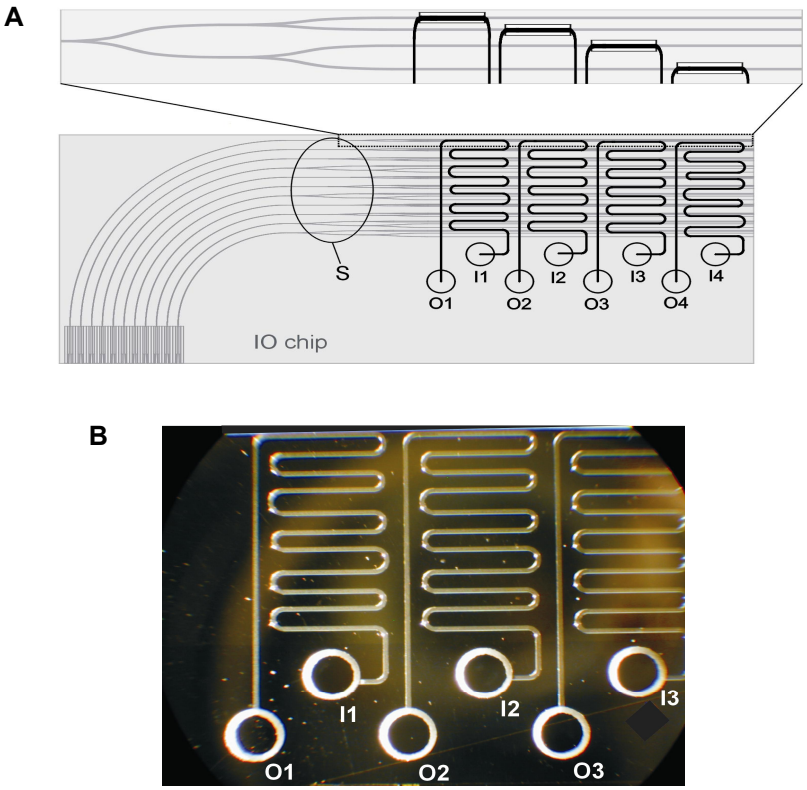


**Fig. 4. Hybrid sensing platform consisting of a glass microfluidic system to a four-channel YI chip.** (A) Top-view of the microfluidic system. The optical chip contains configurations of YI sensor structure, which have different distances between their output channels, resulting in a different length of splitting function, manifested by different starting positions of the first Y-junction (see S in A) [Reproduced with persmission from [14]. Copyright 2005 Elsevier]. (B) Close-view micrograph of microfluidic channel 1, 2, and 3. Ii and Oi indicate the input and output respectively of microfluidic channel i, addressing the ith bundle of sensing windows. Dimensions of the microfluidic sensing system are 63 mm × 24 mm.

## 3. CONCLUSIONS

In this paper we show rapid and ultrasensitive detection of microorganisms, biomarkers and the like using a biosensor based on interferometry and lab-on-a-chip nanotechnology. The sensitivity of the sensor approaches detection of a single virus particle and is, in general, ~ 2 orders of magnitude higher than other label-free sensor techniques such as the well established surface plasmon resonance sensor. Moreover, the sensor is simple, easy to use, and compact, providing excellent possibilities for development of portable point-of-care devices. The detectable concentrations of analytes in a sample approach those required in clinical diagnostics assays, which could be an attractive market for these types of sensors. This is further motivated by the ability to integrate the sensor with efficient microfluidic-based fluid delivery systems resulting in a much faster time response of the sensor. In addition the sensor manufacturing technology is suitable for mass production, allowing important scale-based costs reduction. Our ultimate goal is a point-of-care device consisting of a portable detector and a lab-on-a-chip system that can be used in the field to quickly and easily detect microorganisms from human and animal body fluid and plant samples.

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